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Functional impact of sarcopenia in respiratory muscles

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Abstract

The risk for respiratory complications and infections is substantially increased in old age, which may be due, in part, to sarcopenia (aging-related weakness and atrophy) of the diaphragm muscle (DIAm), reducing its force generating capacity and impairing the ability to perform expulsive non-ventilatory motor behaviors critical for airway clearance. The aging-related reduction in DIAm force generating capacity is due to selective atrophy of higher force generating type IIx and/or IIb muscle fibers, whereas lower force generating type I and IIa muscle fiber sizes are preserved. Fiber type specific DIAm atrophy is also seen following unilateral phrenic nerve denervation and in other neurodegenerative disorders. Accordingly, the effect of aging on DIAm function resembles that of neurodegeneration and suggests possible common mechanisms, such as the involvement of several neurotrophic factors in mediating DIAm sarcopenia. This review will focus on changes in two neurotrophic signaling pathways that represent potential mechanisms underlying the aging-related fiber type specific DIAm atrophy.

Keywords

Diaphragm muscle; Aging; Atrophy; Brain derived neurotrophic factor; Neuregulin; Denervation

1. Introduction

The population of elderly (>65 years of age) individuals is steadily increasing worldwide, e.g., in the USA, the elderly population will more than double by 2030, exceeding 80 million people (Federal Interagency Forum on Aging-Related Statistics, 2012). This demographic shift will bring a multitude of challenges, including an increased economic burden secondary to a greater incidence of chronic disease. Respiratory diseases associated with old age, such as pneumonia (3-fold higher incidence in those >65 years of age) (Chong and Street, 2008; Janssens and Krause, 2004), already account for ~7% of direct healthcare expenditures in the USA amounting to nearly $100 billion (National Institutes of Health: Fact Book Fiscal Year, 2012). Not only do these respiratory diseases diminish the quality of life in old age (Berry et al., 2012), they are also a leading cause of death in the elderly population (Heron, 2011; Xu et al., 2010). Indeed, the elderly are at greater risk for hospitalization and many are admitted to intensive care units because of respiratory failure

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frequently requiring prolonged mechanical ventilation (Creditor, 1993; de Jonghe et al., 2002, 2009; Hamel et al., 2005; Ray et al., 2006; Turrentine et al., 2006). For this reason, understanding the etiology of aging-related respiratory disease is vital to the future development of therapeutic strategies designed to lessen this increasing economic burden (Kirkland and Peterson, 2009).

2. Sarcopenia

Irwin Rosenberg proposed the term ‘sarcopenia’ in 1989 to describe the aging-related decrease of skeletal muscle mass, based on the Greek terms ‘sarx’ for flesh and ‘penia’ for poverty (Rosenberg, 1989). Later, Evans revised this definition of sarcopenia to include an aging-related loss in skeletal muscle strength (Evans, 1995; Evans and Campbell, 1993); a description that aligns with current consensus definitions (Cesari et al., 2012; Cruz-Jentoft et al., 2010; Evans, 2010; Fielding et al., 2011). In humans, the progressive aging-related decline in skeletal muscle mass and strength (sarcopenia) begins at ~30 years of age (similar to other physiologic systems; (Sehl and Yates, 2001)), progressing thereafter at a rate of 0.5–1% of muscle mass lost per year with a more rapid decline in humans >65 years of age (Frontera et al., 2000; Lexell et al., 1988; Nair, 2005). Although the specific mechanisms mediating sarcopenia remain poorly understood, its etiology appears to be multifactorial and sarcopenia can often co-exist with other diseases or conditions that are also associated with cachexia (e.g., inactivity and muscle disuse, cancer, poor nutrition, chronic inflammation, insulin resistance, declining levels of anabolic hormones, etc.) (Biolo et al., 2014; Buford et al., 2010; Evans, 2010; Muscaritoli et al., 2010; Thomas, 2007; Thompson, 2007). In our studies, we are exploring whether sarcopenia involves aging-related alterations in neurotrophic influences affecting the entire motor unit (Fig. 1), which comprises a motor neuron and the muscle fibers it innervates through the neuromuscular junction (NMJ).

3. Muscle fiber type and motor unit classification

The concept of the motor unit as the basic functional element of neuromotor control was first proposed by Sherrington in 1925 (Liddell and Sherrington, 1925). Motor units are classified into specific types based on the mechanical and fatigue properties of the muscle fibers comprising each unit (Burke, 1981; Burke et al., 1971, 1973; Fournier and Sieck, 1988a). Accordingly, fast-twitch (type F) and slow-twitch (type S) motor units are distinguished by the rate of twitch force generation, as well as the presence (type F) or absence (type S) of “sag” in an unfused tetanic force response. Type F motor units are further sub-classified into three distinct types based on their resistance to fatigue during tetanic stimulation: fast-twitch fatigue resistant (type FR); fast-twitch fatigue intermediate (type FInt); and fast-twitch fatigable (type FF). Type S motor units are fatigue resistant. In the diaphragm muscle (DIAm), motor unit types also vary in their contractile strength, with type FInt and FF motor units generating greater peak twitch (P_t) and maximum tetanic (P_o) force compared to type FR and S motor units (Fournier and Sieck, 1988a; Sieck, 1988, 1991, 1994; Sieck et al., 1989a).

Each motor unit type is composed of muscle fibers that are homogeneous with respect to their metabolic properties and contractile protein composition, specifically the expression of

myosin heavy chain (MyHC) isoforms (Enad et al., 1989; Gransee et al., 2012; Greising et al., 2012; Sieck, 1994; Sieck et al., 1989a, 1996). In fact, this relationship forms the basis of muscle fiber type classification (Brooke and Kaiser, 1970; Edstrom and Kugelberg, 1968; Schiaffino et al., 1989; Sieck et al., 1985). Accordingly, type S motor units comprise type I muscle fibers that have higher oxidative capacity and express a $MyHC_{Slow}$ isoform. Type FR motor units comprise type IIa muscle fibers that also have higher oxidative capacity and express the $MyHC_{2A}$ isoform. More fatigable type FInt motor units comprise type IIx muscle fibers that have lower oxidative capacity and express the $MyHC_{2X}$ isoform. Type FF motor units comprise lower oxidative type IIb muscle fibers primarily expressing $MyHC_{2B}$ although co-expression of the $MyHC_{2X}$ isoform in these fibers commonly occurs (Sieck, 1995; Sieck et al., 1989a). The mechanical properties of single muscle fibers reflect the expression of MyHC isoforms (Geiger et al., 1999, 2000, 2001a,b, 2002), which underlies differences in motor unit force generation. In addition, the number of muscle fibers innervated by each motor neuron (innervation ratio) varies, which also contributes to differences in motor unit force generation. Motor unit fatigue resistance is associated with differences in mitochondrial volume density and the oxidative capacity of muscle fiber types (Sieck et al., 1989b, 1996).

Often in pathological conditions, e.g., hypothyroidism (Geiger et al., 2002), spinal muscular atrophy (Ben Hamida et al., 1994) and amyotrophic lateral sclerosis (Baloh et al., 2007), muscle fibers co-express MyHC isoforms obviating unambiguous fiber type classification. In vastus lateralis muscle biopsies from subjects ranging in age from 21 to 66 years of age, we found a significant number of fibers from older subjects co-expressed $MyHC_{Slow}$ and $MyHC_{2A}$ isoforms, as well as $MyHC_{2X}$ and $MyHC_{2A}$ isoforms (Han et al., 2001). These results were consistent with a prior study by Klitgaard et al. (1990) in which they found an increased incidence of co-expression of both $MyHC_{Slow}$ and $MyHC_{2A}$ and $MyHC_{2A}$ and $MyHC_{2B}$ isoforms in medial vastus lateralis and medial biceps brachii muscle fibers from older (68–70 years of age) compared to younger (21–31 years of age) men. In addition to pathological conditions and aging, this type of co-expression of MyHC isoforms also occurs following denervation and removal of neurotrophic influence on muscle fibers (Geiger et al., 2001a); thus, there may be a common underlying mechanism responsible for the loss of matching between motor neurons and muscle fiber type. We believe this common mechanism involves the influence of neurotrophic signaling (see below).

3.1. Motor unit recruitment and frequency coding

Charles Sherrington first proposed that motor units are recruited in an all-or-none fashion, such that force generated by the whole muscle represents the sum of forces produced by the recruitment (recruitment coding) and frequency of activation (frequency coding) of individual motor units (Liddell and Sherrington, 1925; Sherrington, 1925, 1929). Later, Elwood Henneman proposed a mechanism to explain the recruitment of motor units that relates to the intrinsic size-related properties of motor neurons—Henneman's "Size Principle" (Henneman, 1957; Henneman and Olson, 1965; Henneman et al., 1965). According to the Size Principle, both intrinsic membrane resistance and membrane capacitance (Cm) of a motor neuron is size dependent. For a given level of synaptic input (i.e., input current; Ic), the rate of change of membrane potential (dVm/dt—reflecting motor

neuron excitability), is inversely proportional to Cm; such that, $dVm/dt = Ic/Cm$. Thus, for a given level of synaptic input, smaller motor neurons, with a lower Cm are more excitable (increased dVm/dt) compared to larger motor neurons. Motor neurons innervating type S motor units are the smallest and therefore more excitable and recruited first during motor behaviors followed in rank order by motor neurons innervating type FR, FInt, and FF motor units (Burke, 1975; Henneman and Mendell, 1981). Frequency coding of motor units also depends on intrinsic size-related membrane properties of motor neurons (Kernell, 1983, 2003), such that, type S motor units have the lowest onset and peak discharge rates, whereas type FR, FInt and FF motor units display increasingly higher onset and peak discharge rates (Sieck et al., 1984, 2013). The range of discharge rates (onset to peak) of motor neurons generally matches the force-frequency characteristics of motor units, thereby optimizing frequency coding of motor unit force production (Fournier and Sieck, 1988a; Kernell, 2003; Seven et al., 2014; Sieck et al., 1984).

3.2. Diaphragm motor unit recruitment

In 1988, we introduced a model of DIAm neuromotor control across a range of motor behaviors, in which we assumed that DIAm motor unit recruitment order was consistent with Henneman's Size Principle (Sieck, 1988; Sieck and Fournier, 1989). Indeed, it is now clear that DIAm motor units obeyed the Size Principle (Dick et al., 1987; Jodkowski et al., 1987, 1988; Seven et al., 2014). This model of DIAm motor unit recruitment also depends on estimates of the proportion of motor unit types and the forces they contribute. The proportion of different motor unit types can be estimated based on the proportion of different muscle fiber types and measurements of the innervation ratio of each motor unit type (based on glycogen depletion) (Enad et al., 1989; Fournier and Sieck, 1988b). The force generated by each motor unit type depends on the total cross-sectional area (CSA) of the muscle fibers comprising each unit, which can be estimated from fiber type proportion, average fiber CSA and the innervation ratio. In some studies, we directly measured the force generated by different motor unit types by isolating single motor units following dissection of cervical ventral root filaments and graded electrical stimulation (verified by waveform analysis of evoked motor unit action potentials) (Fournier and Sieck, 1988a). In other more recent studies, we measured the specific force of single permeabilized DIAm fibers (type identified by MyHC isoform expression) activated by extracellular Ca^{2+} (Geiger et al., 2000, 2001a, 2002). Using *in vivo* measurements of transdiaphragmatic pressure (Pdi—a surrogate of DIAm force), we determined DIAm force generation across a range of ventilatory and higher force non-ventilatory motor behaviors (Fig. 2). Based on our model of DIAm motor unit recruitment, both eupneic ventilation and ventilation stimulated by a hypoxic-hypercapnic gas mixture (10% O_2, 5% CO_2, balance N_2) require the recruitment of only type S and FR motor units, whereas higher force non-ventilatory motor behaviors require the additional recruitment of more fatigable type FInt and FF motor units (Fournier and Sieck, 1988a; Gransee et al., 2012; Greising et al., 2012; Mantilla et al., 2010; Mantilla and Sieck, 2011; Sieck, 1988, 1989, 1990, 1991, 1994, 1995; Sieck et al., 1989a, 2013; Sieck and Fournier, 1989; Sieck and Gransee, 2012).

3.3. Diaphragm muscle sarcopenia and motor unit recruitment

The presence of sarcopenia is well characterized in limb muscles in both humans (Doherty, 2003; Proctor et al., 1995) and rodent models of aging (Brooks and Faulkner, 1988). Sarcopenia also exists in the DIAm. For example, work from our lab in Fischer 344 rats showed an ~20% reduction in DIAm specific force in old (24 months of age; 50% survival) compared to young (6 months of age; 100% survival) animals (Gosselin et al., 1994). Subsequent work in Fischer 344 (Criswell et al., 1997; Powers et al., 1996; Smith et al., 2002) Fischer 344 × Brown Norway (Criswell et al., 2003) and Wistar (Imagita et al., 2009) rats confirmed an aging-related reduction in DIAm specific force. An aging-related reduction in DIAm specific force has also been reported in golden hamsters (Zhang and Kelsen, 1990). More recently, work by our group used a mouse model (C57BL/6 × 129) of natural aging (6 and 24 months of age, corresponding to 100% and 75% survival, respectively) to investigate DIAm sarcopenia. In these studies, we showed an ~25% reduction in DIAm specific force (Fig. 3A) (Greising et al., 2013, 2015b) together with a selective atrophy of type IIx and/or IIb DIAm fibers (Fig. 3B). Importantly, the CSA of type I and IIa DIAm fibers was not reduced in older mice. In our earlier study (Greising et al., 2013), we also found that in the transgenic *BubR1*$^{H/H}$ mouse model of early onset aging, there was fiber type specific DIAm fiber atrophy and a reduction in specific force characteristic of sarcopenia in the naturally aging mice.

Overall, it appears that DIAm sarcopenia exists and that muscle fiber atrophy and weakness is fiber type specific such that the higher force-generating type IIx and/or IIb fibers are most affected whereas lower force-generating type I and IIa fibers are spared. These results are consistent with work in limb muscles in which it has been reported that aging-related atrophy predominantly affects type IIx and/or IIb fibers, whereas CSA and force-generating capacity of type I and IIa fibers is relatively preserved (Aniansson et al., 1986; Grimby et al., 1982; Larsson et al., 1978; Lexell et al., 1988; Nilwik et al., 2013; Oertel, 1986; Proctor et al., 1995; Tomonaga, 1977). Importantly, an effect of sarcopenia on type IIx and/or IIb fibers would impact the relative contributions of more fatigable type FF and FInt motor units. Thus, lower force motor behaviors would be minimally affected, whereas higher force motor behaviors would be primarily impacted (Fig. 2). For the DIAm, this would involve higher force non-ventilatory behaviors primarily involved in airway clearance.

The functional consequence of DIAm sarcopenia in elderly humans was explored by Tolep et al., where the maximum Pdi generated through a combined expulsive-Mueller maneuver was found to be ~25% lower in older (65–75 years of age) individuals (Tolep et al., 1995). Subsequently, Polkey et al. confirmed aging-related DIAm weakness in subjects 67–83 years old as reflected by a ~14% reduction in Pdi generated by a maximal sniff test (Polkey et al., 1997). Similar to the performance of expulsive, airway clearance maneuvers (e.g., coughing and sneezing), the performance of these maximal inspiratory efforts requires the recruitment of all available motor units, particularly the higher force generating type FInt and FF motor units (Fig. 2) (Fournier and Sieck, 1988a; Greising et al., 2012; Mantilla et al., 2010; Sieck, 1988, 1991, 1994; Sieck et al., 1989a). Accordingly, the aging-related reduction in maximum DIAm strength, does not appear to impact the ability to sustain normal ventilatory behaviors (i.e., eupneic ventilation in normoxia) but does impair the ability to perform

higher force, expulsive airway clearance maneuvers (Greising et al., 2015b). In this way, DIAm sarcopenia may put elderly individuals at an increased risk for developing pneumonia or other respiratory system infections.

4. Aging-related motor neuron loss and sarcopenia

Although the underlying etiology and pathogenesis for the aging-related selective atrophy of type IIx and/or IIb fibers in the DIAm and other muscles remains undetermined, evidence suggests that the entire motor unit (motor neuron, NMJ, and muscle fibers) is involved, which may reflect alterations in neurotrophic influence (Fig. 1). There is an aging-related progressive loss of motor neurons innervating limb muscles (Brown, 1972; Kwan, 2013; Lexell et al., 1988; McNeil et al., 2005; Rosenheimer, 1990; Tomlinson and Irving, 1977) that is correlated with the presence of sarcopenia (Drey et al., 2014). This frank loss of motor neurons results in denervation of muscle fibers, with possible reinnervation by remaining motor neurons (via axonal sprouting), and consequent motor unit expansion (Gordon and Stein, 1982). With axonal sprouting and reinnervation, there is fiber type transition leading to a clustering of fiber types. In a recent study, we found an aging-related increase in fiber type clustering in the mouse DIAm providing indirect evidence of motor neuron loss (Greising et al., 2015c). Importantly, in a number of studies, we have shown that denervation of DIAm fibers induces a decrease in maximum specific force and a selective atrophy of type IIx and/or IIb fibers (Geiger et al., 2001a; Miyata et al., 1995; Zhan et al., 1997), a pattern very similar to aging-related sarcopenia. Thus, motor neuron loss and denervation may at least partially underlie sarcopenia.

In a study, examining the effect of unilateral phrenic nerve denervation on the morphological and mechanical properties of single rat DIAm fibers (Geiger et al., 2001a), we reported that at 14 days after denervation, there was atrophy of type IIx and/or IIb fibers and a decrease in maximum Ca^{2+}-activated force (similar to sarcopenia). Furthermore, there was a decrease in MyHC content per half-sarcomere in type IIx and/or IIb DIAm fibers. Importantly, maximum force was reduced even when normalized for CSA (specific force) as well as MyHC content per half-sarcomere (indicating reduced force per cross bridge). In contrast, unilateral denervation slightly increased the CSA of type I and IIa fibers with no effect on MyHC content per half-sarcomere. However, denervation did induce a decrease in specific force in type I and IIa DIAm fibers, but much less in contrast to that observed in type IIx and/or IIb fibers. It is unlikely that the selective atrophy of type IIx and/or IIb DIAm fibers following unilateral phrenic nerve denervation is due to muscle paralysis and inactivity *per se*, since there is no change in the CSA of type IIx and/or IIb fibers following DIAm paralysis induced by unilateral spinal hemisection at C2 (Miyata et al., 1995; Prakash et al., 1999; Zhan et al., 1997). Accordingly, we concluded that the selective atrophy and reduction of specific force of type IIx and/or IIb DIAm fibers was due to the removal of a neurotrophic influence (Fig. 1). Thus, it is likely that an aging-related disruption in neurotrophic influence (either via motor neuron loss or decreased expression) underlies DIAm sarcopenia (Fig. 4).

4.1. Aging reduces neurotrophic influence on muscle fibers

The importance of neurotrophic interactions in the establishment and maintenance of muscle fiber properties was first demonstrated in 1960 by Buller et al. (1960) in a study in which the nerves innervating the flexor digitorum longus (predominantly fast) and soleus (predominantly slow) muscles were cut and then switched resulting in reinnervation and a reversal of the mechanical properties of these muscles. It is possible that the pattern of neural activation (e.g., frequency of activation) caused the switch in mechanical properties; however, it is now apparent that motor unit-specific neurotrophic factors are primarily responsible. The problem is which ones?

One potential candidate that we are exploring is neuregulin (NRG), which is a member of the epidermal growth factor family of trophic factors that acts through receptor tyrosine kinases of the epidermal growth factor (ErbB) family. To date, six NRG genes (NRG1-6) have been identified, each with multiple splice isoforms (e.g., >30 for NRG-1) that interact with four different ErbB receptors (ErbB1-4) (Buonanno and Fischbach, 2001; Burden and Yarden, 1997; Falls, 2003; Mei and Nave, 2014; Mei and Xiong, 2008; Yarden and Sliwkowski, 2001; Zhu et al., 1995). The number of potential permutations between NRG and ErbB is further increased by ErbB receptor dimerization, which allows for 6 different ErbB receptor complexes (Jones et al., 1999). We have primarily focused on NRG-1 and NRG-2, due to their relevant expression in muscle fibers and motor neurons (Falls et al., 1993; Moscoso et al., 1995; Rimer et al., 2004; Trinidad et al., 2000; Zhu et al., 1995). Neither NRG-1 nor NRG-2 appreciably binds ErbB1, and both are unable to bind ErbB2. Although NRG-1 and NRG-2 can bind ErbB3 and ErbB4, ErbB3 lacks a functional tyrosine kinase domain and requires dimerization with other ErbB receptor complexes for activation. Compared to ErbB3 and ErbB4 homodimers, the ErbB2/3 and ErbB2/4 heterodimers display an ~100 fold greater binding affinity for NRG-1 and NRG-2 (Jones et al., 1999).

We found that activation of NRG/ErbB signaling has an anabolic effect in DIAm and therefore, may have an integral role in the maintenance of muscle mass (Hellyer et al., 2006). The signal transduction initiated from NRG/ErbB binding in muscle is mediated by phosphorylation of the ErbB receptor and subsequent activation of the phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) intracellular pathways (Citri et al., 2003). Indeed, activation of PI3K/Akt and subsequent initiation of mammalian target of rapamycin (mTOR) signaling is a critical regulator of muscle protein synthesis (Argadine et al., 2009; Argadine et al., 2011; Bodine et al., 2001). Although all four ErbB receptors can activate PI3K/Akt/mTOR signaling, the ErbB2/3 receptor heterodimer appears uniquely suited to activating the PI3K/Akt/mTOR pathway in DIAm (Hellyer et al., 2001).

The anabolic effect of NRG/ErbB signaling makes the removal of this neurotrophic factor particularly well suited to mediate the aging-related atrophy of muscle fibers due to a negative protein balance, with a reduction in MyHC content per half-sarcomere and resulting decrease in force generation (Fig. 1). At the present time it is unknown whether there are fiber type specific changes in NRG/ErbB signaling that may contribute to muscle fiber atrophy and weakness characteristic of sarcopenia.

4.2. Neurotrophic signaling at phrenic motor neurons

It is clear that neural-derived trophic influence has a pro-found effect on determining muscle fiber metabolic and mechanical properties within motor units, but neurotrophic factors can also substantially influence properties of the motor neuron as well. Our own studies have focused on brain-derived neurotrophic factor (BDNF), which is a member of the neurotrophin family that includes nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4), all of which act through sub-classes of the high affinity tropomyosin-related kinase (Trk) receptors (Koliatsos et al., 1993; Reichardt, 2006). Acting through its high affinity Trk subtype B receptor (TrkB), BDNF is broadly involved in several aspects of motor unit function, including the regulation of motor neuron survival during embryonic development (pruning process) and possibly during aging (Fig. 1), enhancement of presynaptic release of neurotransmitters and postsynaptic regulation of receptor expression (c.f. Kalinkovich and Livshits, 2015; Raschke and Eckel, 2013; Sakuma et al., 2015).

Recently, we demonstrated a role for BDNF/TrkB signaling in enhancing functional recovery of rhythmic phrenic motor neuron activity following upper cervical spinal cord injury (SCI) (Gransee et al., 2013, 2015; Mantilla et al., 2013, 2014a). Intrathecal infusion of BDNF in the cervical spinal cord promoted recovery after SCI, whereas competitive inhibition of BDNF by intrathecal infusion of TrkB-Fc blunted recovery. Knocking down TrkB receptor expression in phrenic motor neurons by intrapleural injection of siRNA also blunted recovery after SCI, while increasing TrkB receptor expression using an AAV7 vector enhanced recovery. Inhibition of TrkB kinase activity in a knockin $TrkB^{F616A}$ mouse model (reversible inhibition that is sensitive to the phosphoprotein phosphatase-1 derivative, 1NMPP1) also blunted functional recovery after SCI. The postsynaptic effect of BDNF/TrkB signaling at phrenic motor neuron is to enhance excitability through an increased expression of serotonergic (5HT2A) and glutamatergic (NMDA and GluR) receptors (Mantilla et al., 2012).

4.3. Aging effect on neuromuscular transmission

There is substantial evidence of an aging-related remodeling of the NMJ (Deschenes et al., 2010; Fahim and Robbins, 1982; Gutmann and Hanzlikova, 1965; Kung et al., 2014; Miyata et al., 2008; Prakash and Sieck, 1998; Suzuki et al., 2009; Valdez et al., 2010). Previous work from our laboratory showed aging-related remodeling of NMJs in DIAm from 24-month old Fischer 344 rats (75% survival) with motor end-plate fragmentation found predominantly at type IIx and/or IIb fibers (Prakash and Sieck, 1998). Importantly, a similar fiber type specific remodeling of DIAm NMJs was also reported following DIAm paralysis induced by unilateral spinal hemisection at C2 (Prakash et al., 1999). Cardasis and LaFontaine also reported aging-related NMJ remodeling in the rat DIAm (~24 month) with an increased incidence of frank denervation (Cardasis and LaFontaine, 1987). Deschenes et al. (2010) reported that aging-related NMJ remodeling occurred at muscle fibers in the plantaris and soleus muscles in Fischer 344 rats during early stages of aging (21 month; 80% survival). It should be noted that in these muscles, aging-related NMJ remodeling occurred before muscle fiber atrophy. Accordingly, an aging-related disruption of normal neurotrophic signaling, by way of denervation, may induce morphological and mechanical

adaptations of DIAm fibers (predominantly type IIx and/or IIb) that are characteristic of sarcopenia (Fig. 1).

In previous studies, we assessed neuromuscular transmission failure in the DIAm by repetitive stimulation of the phrenic nerve with periodic superimposition of direct muscle stimulation (Kuei et al., 1990; Sieck and Prakash, 1995). In this technique, if neuromuscular transmission failure occurs, the DIAm does not fatigue and there is a greater difference in the forces evoked by nerve vs. direct muscle stimulation (Fig. 5). Using this technique, we demonstrated that significant neuromuscular transmission failure occurs in the DIAm especially at higher rates of stimulation (e.g., >40 Hz). For example, neuromuscular transmission failure accounts for ~43% of rat DIAm force loss at 75 Hz stimulation (Kuei et al., 1990). To assess whether neuromuscular transmission failure was fiber type specific, we used a glycogen depletion technique in which muscle fiber glycogen stores are not depleted in fibers susceptible to neuromuscular transmission failure during repetitive stimulation. Using this technique we demonstrated that type IIx and/or IIb fibers in the rat DIAm are most susceptible to neuromuscular transmission failure (Johnson and Sieck, 1993). Later, it was shown that during repetitive phrenic nerve stimulation, terminals innervating type IIx and/or IIb DIAm fibers demonstrated significantly less synaptic vesicle recycling compared to terminals innervating type I and IIa DIAm fibers (Mantilla et al., 2004a). Furthermore, we showed that during repetitive phrenic nerve stimulation quantal release of acetylcholine (ACh) is depressed to a greater extent at type IIx and/or IIb DIAm fibers, due to a decreased probability of release at nerve terminals (Rowley et al., 2007). With a decrease in quantal ACh release at type IIx and/or IIb DIAm fibers, end-plate potential (EPP) amplitude falls below the safety factor (i.e., ratio of EPP amplitude to action potential activation threshold) and neuromuscular transmission fails (Ermilov et al., 2007).

In hippocampal neurons, exogenous BDNF treatment increases quantal content, and the frequency of miniature excitatory postsynaptic potentials (mEPSPs), indicating an effect on the probability of presynaptic neurotransmitter release (Tyler and Pozzo-Miller, 2001; Tyler et al., 2006). Through this mechanism, BDNF/TrkB signaling improves synaptic transmission (Boulanger and Poo, 1999; Lohof et al., 1993). We also found that at the rat DIAm NMJ, BDNF increases quantal release of ACh (unpublished observations), and thereby improves synaptic transmission (Greising et al., 2015a; Mantilla and Ermilov, 2012; Mantilla et al., 2004b; Zhan et al., 2003). This effect of BDNF/TrkB signaling on synaptic transmission is particularly evident at the DIAm NMJ (Greising et al., 2015a; Mantilla and Ermilov, 2012; Mantilla et al., 2004b, 2014b; Zhan et al., 2003).

In a recent study, we showed an aging-related increase in neuromuscular transmission failure in the mouse DIAm (Fig. 5) (Greising et al., 2015a). Furthermore, we found that aging diminishes the beneficial impact of BDNF/TrkB signaling on neuromuscular transmission. In younger mice and rats (3–6 months of age), BDNF/TrkB signaling significantly improves neuromuscular transmission (Fig. 5) (Greising et al., 2015a; Mantilla et al., 2004b). A similar effect is seen using the BDNF agonist, 7,8-dihydroxyflavone (Mantilla and Ermilov, 2012). In contrast, inhibition of TrkB using the non-specific tyrosine kinase inhibitor, K252a (Mantilla et al., 2004b) or more specifically by inhibiting TrkB tyrosine kinase activity in a knockin $TrkB^{F616A}$ mouse model sensitive to 1NMPP1 (Greising et al., 2015a; Mantilla et

al., 2014b), markedly impairs neuromuscular transmission. However, in older mice (24 months of age), the beneficial effects of BDNF/TrkB signaling on DIAm neuromuscular transmission are largely absent (Fig. 5) (Greising et al., 2015a). Furthermore, in *TrkB*^{F616A} mice at 24 months of age, inhibition of TrkB kinase activity (by 1NMPP1 treatment) has no effect on neuromuscular transmission (Fig. 5). Interestingly, in *TrkB*^{F616A} mice at 18 months of age, BDNF improved neuromuscular transmission, while 1NMPP1-induced inhibition of TrkB kinase activity had no effect on neuromuscular transmission (Fig. 5). Together, these results suggest that with aging there is a reduction in endogenous BDNF production in phrenic motor neurons that precedes alterations in TrkB expression and/or activity (Fig. 1).

5. Conclusion

As the elderly population increases across the world, so will the economic burden associated with a greater incidence of aging-related chronic diseases, particularly the ubiquitous patho-physiological effects of sarcopenia. Unlike sarcopenia of the limb muscles that manifests as systemic weakness, an increased risk for falling and limitations in activities of daily living, sarcopenia of the respiratory muscles rarely contributes to ventilatory failure. Instead, respiratory muscle sarcopenia, particularly of the DIAm, manifests as an impaired ability to generate higher levels of Pdi necessary for expulsive airway clearance behaviors (e.g., coughing and sneezing). Accordingly, DIAm sarcopenia may directly contribute to the significantly increased risk for pneumonia and other respiratory infections common in elderly populations. Unfortunately, the mechanisms underlying sarcopenia remain unknown. However, the similarity in the physiological consequences associated with sarcopenia and unilateral phrenic nerve denervation suggest a common mechanism. With unilateral phrenic nerve denervation, neurotrophic influence on DIAm fibers is removed resulting in atrophy and decreased specific force that is most pronounced in type IIx and/or IIb fibers. Importantly, with aging, there are similar morphological and functional changes in type IIx and/or IIb DIAm fibers that may reflect a progressive removal of neurotrophic influence. In this regard, there is evidence for a loss of phrenic motor neurons with aging. Two potential neurotrophic signaling pathways may be affected by aging. At DIAm fibers, nerve-derived NRG/ErbB signaling exerts an anabolic effect that maintains contractile protein content in type IIx and/or IIb DIAm fibers. The influence of NRG/ErbB signaling may be lost during aging by the loss of phrenic motor neurons. At phrenic motor neurons, BDNF/TrkB signaling may be essential to promote motor neuron survival, possibly by enhancing synaptic transmission. An aging-related decrease in BDNF expression and/or a decrease in TrkB signaling may lead to phrenic motor neuron loss. Together, NRG/ErbB and BDNF/TrkB provide important regulatory signals serving to maintain synaptic transmission, integrity of the neuromuscular junction and muscle fiber mass. Further work aimed at investigating the aging-related impairments in the signaling of these neurotrophic factors may provide important mechanistic insight into the etiology of not only sarcopenia, but potentially other neurodegenerative diseases as well.

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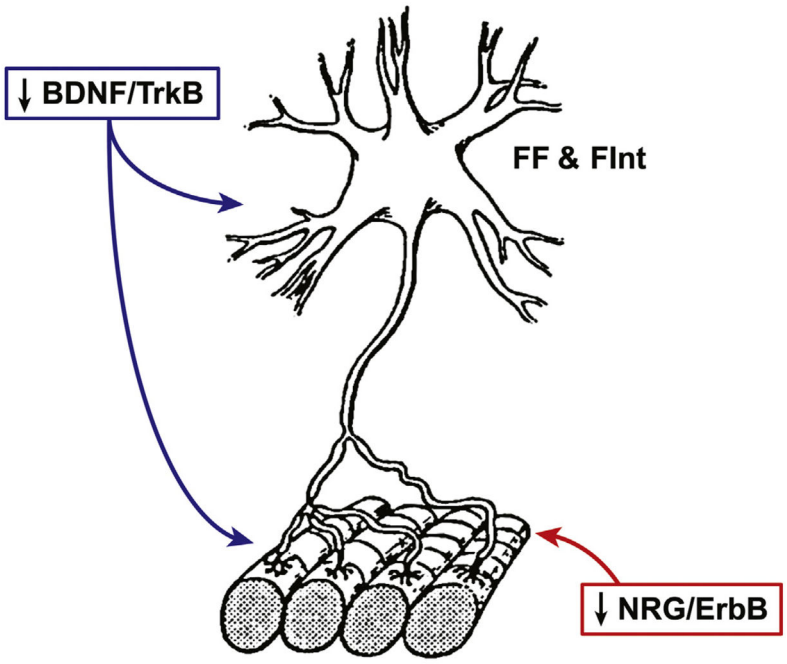

Fig. 1.

Schematic illustrating a fast-twitch fatigable (type FF) and fast-twitch fatigue intermediate (type FInt) motor unit. Muscle fibers corresponding to type FF and FInt motor units are most susceptible to atrophy and weakness secondary to the natural aging process and denervation. Two neurotrophic factors that may be mediating this fiber type specific effect of atrophy and weakness are: (1) brain derived neurotrophic factor (BDNF) acting through the tropomyosin-related kinase receptor B (TrkB) which is involved in motor neuron survival, excitability, and neuromuscular synaptic transmission, and (2) the trophic factor family of neuregulins (NRG) which activate tyrosine kinases of the ErbB receptor family that is released from the motor neuron exerting an anabolic effect on muscle fibers and is implicated in the matching of motor neuron to muscle fiber properties.

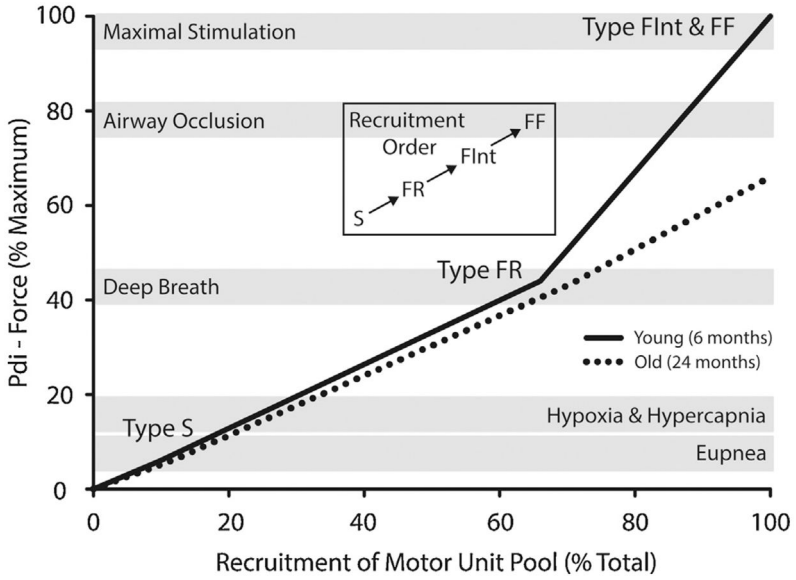

Fig. 2.

Model of diaphragm muscle motor unit recruitment during ventilatory and non-ventilatory behaviors in mice at 6 and 24 months of age. Motor units are assumed to be recruited in an orderly fashion in rank order beginning with slow-twitch (Type S), followed by fast-twitch fatigue resistant (Type FR), fast-twitch fatigue intermediate (Type FInt), and finally fast-twitch fatigable (Type FF) motor units. Ventilatory behaviors can be accomplished without the recruitment of more fatigable motor units (i.e., FInt and FF). Recruitment of these motor units is only required during more forceful non-ventilatory behaviors that are associated with clearing the airways. Data adapted from (Greising et al., 2015b).

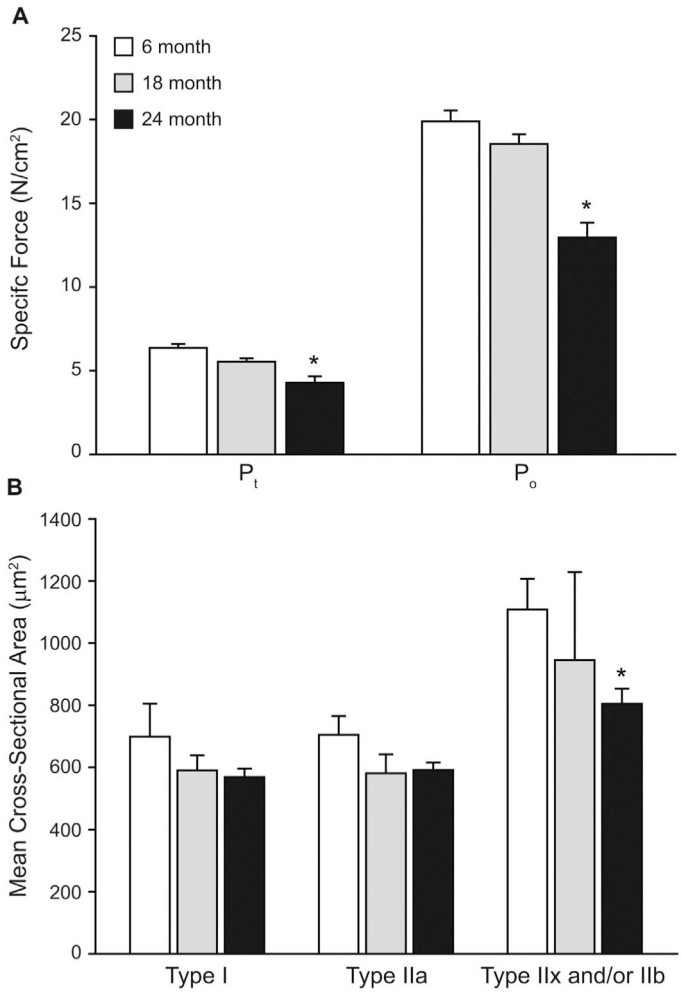

Fig. 3.

Diaphragm muscle sarcopenia is event by ~75% survival in mice. (**A**) Maximal isometric twitch force (P_t) and maximal tetanic force (P_o), normalized to physiological cross-sectional area, of midcostal diaphragm muscle (DIAm) of mice across the lifespan (aged 6, 18 and 24 months). There is a significant (*) loss of force by 24 months of age but not between 6 and 18 months. Reprinted with permission from (Greising et al., 2015a). (**B**) Fiber cross-sectional area (CSA) of the DIAm of across the lifespan. There is a significant (*) loss of type IIx and/or IIb DIAm fiber CSA by 24 months of age. Data at 6 and 24 months of age adapted from (Greising et al., 2013), data at 18 months of age is pilot ($n = 4$). Mean ± SE.

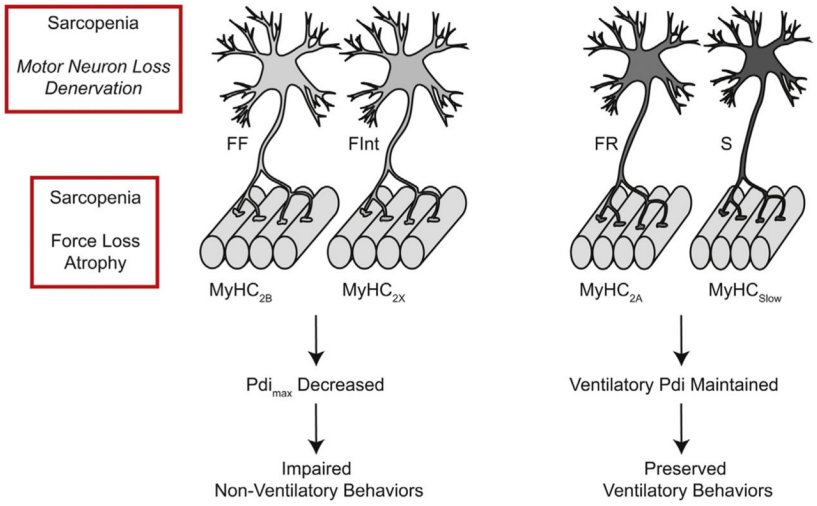

Fig. 4.

Schematic illustrating the aging-related functional consequences of diaphragm muscle (DIAm) fiber type specific sarcopenia (atrophy and weakness). In this model, DIAm sarcopenia is predominantly present in the fast-twitch fatigable (type FF) and fast-twitch fatigue intermediate (type FInt) motor units, which in turn are associated with type IIb and IIx DIAm fibers that express the $MyHC_{2B}$ and $MyHC_{2X}$ isoforms, respectively. In contrast, DIAm sarcopenia is less evident in the fast-twitch fatigue resistant (type FR) and slow (type S) motor units, which in turn are associated with type IIa and I DIAm fibers that express the $MyHC_{2A}$ and $MyHC_{Slow}$ isoforms, respectively. The functional consequences of this fiber type specific effect of DIAm sarcopenia is a decrease in the maximum transdiaphragmatic pressure (Pdi_{max}) the DIAm is capable of generating, and therefore an impaired ability to perform expulsive, high force non-ventilatory behaviors (i.e., coughing and sneezing). However, the Pdi required to perform ventilatory behaviors remains preserved.

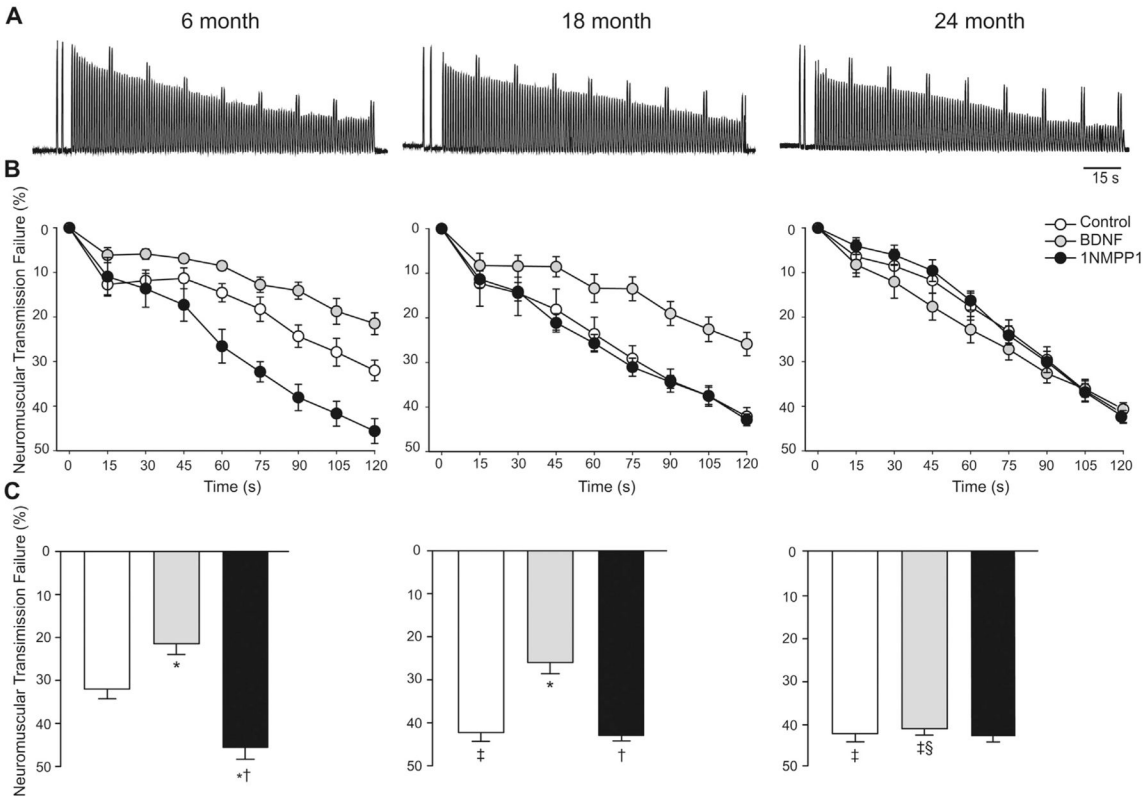

Fig. 5.

Across the lifespan in mice (aged 6, 18 and 24 months) the contribution of neuromuscular transmission failure to diaphragm muscle fatigue over a 2-min period of repetitive nerve stimulation and superimposed intermittent muscle stimulation. (**A**) Representative tracings for control (vehicle-treated) diaphragm muscle-phrenic nerve preparations of each age group. (**B**) Time course of neuromuscular transmission failure during repetitive stimulation in control, brain derived neurotrophic factor (BDNF), or 1NMPP1-treated (inhibition of TrkB kinase activity) preparations at each age group, all for 30 min in vitro. In all age and treatment groups, there is progressively greater neuromuscular transmission failure over time. (**C**) Neuromuscular transmission failure following 2 min of repetitive stimulation in control, BDNF- and 1NMPP1-treated groups. *Significantly different from control at the same age; †significantly different from BDNF at the same age; ‡significantly different from 6 months within the same treatment; §significantly different from 18 months within the same treatment. Mean ± SE. Reprinted with permission from (Greising et al., 2015a).