Diaphragm Plasticity in Aging and Disease: Therapies for Muscle Weakness go from Strength to Strength

Sarah M. Greising[1,2], Coen A.C. Ottenheijm[3,4], Ken D. O'Halloran[5], & Esther Barreiro[6,7*]

[1]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester MN, USA; [2]School of Kinesiology, University of Minnesota, Minneapolis MN, USA; [3]Department of Physiology, VU University Medical Center, Amsterdam, Netherlands; [4]Cellular and Molecular Medicine, University of Arizona, Tucson AZ, USA; [5]Department of Physiology, University College Cork, Cork, Ireland; [6]Pulmonology Department-Muscle Wasting and Cachexia in Chronic Respiratory Diseases and Lung Cancer Research Group, IMIM-Hospital del Mar, Parc de Salut Mar, Health and Experimental Sciences Department (CEXS), Universitat Pompeu Fabra (UPF), Barcelona Biomedical Research Park (PRBB), Barcelona, Spain; [7]*Centro de Investigación en Red de Enfermedades Respiratorias* (CIBERES), *Instituto de Salud Carlos III* (ISCIII), Barcelona, Spain.

***Corresponding Author:** Dr. Esther Barreiro, Muscle Wasting and Cachexia in Chronic Respiratory Diseases and Lung Cancer Research Group, IMIM-Hospital del Mar, PRBB Building, Dr. Aiguader, 88, E-08003 Barcelona, Spain, Telephone: +34 93 316 0385, Fax: +34 93 316 0410, e-mail: ebarreiro@imim.es

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Introduction

The diaphragm is the main inspiratory muscle and is required to be highly active throughout the lifespan. The diaphragm muscle must be able to produce and sustain various behaviors that range from ventilatory to non-ventilatory such as those required for airway maintenance and clearance. Throughout the lifespan various circumstances and conditions may affect the ability of the diaphragm muscle to generate force and in turn the diaphragm muscle may undergo significant weakness and dysfunction. For example, hypoxic stress, critical illness, cancer cachexia, chronic obstructive pulmonary disorder (COPD), and age-related sarcopenia all represent conditions in which significant diaphragm muscle dysfunction exists.

This perspective review article presents several interesting topics involving diaphragm plasticity in aging and disease that were presented at the International Union of Physiological Sciences (IUPS) Conference in 2017. During the lecture respiratory physiologists gave overviews of the function of the diaphragm muscle and the effects of various comorbidities on the respiratory system. Furthermore each speaker presented current therapeutic options or targets that have shown promise in mitigating the deleterious effects imposed on the diaphragm muscle. Each section below summarizes individual presentations given during this lecture at IUPS including a brief concluding summary for each section. Furthermore, a general concluding paragraph is also given at the end of the review along with the future perspectives. The goal of the lectures and this perspective review is to maximize the broad and collective research impact on diaphragm muscle dysfunction, in the search for transformative treatment approaches to improve the diaphragm muscle health throughout the lifespan (Fig. 1).

Hypoxia-induced diaphragm dysfunction: Strengthening the case for antioxidant intervention in respiratory patients

The diaphragm muscle: Plasticity in the pump of life

The diaphragm is the primary muscle of breathing, pivotal in the breath-by-breath control of respiratory homeostasis. Akin to other striated muscles, the diaphragm retains a remarkable capacity for plasticity in health and disease. Intrinsic malleability in the structure-function relationship of the diaphragm may confer dynamic flexibility supporting respiratory performance in the course of physiological stressors such as development and exercise. Conversely, respiratory muscle remodeling in response to pathophysiological stressors can have deleterious consequences for respiratory mechanics, potentially perpetuating respiratory morbidity, for example in disease. Arguably, diaphragm atrophy and weakness in the critical care setting is the exemplar of egregious diaphragm muscle plasticity, with expedient and profound consequences for physiological performance (49). It is also very well established that diaphragm remodeling and dysfunction presents in several chronic respiratory diseases, perhaps most notably in COPD (7). Moreover, sarcopenia and weakness are evident in the aged diaphragm (38). Whatever the driver of dysfunction, impaired diaphragm performance is associated with poor prognosis for patients.

Oxygen homeostasis is critical to diaphragm function. Yet, despite the clinical relevance, the effects of hypoxia on the intrinsic properties of the diaphragm are under-explored and consequentially relatively poorly understood. Oxygen deficit presents in many guises in physiological and pathophysiological scenarios (e.g. exercise, altitude, surgery, disease) with often considerably diverse paradigms of exposure. It is therefore unsurprising that hypoxia-dependent plasticity in respiratory muscles can span a spectrum of change from phenotypic responses that appear to confer at least compensatory (e.g., force preservation in the face of a stressor) and perhaps improved measures of performance, to overt changes that are clearly deleterious for muscle, and hence system performance (59, 76). Pattern, intensity and duration of hypoxic exposure are key determinants of functional outcomes. Muscle-specific effects related to intrinsic differences in structure, function, and metabolic signatures are also recognized. Emerging evidence reveals that age and sex are also influential factors shaping respiratory muscle responses to hypoxia, with evidence of muscle-specific early-life susceptibility to hypoxia, and female advantage in adult animals due to protective effects of estrogen. Phenotypic change in muscle in response to perturbations in oxygen availability, often referred to as adaptive and maladaptive plasticity, reveals that hypoxia is much more than a mere symptom of respiratory morbidity. Rather, hypoxic stress has a capacity to exert major influence on respiratory muscle form and function with resultant consequences for respiratory system performance. This short article provides an overview of studies in rodent models of chronic hypoxia, which have provided insight to characteristics and mechanisms of hypoxia-induced respiratory muscle plasticity. The findings may have relevance to human diaphragm function at high altitude and in hypoxic respiratory disease.

The hypoxic diaphragm: Adaptive plasticity

There is evidence of apparent improved functional performance, termed adaptive or compensatory plasticity in response to hypoxia in diaphragm muscle, responses which serve to protect against oxygen deficit, or limit the deleterious consequences of hypoxic stress on muscle performance (hypoxic tolerance). There appears to be relative resilience in rodent diaphragm, compared with other muscles, in response to hypoxia, whether presented as a chronic sustained exposure mimicking altitude and pulmonary disease, or as a chronic intermittent stimulus modeling sleep-disordered breathing. Rat and mouse diaphragm fatigue tolerance is preserved or increased in response to 4-6 weeks of sustained hypoxia, whereas increased fatigue is revealed in other striated muscles (31, 32, 72). Chronic sustained hypoxia increases diaphragm Na^+/K^+ ATPase pump content (72) and mitochondrial aerobic efficiency (31), and results in myofiber atrophy with increased capillary length to fiber perimeter ratio (32), responses peculiar it seems to diaphragm compared with other striated respiratory and non-respiratory muscles (32, 72). Exposure to chronic sustained hypoxia also increases rat diaphragm muscle tolerance of subsequent acute severe hypoxic stress, insofar as force-generating capacity is better preserved during severe hypoxia in chronic hypoxic diaphragm compared with control (58). It is important to note however, that fatigue is commonly assessed as the time-dependent run down in force-generating capacity relative to pre-fatigue force. However, basal force is often reduced following chronic hypoxic exposure, complicating the interpretation of improved fatigue tolerance in the wider context of muscle performance. Early life exposure to sustained hypoxic stress increases rat pharyngeal dilator muscle fatigue, whereas diaphragm fatigue tolerance is preserved (14). A similar outcome is noted in early life exposure to chronic intermittent hypoxia, with evidence of persistent pharyngeal dilator muscle weakness, but maintenance of diaphragm force-generating capacity (70, 71). The few studies that have explored this issue paint a portrait of an essential pump with properties that protect from the insidious effects of hypoxia.

The hypoxic diaphragm: Maladaptive plasticity

Whereas adaptive plasticity is portrayed in rodent diaphragm muscle in response to chronic hypoxia, contemporaneous maladaptive plasticity has also been revealed (59, 76). Chronic hypoxia causes rat and mouse diaphragm weakness (61, 72), consistent with evidence of diaphragm myofiber atrophy (31, 72). It is also established that chronic sustained hypoxia decreases type IIa fiber specific force (20), revealing fiber dysfunction in addition to fiber atrophy. The significance of these observations is that diaphragm weakness is recognized as having prognostic value in the management of respiratory patients. Chronic sustained hypoxia was shown to cause progressive and extensive protein oxidation, and metabolic remodeling culminating in a p38 MAP kinase/FOXO-3a/20S proteasome-dependent atrophic response causing diaphragm weakness in mice (61). Curiously, chronic intermittent hypoxia causes rat diaphragm weakness and fatigue without overt oxidative stress (93), notwithstanding that other studies have revealed oxidant stress following chronic intermittent hypoxia in muscle and other tissues (59, 76). Deleterious effects of chronic sustained hypoxia and chronic intermittent hypoxia extend to pharyngeal dilator muscles (62, 97), which could serve to further compromise the integrative control of breathing. The experimental evidence points to differential outcomes that depend upon pattern, duration and intensity of hypoxia, as well as age and sex (59, 60, 76, 77). The time-dependent elaboration of diaphragm dysfunction in response to sustained hypoxia over 1-6 weeks of exposure reveals a temporal oxidative stress (61), due to progressive pro-oxidant production and a loss of antioxidant capacity. Therefore the threshold or tipping point in the transition from adaptation to maladaptation appears to be related to the oxidant:antioxidant balance, with muscle dysfunction arising during net oxidation. Oxidation can increase or decrease calcium sensitivity of the contractile apparatus, which might explain the capacity for divergent outcomes in skeletal muscle performance in response to hypoxia-dependent oxidative stress. S-glutathionylation of the fast troponin I isoform increases rat and human fast fibre sensitivity to calcium (74), enhancing muscle performance. Conversely, excess H_2O_2 and downstream hydroxyl radicals depress fast fibre calcium sensitivity, with endogenous glutathione levels serving as an important gatekeeper of redox status and muscle performance (75). Future studies should determine calcium sensitivity of the myofilaments in hypoxic diaphragm, and establish the extent of oxidation in myofibrillar proteins.

It appears that physiological trade-offs are at play in respect of respiratory muscle responses to hypoxic stress. Diaphragm weakness is a hallmark of hypoxic exposure perhaps at the expense of cellular strategies providing homeostatic defense and improved tolerance of oxygen lack. In the light of all considerations, the data suggest that hypoxia can perpetuate respiratory morbidity through deleterious effects on diaphragm muscle performance.

Antioxidant supplementation prevents hypoxia-induced diaphragm dysfunction

Dietary supplementation with antioxidants ameliorates or completely prevents respiratory muscle dysfunction following chronic intermittent hypoxia (79). N-acetyl cysteine supplementation is the most effective intervention in preventing rat diaphragm weakness and fatigue elaborated by chronic intermittent hypoxia (93). N-acetyl cysteine is also effective in preventing chronic sustained hypoxia-induced airway dilator (62) and diaphragm (61) dysfunction in mice. Antioxidant supplementation prevented respiratory muscle protein carbonylation following 6 weeks of sustained hypoxia (61, 62). Moreover, mouse diaphragm weakness following just 8 hours of exposure to sustained hypoxia (79) is reversed by N-acetyl cysteine pre-treatment (78). A comparison of the effects of antioxidants on respiratory muscle in animal models of hypoxic stress is provided in a recent review article (76). The findings of these studies provide strong rationale for a comprehensive assessment of N-acetyl cysteine supplementation intervention in human trials.

Conclusions

The multi-faceted stimulus of oxygen deficit can drive adaptive and maladaptive outcomes for respiratory muscle performance. Hypoxia is a feature of high altitude and it commonly presents as a consequence of respiratory morbidity, with a capacity whatever the cause to contribute further to impaired respiratory performance and perhaps the spiral of disability characteristic of chronic respiratory diseases. Altered redox signaling is pivotal in driving diaphragm plasticity in response to hypoxic stress. The experimental evidence in animal models clearly demonstrates the efficacy of N-acetyl cysteine supplementation in preventing hypoxia-induced diaphragm dysfunction. On this basis, there is adequate justification to explore the potential of adjunctive antioxidant therapies for respiratory muscle dysfunction in human respiratory disease.

Diaphragm fiber weakness in the critically ill

Diaphragm weakness in critically ill patients

Patients with critical illness experience substantial skeletal muscle weakness and physical disability. This leads to functional impairment of survivors of the Intensive Care Unit (ICU), an impairment that can last for years (11, 47, 91). In particular, weakness of the diaphragm – the main muscle of inspiration – is of major concern in critically ill patients: it prolongs ventilator dependency, increases morbidity and duration of hospital stay, and it is associated with long term functional limitations after hospital discharge (12, 19, 27, 35).

Diaphragm weakness in mechanically ventilated critically ill patients has been established with non-invasive measurements; ultrasound revealed reduced motion and thinning of the diaphragm (22, 36, 44, 54), and by magnetic stimulation of the phrenic nerves a reduced capacity to generate pressure was observed (21, 35, 46, 52). The cellular changes that underlie diaphragm weakness in critically ill patients are unclear. Changes in phrenic nerve function, in neuromuscular transmission, or in the contractility of individual muscle fibers all may explain the reduction in pressure generation by the diaphragm.

A plethora of data suggests that changes in the contractility of individual diaphragm muscle fibers play a major role. Critical illness-associated phenomena, such as mechanical ventilation-induced diaphragm inactivity (55, 90, 92), malnutrition (57), and inflammation (86) are associated with contractile weakness of diaphragm fibers and activation of proteolytic pathways within diaphragm fibers in animal models. Whether these findings translate to humans is unknown, although several studies (51, 56), but not all (50), in brain dead organ donors who received mechanical ventilation prior to organ harvest revealed atrophy and activation of the ubiquitin-proteasome pathway in diaphragm muscle fibers. However, brain dead organ donors do not exhibit the clinical features of critically ill patients; complete absence of neural activation of the diaphragm, metabolic stress and brain ischemia differentiates them. Consequently, it is unknown whether these findings translate to critically ill patients.

Studies on individual diaphragm fibers of critically ill patients

To fill this void, recent studies aimed to study the contractile function of diaphragm fibers of critically ill patients. We obtained diaphragm biopsies of critically ill patients who received mechanical ventilation prior to surgery, and compared these biopsies with those obtained from patients undergoing resection of an early lung malignancy (controls). The size and the contractile strength of individual muscle fibers were determined. Both slow-twitch and fast-twitch diaphragm muscle fibers of critically ill patients had a ~25% smaller cross sectional area, and a >50% lower absolute contractile force (49), (note that the contractile properties of individual diaphragm fibers depend on fiber type (33, 34), and therefore data from slow- and fast-twitch fibers are reported separately). Additionally, we evaluated critical components of the ubiquitin-proteasome pathway, and performed proof-of-concept studies in MuRF-1 knockout mice to evaluate the role of this pathway in the development of contractile weakness during mechanical ventilation (49). Markers of the ubiquitin-proteasome pathway, in particular MuRF-1, a muscle – specific ubiquitin ligase, were significantly upregulated in the diaphragm of critically ill patients. MuRF-1 deficient mice were protected against the development of diaphragm contractile weakness during mechanical ventilation, indicating that MuRF-1 upregulation in the diaphragm of critically ill patients might play an important role in the development of fiber atrophy and contractile weakness.

The mechanisms that trigger proteolysis in diaphragm muscle fibers of critically ill patients are incompletely understood. Studies on animal models and on brain dead organ donors suggest that these mechanisms include oxidative stress, induced by mitochondrial alterations during mechanical ventilation (56, 69, 80). For instance, the influx of energetic substrates, such as lipid and glucose, might exceed the demand of diaphragm muscle fibers during a sudden drop in substrate utilization rate during mechanical unloading of the diaphragm in brain dead organ donors and mechanically ventilated rodents (80, 81). This state of metabolic oversupply then promotes mitochondrial fission, mitochondrial dysfunction and increase oxidative stress (53). Surprisingly, recent studies from our group on thirty-six mechanically ventilated critically ill patients, whom displayed significant atrophy and contractile weakness of diaphragm muscle fibers, revealed absence of mitochondrial dysfunction, oxidative stress, and metabolic oversupply in diaphragm fibers (98). These findings suggest that mitochondria and redox status should not be the primary target of therapy aimed at preserving diaphragm function in critically ill patients.

Targeting the diaphragmatic sarcomere

Recent studies tested the ability of troponin activators to improve diaphragm fiber contractility in critically ill patients. These compounds target the sarcomere, the smallest contractile unit in muscle, and in particular those in fast-twitch muscle fibers. These fast-twitch diaphragm fibers of critically ill patients were of particular interest as they not only have reduced maximal force, but they also require more calcium to generate submaximal force (48). To test whether this reduced calcium-sensitivity of force can be restored, diaphragm fibers of critically ill patients were exposed to the fast skeletal troponin activator CK-2066260. Fast skeletal troponin activators increase the affinity of the troponin complex to Ca^{2+} (87). Compared to vehicle, 5μM of CK-2066260 significantly increased the calcium sensitivity of diaphragm fibers, both in controls and in critically ill patients. Importantly, at physiological calcium concentrations, CK-2066260 restored the contractile force of fast-twitch diaphragm fibers of critically ill patients back to levels observed in untreated fibers from controls (48).

Conclusions

To date, no drug is approved to improve respiratory muscle function in mechanically ventilated critically ill patients. Administration of troponin activators might prove an elegant strategy to restore diaphragm fiber strength. These compounds improve contractility at calcium concentrations which reflect activation during daily live activities (87). And since ~50% of fibers and total fiber area in the human diaphragm consists of fast-twitch fibers, fast skeletal troponin activators might significantly improve in vivo diaphragm strength. Similarly, studies with levosimendan, a commercially available calcium sensitizer that targets sarcomeric troponin in slow-twitch muscle fibers, showed improved neuromechanical efficiency and contractile function of the diaphragm in healthy controls (25). These findings underscore the therapeutic potential of troponin activators.

Age-related sarcopenia in the diaphragm muscle

Aging and sarcopenia

The aging population is increasing steadily both in the USA and worldwide. In the USA alone, there are currently 48 million people over the age of 65, and this is expected to increase to about 88 million by 2050 (45). Aging alone is related to increased incidence of chronic diseases, and a single intrinsic risk factor underlying many disease states. For example, cardiovascular disease and osteoporosis have a significantly increased prevalence with age. Sarcopenia is the age-related disorder of skeletal muscle, and is associated with the loss of muscle mass and function (30). To date, sarcopenia has been well defined and investigated in limb muscles but less so in the diaphragm muscle (13). In a series of studies we established the effects of diaphragm muscle sarcopenia (26, 38, 39, 41), investigated mechanistic drivers (37, 42), and evaluated possible therapeutic approaches to target age-related changes to the diaphragm muscle (43).

The two main components of sarcopenia were investigated in the diaphragm muscle of mice at ages of 100%, 90%, and ~75% survival (38, 39, 41). The first component investigated was the force generating capacity of the diaphragm muscle. The old mice at 75% survival had ~34% less force generating capacity (i.e., maximal specific force) than young mice (100% survival) and those in early old age (90% survival). Significant force loss occurred in mice between 90 and 75% survival, a window of only six months (18 – 24 months of age). Loss of maximal diaphragm muscle force is expected to impact the ability to perform non-ventilatory behaviors related to airway clearance. The second component of sarcopenia investigated was the type specific cross-sectional area of diaphragm muscle fibers. There is a loss of muscle fiber size of the type IIx and/or IIb muscle fibers, but not in type I and IIa diaphragm muscle fibers between 100% and 75% survival in mice (38, 41). Collectively this extent of sarcopenia is expected to significantly impair the ability of the diaphragm muscle to accomplish a broad range of behaviors in old age, and limit the ability to maintain airways clear. The overarching goal of this on-going work has been to understand if sarcopenia is related to trophic changes throughout the motor unit and to evaluate mitigating approaches for age-related diaphragm neuromuscular transmission failure and sarcopenia.

Diaphragm motor unit recruitment

Motor units are composed of an individual motor neuron that innervates a group of muscle fibers through a neuromuscular junction on each fiber, and the motor unit is known as the basic unit of neuromotor control (63). Individual motor units are classified as fast-twitch (type F) and slow-twitch (type S) based on specific mechanical and fatigue properties of the muscle fibers. The type F motor units are further categorized into fast-twitch fatigue resistant (type FR), fast-twitch fatigue intermediate (type FInt), and fast-twitch fatigable (type FF) based on specific metabolic capacities of the muscle fibers. The type FInt and FF motor units comprise type IIx and IIb muscle fibers, primarily expressing myosin heavy chain $MyHC_{2X}$ and $MyHC_{2B}$, respectively, which are the fibers that display the preferential age-related loss in cross-sectional area.

The diaphragm muscle must be able to accomplish ventilatory behaviors such as normal room air breathing (eupnea) and during stimulation from increased chemical drive (hypoxia-hypercapnia or exercise). Additionally, the diaphragm muscle must also perform non-ventilatory behaviors that require greater force and are related to maintenance of airway patency and airway clearance (e.g., sneezing, gagging and coughing). Using a well-established model of diaphragm muscle neuromotor control across a range of diaphragm behaviors (94, 96) it is known that ventilatory behaviors require only the type S and FR motor units, while non-ventilatory behaviors also require type FInt and FF (66, 67, 95). Knowing that diaphragm muscle sarcopenia selectively atrophies the more forceful type IIx and/or IIb fibers, we investigated if there was an age-related impairment in maximal behaviors using transdiaphragmatic pressure (Pdi) measurements, a surrogate for diaphragm muscle force, in mice at ages of 100% and ~75% survival (39, 40). Consistent with the large reserve capacity for force generation by the diaphragm muscle, aging did not affect the ability to generate forces necessary for ventilatory behaviors, however there was significant age-related impairment of more forceful non-ventilatory behaviors.

Trophic influences

The exact mechanisms responsible for diaphragm muscle sarcopenia remain unclear, but it is possible that they may be related to changes throughout the neuromuscular system. The first step in understanding age-related trophic changes was to evaluate effects at the neuromuscular junction. In a series of studies, we investigated neuromuscular transmission using a global measure of force generation by the diaphragm muscle in response to nerve and direct muscle activation during aging. In dissimilarity to aging effects on maximal force generation, alterations in diaphragm neuromuscular transmission were evident in mice by early old age with no further change into old age (90 vs 75% survival, respectively) (37). These findings indicate that the changes in neuromuscular transmission precede the loss of diaphragm muscle force or significant muscle fiber atrophy.

There are various possible trophic influences at the neuromuscular junction and it is understood that neurotrophins can acutely enhance neuromuscular transmission. It is unclear if age-related alterations in neurotrophins could help to explain the detrimental effects of sarcopenia on neuromuscular transmission. The neurotrophin brain-derived neurotrophic factor (BDNF) acting through its high affinity tropomyosin-related kinase receptor subtype B (TrkB) receptor is known to play an important role in the development and maintenance of adult neuromuscular junctions and has an important role in neuromuscular transmission (65, 68). Furthermore, in young diaphragm muscles it is known that enhancing BDNF signaling can improve neuromuscular transmission, while inhibition of TrkB kinase activity can impair neuromuscular transmission (68).

To investigate possible age-related alterations in trophic interactions throughout the motor unit we investigated the effects of BDNF/TrkB signaling in the aging diaphragm muscle and neuromuscular junction. In early old age (~90% survival), increased BDNF improved neuromuscular transmission, while inhibition of TrkB kinase activity had no effect. However, in old age (~75% survival) neither increased BDNF nor the inhibition of TrkB kinase activity had any effect on diaphragm neuromuscular transmission. This suggested that there may be reductions in endogenous BDNF in the aging diaphragm muscle which likely preceded reductions in TrkB kinase activity (37). Second, examining the early old age time point, given that neuromuscular transmission was impaired without evidence of sarcopenia, a detailed morphological investigation of the neuromuscular junction was conducted. Using a knockin $TrkB^{F616A}$ mouse model allowing for reversible inhibition that is sensitive to the phosphoprotein phosphatase-1derivative (1NMPP1) (18) BDNF/TrkB signaling was disrupted for one week. The inhibition of TrkB kinase activity in early old age increased the proportion of denervated neuromuscular junctions in the diaphragm muscle (42). Collectively, the early old age time point indicated loss of neuromuscular transmission and a period of susceptibility during early old age in which BDNF/TrkB signaling at diaphragm neuromuscular junctions supports the maintenance of neuromuscular junctions structure and muscle innervation, prior to the onset frank sarcopenia.

In efforts to mitigate both diaphragm muscle sarcopenia and neuromuscular transmission failure, BDNF/TrkB signaling was targeted therapeutically starting at early old age. Previously, in young adult mice the highly selective BDNF analog and TrkB agonist, 7,8-dihydroxyflavone (7,8-DHF) was shown to acutely improve diaphragm neuromuscular transmission. As such, chronic treatment was investigated starting at early old age for a six month period. However, contrary to the anticipated effects, chronic treatment with 7,8-DHF was not able to mitigate diaphragm muscle sarcopenia or impairments in neuromuscular transmission (43). While the therapeutic approach with 7,8-DHF was not effective in mitigating age-related dysfunction it is possible that targeting BDNF/TrkB signaling may still be an effective treatment approach if started earlier.

Conclusions

Our work has sought to understand neuromuscular dysfunction in the aging diaphragm muscle by understanding the impact of age-related impairments in BDNF/TrkB signaling. The most salient findings of this on-going work have been the evidence for a time course of age-related changes in neuromuscular activity and trophic signaling. The reduction in BDNF availability likely precedes a loss of TrkB receptor expression and occurs when there are reversible changes in neuromuscular transmission but not yet evidence of sarcopenia. This work supports ongoing investigations of the mechanisms underlying disrupted trophic signaling at the neuromuscular junction and highlights the importance of understanding the role of motor neuron and neuromuscular junction dysfunction in the pathogenesis of sarcopenia.

Diaphragm dysfunction in cancer cachexia: mechanisms and therapies

Skeletal muscle dysfunction in chronic respiratory diseases

In patients with chronic respiratory conditions, dysfunction of the ventilatory and limb muscles is frequently observed as a relevant systemic manifestation of these diseases. Other conditions such as chronic heart failure, cancer cachexia, and sepsis may also induce both respiratory and peripheral muscle dysfunction and mass loss in patients as well as in animal models (2, 3, 6, 64, 73, 99). Skeletal muscle dysfunction is of multifactorial etiology . Several factors intrinsic to both the host (whether patient or animal) and the condition itself and biological mediators interact together to alter the function of the muscles characterized by a decline in either the strength or endurance properties. Interestingly, the mechanical factors, mainly due to the increased inspiratory loads of patients with chronic respiratory diseases, contribute to a great extent to the ventilatory muscle dysfunction described in these patients, while those factors are irrelevant in the etiology of the dysfunction of locomotor muscles.

The most relevant factors and biological mediators that contribute to respiratory and limb muscle dysfunction in patients and animal models have been extensively reviewed in several review articles (2, 3, 6, 64, 73, 99). Briefly, factors such as hypoxia, hypercapnia, acidosis, cigarette smoking, metabolic disorders, nutritional abnormalities, aging, genetic predisposition, drugs, comorbidities, systemic inflammation, and inactivity have been shown to contribute to muscle mass loss and impaired function in animal models and patients (2, 3, 6, 64, 73, 99). In the respiratory muscles, mainly the diaphragm, the contribution of the alterations in the thorax geometry and the increased inspiratory loads to which chronic respiratory patients are continually exposed, are major contributors to the reported ventilatory muscle dysfunction, especially in the initial stages (2, 3, 6, 64, 73, 99). Furthermore, biological mediators such as oxidative stress, increased protein breakdown and muscle protein degradation, poor anabolism, mitochondrial alterations, and epigenetic events are counted among the most relevant mechanisms shown to be involved in the skeletal muscle dysfunction of the patients and animal models (2, 3, 6, 28, 29, 64, 73, 82-85, 99). Moreover, in the early stages of respiratory muscle dysfunction, several biological adaptations take place in the diaphragm muscle as a result of the increased inspiratory loads intrinsic to chronic respiratory disease (3, 6). A rise in capillary contacts, shorter sarcomere length, increased oxidative capacity, slow-twitch fiber proportions, in myoglobin and in mitochondrial content are the most relevant adaptive mechanisms described in the diaphragm of patients with chronic respiratory conditions (3, 6). The adaptive mechanisms counterbalance the deleterious effects inherent to the host and disease up to a certain point. When the harmful conditions (e.g., hypoxia and increased metabolic and oxygen demands) outweigh the adaptive scenario, respiratory muscle dysfunction occurs in the patients with chronic respiratory conditions (3, 6).

Respiratory muscle dysfunction: Biological mechanisms and therapeutic targets in several conditions

Sepsis. A decline in diaphragm force generation has been consistently observed in endotoxemic mice and rats in many different studies. Our group demonstrated that heme oxygenase activity is involved in muscle performance in normal and endotoxemic rats as its selective inhibition with chromium-mesoporphyrin IX induced a further decline in muscle force generation of diaphragm muscle strips (4). The antioxidant N-acetyl cysteine was shown to induce an improvement in respiratory muscle function (*in vivo* measurements) in septic rats together with a rise in superoxide dismutase activity and a decline in diaphragm oxidative stress (10).

Cigarette smoking models. Chronic exposure to cigarette smoking induced a significant rise in protein oxidation levels, especially of creatine kinase, in the diaphragm of guinea pigs (8) and mice (5). Moreover, it also induced a significant decline in body weight gain over time in both animal models, suggesting that the compounds of cigarette smoke may influence body compartments and composition in these animal models (5, 8).

Hypoxia. Exposure to chronic intermittent hypoxia also induced a decline in body weight gain in rats after two weeks (23). Levels of proinflammatory cytokines were greater in the diaphragm and plasma of the animals exposed to chronic intermittent hypoxia for 15 days (23). Moreover, levels of superoxide anion were also significantly increased in the mitochondria and membrane compartments of the diaphragm muscle and plasma of the rats exposed to the 15-day period of chronic intermittent hypoxia (23). Treatment of the animals with the antioxidant N-acetyl cysteine induced a significant reduction in superoxide anion levels as measured by chemiluminescence in both diaphragm muscle and systemic levels of the rats exposed to hypoxia compared to non-exposed animals (23). Treatment with the anti-tumor necrosis factor (TNF)-alpha antibody of the rats exposed to chronic intermittent hypoxia reduced levels of superoxide anion in the diaphragm muscle compartments (membrane and mitochondria) and plasma, while it only induced a significant decline in the systemic levels of proinflammatory cytokines, but not in muscles (23).

Inspiratory threshold loading models. The diaphragm was also analyzed in rats exposed to several degrees of inspiratory threshold loading in another investigation(23, 24). A decline in body weight gain was observed in the rats exposed to the greatest loads (70% of maximal inspiratory pressure, MIP) (24). Superoxide anion levels were greater in the mitochondrial and membrane compartments of the diaphragm of the 70%-MIP rats and treatment with either N-acetyl cysteine or anti-TNF-alpha antibody induced a significant decline in those levels even below control levels (24).

Chronic heart failure. Experimental chronic heart failure (CHF) induced as a result of a single dose of monocrotaline injection in rats, which experienced a significant reduction in body weight gain and muscle mass loss (9). Treatment with either bortezomib (proteasome inhibitor) or N-acetyl cysteine attenuated these deleterious effects on the animals after one month (9). Levels of proteolysis (tyrosine release) were also increased in the diaphragm and limb muscles of the CHF rats and treatment with bortezomib and N-acetyl cysteine significantly reduced those levels (9).

Diaphragm dysfunction in cancer-induced cachexia and potential therapies

Several therapeutic strategies to treat muscle wasting in models of cancer-induced cachexia have been tested in animals in a great variety of studies. In this regard, in this section the effects of different therapeutic agents will be subsequently described in experimental models of lung cancer-induced cachexia.

Models of lung cancer carcinogenesis in mice. Our group has published several studies focused on the elucidation of the underlying biological mechanisms of muscle mass loss in respiratory and limb muscles in animal models of cancer cachexia. Body weight gain and muscle mass were significantly reduced in mice exposed to chronic lung carcinogenesis with urethane for several time-points (88). Increased muscle injury and apoptosis along with a decline in the content of specific structural and functional muscle proteins were seen in the diaphragm of the cancer-induced cachectic mice compared to non-exposed control animals (88).

Models of lung cancer cell inoculation in wild type mice. In another model of lung cancer cell inoculation in mice, treatment with several agents such as specific inhibitors of nuclear factor (NF)-κB and mitogen-activated protein kinases (MAPK) pathways, but not bortezomib, attenuated the loss in body weight gain and diaphragm and gastrocnemius muscle mass and reduced limb strength in the animals (16). Levels of proteolysis (tyrosine release) were attenuated in the diaphragm and limb muscles in response to treatment with either the proteasome, MAPK, or NF-κB inhibitors after one month of study (16).Cachexia-induced NF-κB signaling was also attenuated as a result of treatment of the animals with either bortezomib for the corresponding NF-κB inhibitor (16). In the same animal model of lung cancer-induced cachexia, a decline in the mitochondrial respiratory chain complexes I and IV and oxygen consumption was observed in the diaphragm and gastrocnemius of the cachectic mice compared to control non-cachectic animals (28). Importantly, treatment with either NF-κB or MAPK inhibitors improved, especially in the diaphragm, mitochondrial respiratory chain activity and oxygen consumption in the cachectic mice (28). The proteasome inhibitor bortezomib, however, did not elicit any significant improvement in those parameters in the cancer cachectic mice (28). Lately, our group has also demonstrated that the $beta_2$ agonist formoterol exerted its beneficial through reduced oxidative stress (89) and atrophy signalling levels (1) in both diaphragm and especially the gastrocnemius muscle in rats with cancer-induced cachexia.

Model of lung cancer cell inoculation in genetically deficient mice. The effects of poly(ADP-ribose) polymerases (PARP) on the process of muscle mass loss and wasting in cancer-induced cachexia has also been recently explored. In fact, PARP activity was increased in the diaphragm and gastrocnemius of the cancer-induced cachexia mice compared to the non-cachectic control animals (17). Interestingly, in cancer cachectic mice deficient for either Parp-1 or Parp-2 genes, the decrease in total body weight gain, muscle weights, and limb strength was attenuated compared to cachectic wild type animals (15, 17). Moreover, the alterations observed in several epigenetic mechanisms, namely reduced muscle-specific microRNA expression and histone deacetylase levels, of the diaphragm and gastrocnemius muscles in the *Parp1*$^{-/-}$ and *Parp2*$^{-/-}$ cachectic mice were also partially attenuated compared to those detected in the wild type cachectic rodents (15). In the same model of PARP-1 and PARP-2 deficient mice, the increased levels of oxidative stress, proteolysis (tyrosine release assay), ubiquitin-proteasome pathway markers, and atrophy signaling pathways were also attenuated in the cancer cachectic animals compared to the cachectic wild type mice (17). Additionally, the reduction in myosin protein levels detected in the diaphragm of cachectic wild type animals was also attenuated particularly in the *Parp2*$^{-/-}$ cachectic mice (17).

Conclusions

In conclusion, several therapies have been demonstrated to be very efficient for the treatment of muscle wasting in several models of cachexia including cancer. The antioxidant N-acetyl cysteine and inhibitors of atrophy signaling pathways have shown an important attenuation of the deleterious effects leading to muscle mass loss. Moreover, PARP activity seems to play a relevant role in the process of muscle wasting, which could also be the basis for the use of PARP inhibitors in models of cancer-induced cachexia that could eventually be translated into the clinics.

Conclusions and Future Directions

The goal of this IUPS symposium and perspective review was to highlight our collective research efforts to understand novel approaches for various forms of diaphragm muscle dysfunction (Fig. 1). Collectively the results presented at IUPS indicate that while no specific treatment option is currently approved to target global diaphragm muscle dysfunction, there are several therapeutic strategies underway. Specifically, therapies have been demonstrated to be very efficient for the treatment of various models of diaphragm muscle dysfunction.

First, the antioxidant N-acetyl cysteine and inhibitors of atrophy signaling pathways have shown an important attenuation of the deleterious effects leading to muscle mass loss. Second, PARP activity seems to play a relevant role in the process of muscle wasting, which could also be the basis for the use of PARP inhibitors in models of cancer-induced cachexia that could eventually be translated into the clinics. Third, targeting of calcium concentrations via troponin activators may be transformative to critically ill ventilator-dependent patients. Forth, altered redox signaling plays a pivotal in driving diaphragm plasticity in response to hypoxic stress. The experimental evidence in animal models clearly demonstrates the efficacy of antioxidant supplementation in preventing hypoxia-induced diaphragm dysfunction. Finally, in models of aging currently understood disruptions in trophic signalling provide a possible target for novel therapies although no specific therapies have been identified yet.

At present more research is needed in order to move these possible therapies to the clinic. Furthermore, work to identify additional novel therapeutic targets may help mitigate the deleterious effects of the loss in diaphragm function in chronic respiratory diseases, cancer as well as in critical illness and aging.

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Sarah M. Greising, Ph.D.

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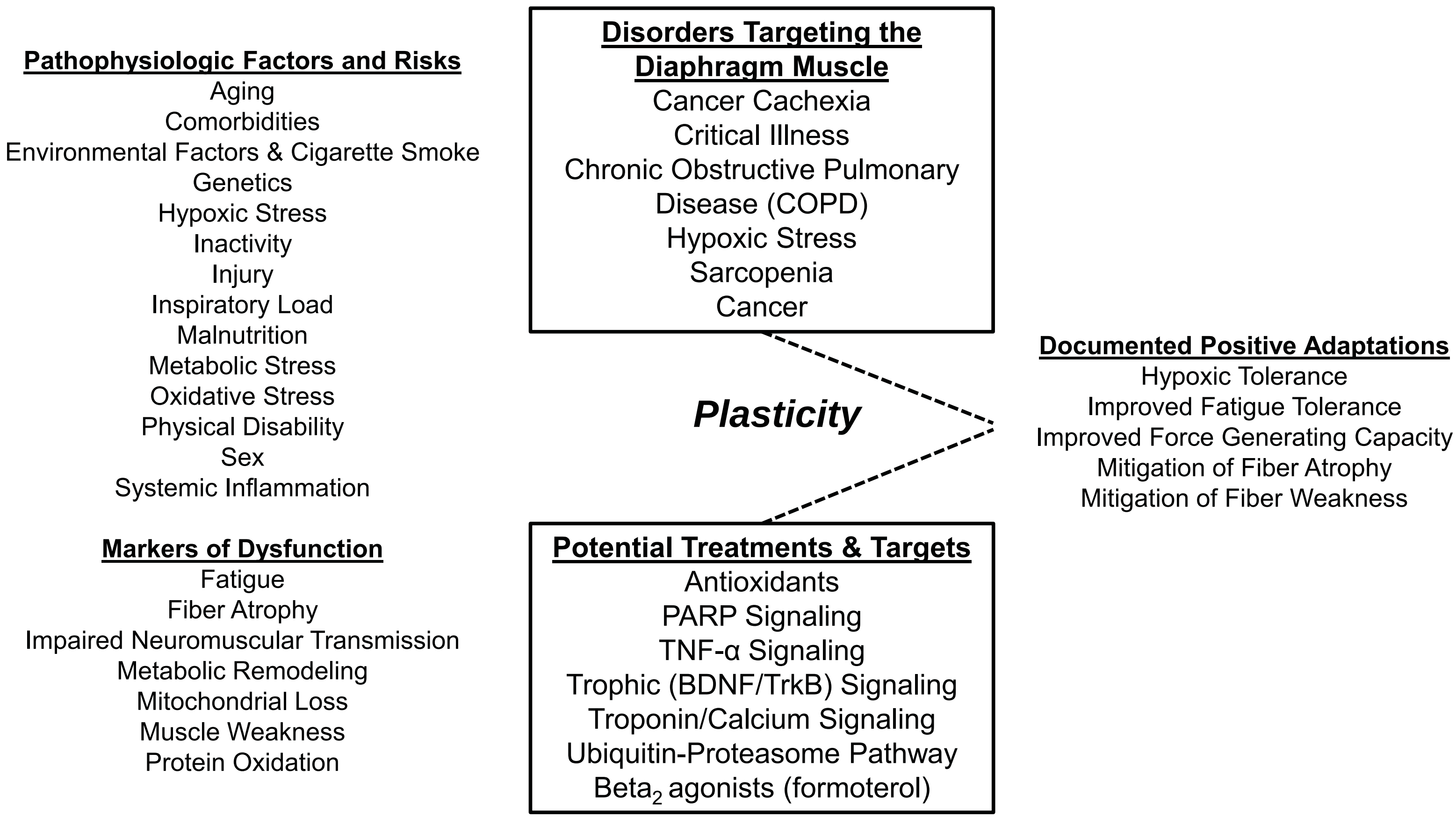
Figure Legend

Figure 1. Framework of the collective research impact on diaphragm muscle dysfunction resented at the IPUS 2017. This symposium sought to understand possible treatment approaches and targets to improve the diaphragm muscle health throughout the lifespan and across chronic diseases and disorders. Throughout this perspective review we present various biological factors, markers of dysfunction, and positive adaptations to potential treatments in models of hypoxic stress, critical illness, cancer cachexia, chronic obstructive pulmonary disorder (COPD), and age-related sarcopenia all of which present significant diaphragm muscle dysfunction.

BDNF/TrkB: brain-derived neurotrophic factor / tropomyosin-related kinase receptor subtype B

PARP: poly(ADP-ribose) polymerases

TNF-α: tumor necrosis factor-alpha


Pathophysiologic Factors and Risks
Aging
Comorbidities
Environmental Factors & Cigarette Smoke
Genetics
Hypoxic Stress
Inactivity
Injury
Inspiratory Load
Malnutrition
Metabolic Stress
Oxidative Stress
Physical Disability
Sex
Systemic Inflammation
Markers of Dysfunction
Fatigue
Fiber Atrophy
Impaired Neuromuscular Transmission
Metabolic Remodeling
Mitochondrial Loss
Muscle Weakness
Protein Oxidation
Disorders Targeting the Diaphragm Muscle
Cancer Cachexia
Critical Illness
Chronic Obstructive Pulmonary Disease (COPD)
Hypoxic Stress
Sarcopenia
Cancer
Plasticity
Potential Treatments & Targets
Antioxidants
PARP Signaling
TNF-α Signaling
Trophic (BDNF/TrkB) Signaling
Troponin/Calcium Signaling
Ubiquitin-Proteasome Pathway
Beta$_2$ agonists (formoterol)
Documented Positive Adaptations
Hypoxic Tolerance
Improved Fatigue Tolerance
Improved Force Generating Capacity
Mitigation of Fiber Atrophy
Mitigation of Fiber Weakness