

Diaphragm Muscle Fiber Dysfunction in Chronic Obstructive Pulmonary Disease

Toward a Pathophysiological Concept

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Inspiratory muscle weakness in patients with chronic obstructive pulmonary disease (COPD) is of major clinical relevance; maximum inspiratory pressure generation is an independent determinant of survival in severe COPD. Traditionally, inspiratory muscle weakness has been ascribed to hyperinflation-induced diaphragm shortening. However, more recently, invasive evaluation of diaphragm contractile function, structure, and biochemistry demonstrated that cellular and molecular alterations occur, of which several can be considered of pathologic nature. Although the fiber-type shift toward oxidative type I fibers in COPD diaphragm is regarded as beneficial, rendering the overloaded diaphragm more resistant to fatigue, the reduction of diaphragm fiber force generation *in vitro* likely contributes to diaphragm weakness. The reduced diaphragm force generation at single-fiber level is associated with loss of myosin content. Moreover, the diaphragm in COPD is exposed to oxidative stress and sarcomeric injury. The current Pulmonary Perspective postulates that the oxidative stress and sarcomeric injury activate proteolytic machinery, leading to contractile protein wasting and, consequently, loss of force-generating capacity of diaphragm fibers in patients with COPD. Interestingly, several of these presumed pathologic alterations are already present early in the course of the disease (GOLD I/II), although these patients do not appear to be limited in their daily-life activities. Therefore, investigating *in vivo* diaphragm function in mild to moderate COPD should be the focus of future research. Treatment of diaphragm dysfunction in COPD is complex because its etiology is unclear, but recent findings show promise for the use of proteasome inhibitors in syndromes associated with muscle wasting, such as the diaphragm in COPD.

Keywords: chronic obstructive pulmonary disease; diaphragm; muscle wasting; contractile dysfunction; titin

Inspiratory muscle weakness is of clinical importance in patients with chronic obstructive pulmonary disease (COPD). For instance, dyspnea is the most disabling symptom, and although the pathophysiology of dyspnea is complex, an imbalance between the load imposed on the respiratory muscles and the ability to sustain this load may play a role in the sensation of dyspnea (1). Hypercapnic respiratory failure due to inspiratory muscle weakness (2) is associated with morbidity in these pa-

tients (3), and maximum inspiratory pressure is an independent determinant of survival in patients with severe COPD (4).

The majority of studies dealing with inspiratory muscle weakness in COPD have focused on the diaphragm, mainly because this is the principal muscle of inspiration. Although it is well established that patients with COPD generate less transdiaphragmatic pressure than healthy subjects (5), the underlying causes are still under debate. Traditionally, diaphragm weakness has been ascribed to hyperinflation-induced diaphragm shortening, which places the diaphragm at a mechanical disadvantage (6). However, several later studies indicate involvement of other phenomena, such as intrinsic diaphragm alterations (7, 8).

A decade ago, Levine and colleagues (9) published a landmark paper showing a fiber-type shift in the diaphragm muscle of patients with severe COPD. This fiber-type shift toward more oxidative type I fibers is regarded as a beneficial adaptive response to increased diaphragm loading, because it renders the diaphragm less susceptible to fatigue. Since then, several investigators have studied the effects of COPD on functional, biochemical, and morphological characteristics of the diaphragm.

This perspective presents an overview of the alterations in the diaphragm muscle of patients with COPD and provides a pathophysiological concept for understanding these alterations. Finally, we will discuss potential strategies to improve diaphragm muscle function.

MOLECULAR AND CELLULAR CHANGES OF THE DIAPHRAGM IN COPD

Diaphragm Fiber-type Shift toward Oxidative Type I Fibers in COPD

It is well established that the diaphragm of patients with severe COPD (8–14) has an increased proportion of type I, slow-twitch, fatigue-resistant fibers, whereas the proportion of fast, fatiguing fibers (type II) is decreased. Less well established is that this diaphragm fiber-type shift already occurs in mild-to-moderate COPD (15, 16). The increase of oxidative capacity and mitochondrial electron transport chain function in the diaphragm occurs in line with the progression of COPD and reflects the shift toward more oxidative type I fibers.

Dysfunction of Diaphragm Single Fibers in COPD

Single-fiber contractile properties. Human diaphragm muscle biopsies do not allow dissection of intact fibers, due to their length. Therefore, fiber ends are not sealed by their tendons, which disrupts normal excitation–contraction coupling. However, skinned fibers provide an excellent *in vitro* model for the evaluation of myofilament function of the human diaphragm. In

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skinned fibers, the membranous structures are made highly permeable, which enables activation of the myofilaments with exogenous calcium. Data from Levine and coworkers (7) indicate reduced force generation ($\sim 35\%$) in skinned diaphragm fibers from patients with severe COPD. Interestingly, our group has shown that diaphragm fiber dysfunction is not restricted to severe COPD because maximum force generation of skinned diaphragm fibers was markedly reduced ($\sim 25\%$) in patients with only mild to moderate COPD (FEV_1 , $\sim 70\%$ predicted) (17). The maximum force generated by a muscle fiber is strongly dependent on myosin content. The reduction of maximum force generated by COPD diaphragm fibers was associated with an approximate 30% loss of myosin content (Figure 1) (17).

It should be noted that, *in vivo*, the diaphragm does not perform maximum isometric contractions, but shortens against a submaximal load. Thus, submaximal and kinetic parameters of muscle function provide more relevant information. Recent data show that, at a certain calcium concentration, the generated force relative to maximum force is less in fibers from patients with COPD compared with patients who do not have COPD (17). This appears to be an important finding, because it implies that higher cytosolic calcium is needed to maintain equivalent force generation, leading to increased ATP consumption by the Ca^{2+} -ATPases. Alternatively, at a set submaximum activation, reduced calcium sensitivity could compromise *in vivo* diaphragm force generation.

Single-fiber passive properties. For optimal contractile function, passive-elastic structures in the sarcomere are indispensable

(18). Therefore, passive force characteristics provide important information on muscle function. Recently, it was demonstrated that diaphragm fibers from patients with mild to moderate COPD generate less passive tension upon fiber stretch compared with fibers from patients without COPD (15, 19). Passive tension in these fibers is mainly determined by the properties of titin. Titin spans the half-sarcomeric distance from the Z-line to the M-line, thus forming a third sarcomeric filament, apart from the myosin and actin filaments. In the I-band region, titin's elastic PEVK (Pro-Glu-Val-Lys) region functions as a molecular spring that develops passive tension upon stretch. Titin gene transcript studies revealed increased expression of exons coding for the PEVK region in COPD diaphragm, resulting in an elongated extensible titin segment (15) (Figure 2). These data strongly suggest that alternative splicing of the titin gene reduces the passive tension generated by diaphragm fibers from patients with COPD. Because these changes in titin stiffness were present in patients with COPD without chronic hyperinflation (15), diaphragmatic adaptations due to chronic diaphragm shortening (i.e., dropout of sarcomeres) are not expected to alter the *in vivo* stress on titin. Interestingly, in contrast to myosin and nebulin, titin content appeared to be preserved in the diaphragm of these patients (15). Apparently, the diaphragm of patients with mild to moderate COPD is subject to selective loss of proteins and qualitative changes within molecules.

Diaphragm Atrophy: Not Restricted to Severe COPD

Although cross-sectional area of diaphragm fibers is reduced in patients with severe COPD compared with that in patients without COPD (9, 20), this has not been found in patients with mild or moderate COPD (16, 21–24). Nevertheless, cross-sectional area is not a sensitive marker for muscle atrophy. Indeed, recent data show loss of myosin in the diaphragm fibers in mild to moderate COPD (15, 17). In these patients, the myosin content per half sarcomere in both type I and IIa diaphragm fibers was markedly reduced ($\sim 30\%$), whereas fiber cross-sectional area remained unchanged (17). As expected, this reduction in myosin content was accompanied by decreased maximum force per cross-sectional area.

Striated muscle atrophy may be the result of increased proteolysis and/or reduced protein synthesis. Few data are available on the effects of COPD on diaphragm protein synthesis, although Nguyen and colleagues (12) reported lower expression of neonatal and embryonic myosin heavy chains in diaphragm muscle of patients with severe COPD, which would be in line with decreased myosin heavy chain synthesis. However, several recent studies show activation of proteolytic pathways in the diaphragm of patients with COPD (17, 25). During atrophy, the bulk of myofibrillar protein degradation occurs via the ubiquitin–proteasome pathway (26, 27). Proteolysis by this pathway is highly selective and precisely regulated. Proteins degraded via this pathway are labeled first with ubiquitin through the action of specific ligases. Ubiquitin-conjugated proteins are subsequently recognized, bound, and degraded by the 20S proteasome (26). Recent work demonstrated activation of the ubiquitin–proteasome pathway in the diaphragm of patients with mild-to-moderate COPD, as indicated by increased proteasome activity, elevated mRNA levels of the ubiquitin ligase MAFbx (25), and elevated levels of ubiquitin-conjugated proteins (17). These findings strongly suggest accelerated protein degradation via this proteolytic pathway. Activation of the endoprotease caspase-3 is an initial step in myofilament proteolysis by cleavage of myosin and actin (28). In this way, activated caspase-3 yields fragments that are degradable by the ubiquitin–proteasome pathway. Indeed, caspase-3 activity is increased in the diaphragm of patients

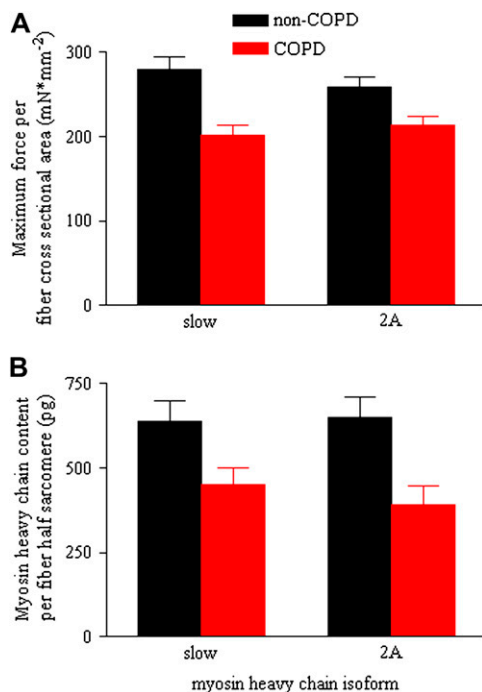


Figure 1. (A) Maximum calcium-activated force generation of skinned diaphragm fibers, normalized to fiber cross-sectional area, is reduced in patients with mild to moderate chronic obstructive pulmonary disease (COPD) compared with patients without COPD. (B) The reduced force generation of both slow type and type 2A fibers from patients with COPD is associated with reduced myosin heavy chain content per half sarcomere in those fibers compared with diaphragm fibers from patients without COPD. Myosin heavy chain content per half sarcomere estimates the number of cross-bridges in parallel per diaphragm fiber. Adapted by permission from Reference 17.

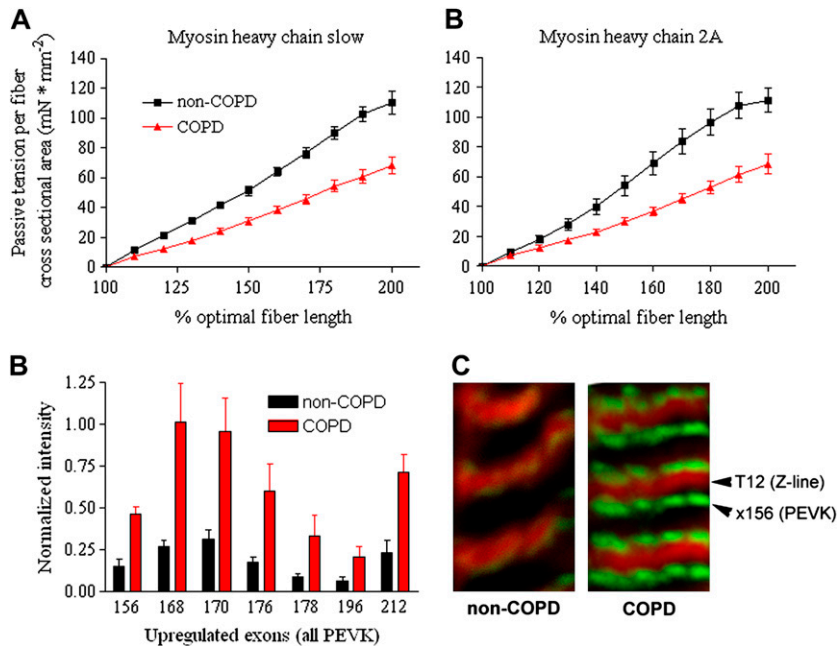


Figure 2. (A) Passive tension generated by slow type and type 2A fibers from patients with mild-to-moderate chronic obstructive pulmonary disease (COPD) is lower at all fiber lengths compared with fibers from patients without COPD. (B) Analysis of diaphragm transcripts of patients with and without COPD by a 50-mer oligonucleotide array containing 385 sequence-verified probes representing all of titin's human gene exons revealed up-regulation of seven exons in diaphragm from patients with COPD when compared with diaphragm from patients without COPD; all up-regulated exons code for the extensible PEVK (Pro-Glu-Val-Lys) segment of titin. (C) Confocal immunofluorescence analysis of antibody x156, directed against the PEVK domain encoded by exon 156. Double-staining with antibodies x156 and T12 (antibody directed against a non-up-regulated titin domain near the sarcomere's Z-line) revealed increased staining intensity of x156 in the sarcomere's I-band region in diaphragm cryosections from patients with COPD (green), whereas staining intensity of T12 was comparable between both groups (red). These data suggest increased protein expression of exon 156 in COPD diaphragm. Adapted by permission from Reference 15.

with mild to moderate COPD compared with patients without COPD (25).

Diaphragm Injury in COPD

Increased sarcomere disruption and Z-line misalignment have been found in the diaphragm of patients with moderate to severe COPD, as well as other abnormal features such as internal nuclei

and small angular fibers (24, 29). In patients with mild to moderate COPD (FEV_1 , $\sim 60\%$ predicted), diaphragm cross sections showed no signs of injury, although sarcomere length appeared shorter (22). The latter was proposed to be the result of hyperinflation-induced diaphragm shortening. Interestingly, compared with patients without COPD, diaphragms from patients with moderate to severe COPD were found to be three times more

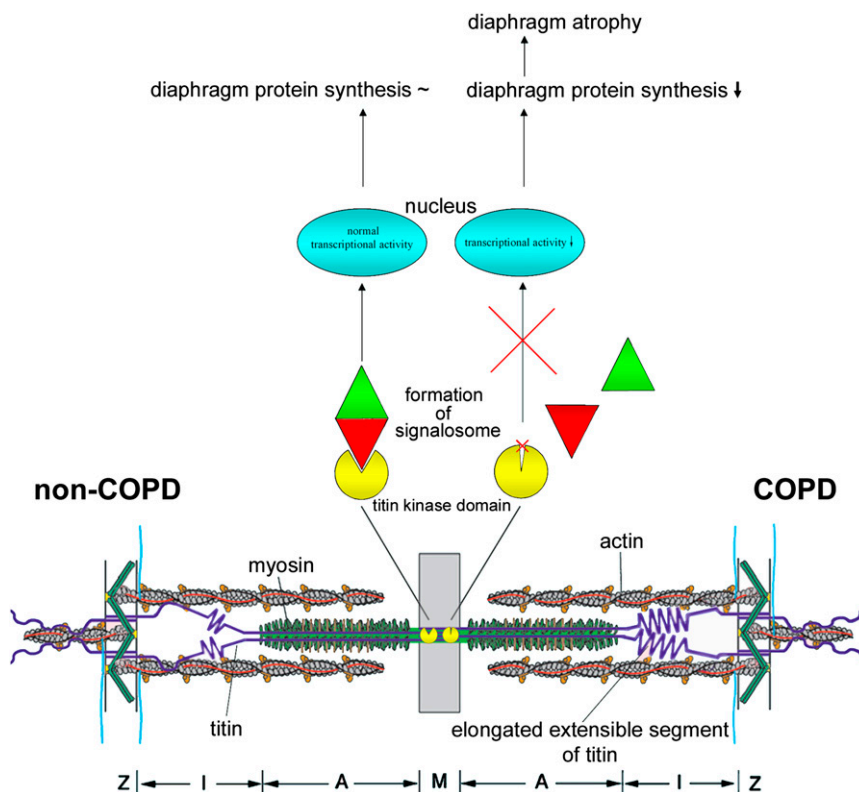


Figure 3. Titin and diaphragm atrophy in chronic obstructive pulmonary disease (COPD). The sarcomere is mainly composed of the thin (mostly actin) filaments, the thick (mostly myosin) filaments, and the giant filamentous molecule titin. Single titin molecules span the half sarcomere from the Z-line to the M-line. In the I-band region of the sarcomere, titin has an extensible segment that develops passive tension upon stretch. Previous work demonstrated that alternative splicing of the titin gene resulted in an elongated extensible segment reducing titin-based mechanical tension in COPD diaphragm. During contraction or passive tension, the titin kinase domain in the M-line is stressed. This mechanical tension opens the active site of the titin kinase domain and triggers the assembly of a signalosome that communicates with the nucleus and thereby regulates muscle gene expression. We hypothesize that the elongated extensible titin segment in COPD diaphragm reduces the mechanical stress on the titin kinase domain and results in impaired communication with the nucleus through preventing signalosome formation. The loss of this signaling pathway leads to diminished transcriptional activity and protein synthesis, resulting in diaphragm atrophy. The right half sarcomere represents a "COPD diaphragm sarcomere" including the elongated extensible titin segment; the left half sarcomere represents a non-COPD diaphragm, or normal sarcomere.

susceptible to additional sarcomere disruption when breathing against inspiratory loads (24).

INTERPRETATION AND INTEGRATION OF CHANGES IN COPD DIAPHRAGM: GENERATION OF A PATHOPHYSIOLOGICAL CONCEPT

The fiber-type shift toward more oxidative type I fibers is regarded as beneficial because it renders the overloaded COPD diaphragm more resistant to fatigue (9). Indeed, unlike control subjects, patients with severe COPD do not develop diaphragmatic contractile fatigue during maximal voluntary ventilation (30), and diaphragmatic contractile fatigue is an uncommon event after exhaustive exercise in severe COPD (31, 32). In contrast, the loss of myosin and force-generating capacity in diaphragm single fibers, and the elevated sarcomeric injury and oxidative stress, constitute alterations in the diaphragm of these patients that can be regarded as pathologic (7, 8, 17, 24, 29), and are suggested to greatly impair diaphragm isometric force generation *in vivo* (7, 8).

Diaphragm Weakness in COPD: *In Vivo* versus *In Vitro*

Based on their skinned fiber contractile data, Levine and colleagues (7) calculated that patients with severe COPD would generate 60% of normal maximal transdiaphragmatic pressure. Because maximal transdiaphragmatic pressure in patients with severe COPD is reduced to approximately 65% of control value (6, 33–35), they proposed that changes at the cellular and molecular level in the diaphragm can fully account for the reduced diaphragm strength *in vivo* (7). This proposition seemingly contradicts previous findings by Similowski and coworkers (6) suggesting that weakness of the diaphragm could be explained by hyperinflation-induced diaphragm shortening, which places the diaphragm on a suboptimal position on its pressure–length relationship. In fact, at equivalent lung volumes, the generated transdiaphragmatic pressures were even higher in some patients with COPD compared with healthy subjects. It should be noted, however, that the capacity to generate negative intrathoracic or transdiaphragmatic pressure is an indirect measure of inspiratory muscle strength (these methods do not solely depend on muscle function, but on nerve function and neuromuscular transmission as well), and that direct determination of diaphragm muscle force-generating capacity *in vivo* is not possible. It is hard to imagine that diaphragm fibers with markedly reduced myosin content (~65% of control values), as found in mild to moderate COPD (FEV₁, ~70% predicted) (15, 17), do not compromise diaphragm function *in vivo*. Therefore, to elucidate the apparent discrepancy between *in vivo* and *in vitro* diaphragm function, investigations into transdiaphragmatic pressure generation in patients with mild to moderate COPD are needed, together with studies into the potential role of factors that affect inspiratory muscle output upstream of the muscle, such as central drive, nerve function, and neuromuscular transmission.

Diaphragm Atrophy in COPD: The Relationship among Proteolysis, Oxidative Stress, and Sarcomeric Injury

The current knowledge suggests that accelerated proteolysis via the ubiquitin–proteasome pathway contributes to loss of myosin in COPD diaphragm, and constitutes an initial step in the pathogenesis of diaphragm weakness. Chamberlain (36) addressed the role of selective degradation of myosin by the ubiquitin–proteasome pathway in cachexia. Because preferential loss of myosin would increase the average distance between myosin and actin filaments, this might induce reduction of fiber size later in the course of the disease to restore filament lattice spacing needed for optimal contractile function. Indeed, loss of myosin

precedes reduction of fiber cross-sectional area in COPD diaphragm. Although speculative, these findings support the hypothesis that loss of myosin is an initial step in diaphragm fiber atrophy in COPD.

The increased susceptibility of the COPD diaphragm to inspiratory loading–induced sarcomeric injury (24) appears to be an important finding because it suggests that, during exacerbations of COPD, when patients face acute diaphragm loading, sarcomeres may develop additional injury. This could further compromise diaphragm function and consequently result in respiratory failure. Indeed, *post mortem* analysis of the diaphragm in patients with COPD suggests that acute-on-chronic loading of the respiratory muscles enhances diaphragm fiber injury and fibrosis (21). Titin is a major player in maintaining the structural and mechanical stability of sarcomeres during activation, by maintaining myosin filaments in a central position (18). Therefore, reduced stiffness of titin, as described earlier, is likely to induce structural instability of sarcomeres in COPD diaphragm, and could result in misalignment of myosin filaments and inability of the muscle fiber to resist sarcomere length inhomogeneity during activation. This overstretching of sarcomeres might contribute to the sarcomeric injury that occurs in the diaphragm of patients with severe COPD.

In addition, we postulate that the elevated levels of oxidized sarcomeric proteins in the diaphragm of patients with severe COPD (8) contribute to the increased susceptibility to diaphragm injury. Oxidized proteins have impaired structural integrity and function, and are known substrates for proteasomal degradation (37–39). These subtle structural modifications might be already present in patients with mild to moderate COPD, and play a role in the observed contractile protein dysfunction and the loss of myosin by accelerating proteasome-mediated proteolysis. Although in patients with moderate COPD the levels of oxidized diaphragm proteins did not appear to be elevated (8, 16), it should be noted that the detection of oxidized proteins is dependent on the sensitivity of the essays to measure such damage (40). Future studies using highly sensitive proteomic analysis should test this hypothesis.

Regeneration after Diaphragm Injury in COPD?

In general, muscle injury is followed by an inflammatory response and subsequent regeneration to prevent loss of muscle mass. Previous studies reported no evidence of inflammatory cells in the diaphragm of patients with COPD, although extensive fiber injury was evident (24, 29). Also, Nguyen and colleagues (12) found reduced expression of embryonic/neonatal myosin heavy chains in the diaphragm of patients with severe COPD. Considering that, normally, injured and regenerating muscle shows elevated developmental myosin heavy chain isoforms (41), elevated expression of embryonic/neonatal isoforms would be expected in COPD diaphragm. Although speculative, these data suggest an inadequate response to diaphragm injury in patients with COPD. A key feature of muscle regeneration after injury is activation of satellite cells and their subsequent proliferation into myoblasts and differentiation into new muscle tissue, replacing the injured/degenerated tissue (41). Considering their important role, disturbed proliferation and/or differentiation of satellite cells in COPD diaphragm might be involved in the proposed inadequate response to diaphragm injury in COPD. With this in mind, future studies investigating the proliferation and differentiation capacity of isolated satellite cells could shed more light on their role in diaphragm regeneration and atrophy in COPD.

A Weakened Giant Protein: Reducing Protein Synthesis in COPD Diaphragm?

The knowledge regarding diaphragm protein synthesis in COPD is very limited, but recent evidence suggests that it could be

negatively affected by the sarcomeric protein titin (42). Titin has a multitude of functions that go beyond its previously discussed mechanical role. In particular, recent studies indicate that titin is an important player in the maintenance of striated muscle mass. Titin functions as a stretch sensor, with titin-based stiffness regulating muscle remodeling and gene expression, through a signaling pathway linking activity of titin's kinase domain to nuclear transcriptional activity (42). Reduced mechanical strain on the titin kinase domain resulted in down-regulation of muscle-specific gene transcription, and was suggested to cause loss of contractile protein and muscle weakness in patients with hereditary myopathy with early respiratory failure (42). It is a very attractive hypothesis that the reduced stiffness of titin in the diaphragm of patients with mild to moderate COPD reduces its kinase domain activity, thereby inducing loss of signaling between titin and the nucleus, resulting in decreased transcription of muscle-specific genes (Figure 3). This could affect the production of contractile proteins, and contribute to the loss of myosin in the diaphragm of these patients, especially because diaphragm protein turnover appears to be elevated. Evaluating titin kinase activity and the distribution of sarcomeric and nuclear components of this signaling pathway in COPD diaphragm could give direction to the proposed hypothesis.

SPECULATIONS ON THE ETIOLOGY OF DIAPHRAGM WEAKNESS IN COPD

A major goal for future studies will be to identify the triggers responsible for the pathologic alterations in COPD diaphragm. This is especially challenging because several of these alterations are already present early in the course of the disease. We postulate two hypotheses describing potential triggers and proposed pathways leading to diaphragm atrophy and weakness. These two hypotheses are simplified in a schematic representation shown in Figure 4, and are further discussed below.

Chronic Diaphragm Loading Leading to Weakness: A "Mechanical Hypothesis"

A potential trigger for the described diaphragmatic changes in patients with COPD is increased contractile activity of the diaphragm. Short-term loaded breathing until task failure is known to impair diaphragm contractility in healthy humans (43). It was proposed that free radicals were involved because treatment with *N*-acetylcysteine, a molecule with antioxidant properties, attenuated diaphragm fatigue in these subjects (43). Free radicals have been shown to structurally modify proteins, impair contractile protein function, and activate the ubiquitin–proteasome pathway in a dose- and time-dependent manner (37, 44). In patients with severe COPD, diaphragm contractile activity is chronically increased (45), and recent data indicate oxidative stress in the diaphragm of these patients. Surprisingly, very little is known about the contractile activity of the diaphragm in patients with only mild to moderate COPD. Markers for oxidative stress were not elevated in the diaphragm of these patients, but, as mentioned previously, oxidative protein modification could be too subtle for detection with the applied assays. However, the fiber-type shift toward type I fibers in some patients with mild to moderate COPD (15, 16) is in line with elevated contractile activity of the diaphragm in these patients. Therefore, it is important that future studies are directed at determining diaphragm contractile activity in patients with mild or moderate COPD. These data should indicate whether or not altered diaphragm contractile activity might play a role in the development of diaphragm weakness in COPD.

Systemic Inflammation/Oxidative Stress Leading to Diaphragm Weakness: A "Systemic Hypothesis"

At rest, circulating levels of cytokines (e.g., IL-6 and tumor necrosis factor- α) are elevated in COPD (46–48), with some studies suggesting a relation with nutritional depletion. Animal studies have shown that cytokines initiate striated muscle injury, impair contractile protein function (49), and stimulate proteolysis through the ubiquitin–proteasome pathway *in vitro* (50, 51). Circulating cytokines are proposed to initiate cachexia-associated muscle wasting by selective targeting of myosin (36). Interestingly, a recent study showed that physical exercise induces an exaggerated systemic inflammatory (IL-6 production) and oxidative (free radical production by circulating neutrophils, lipid peroxidation, oxidized glutathione/reduced glutathione [GSSG/GSH] ratio) response in nutritionally depleted patients with moderate COPD (FEV₁, ~ 53% predicted), and to a lesser extent in nondepleted patients with moderate COPD (FEV₁, ~ 54% predicted) (52). This response might be harmful, because the elevated cytokines and oxidative stress may contribute to the observed alterations in the diaphragm. It would be of interest to investigate if this abnormal exercise-induced inflammatory/oxidative response is also present in mild to moderate COPD. It should be noted that, if a systemic etiology is proposed, alterations in the diaphragm and peripheral muscles are expected to share a high degree of similarity and, to a certain extent, develop simultaneously. This notion is based on the assumption that the different contractile pattern of the previously mentioned muscles does not affect their response to a systemic trigger; although no studies have confirmed this assumption, it also has not been rejected. However, the diaphragm and peripheral muscles appear differentially affected in COPD (e.g., a fiber-type shift in opposite direction in peripheral muscles compared with the diaphragm [9, 53], increased apoptosis in peripheral muscles [54], but not in the diaphragm, and whereas diaphragm injury is a common feature in patients with severe COPD [24], profound peripheral muscle injury has not been reported in these patients), and the diaphragmatic alterations appear to precede the peripheral muscle changes (16). The source for the exercise-induced cytokines remains to be identified. Initially, they had been attributed to bronchial inflammation; however, recent data indicate differential regulation of the cytokine response in induced sputum and plasma in patients with COPD (55), which may suggest additional origins for COPD-associated cytokinemia. The diaphragm provides an alternative candidate. In addition to elevated production of free radicals, diaphragm loading induces expression of cytokines in rat (56), and presumably human (57), diaphragm fibers. It could be speculated that the expression of cytokines is increased in the diaphragm of patients with mild to moderate COPD, and induces the observed sarcomeric injury and the protein modification and subsequent degradation but also the differential response of diaphragm and peripheral muscles. Therefore, evaluation of the cytokine profile in the diaphragm of patients with mild or moderate COPD will advance our understanding of the etiology of the cellular and molecular changes in COPD diaphragm.

CLINICAL PERSPECTIVE

Considering the clinical relevance of inspiratory muscle weakness in patients with COPD, counteracting diaphragm weakness is of major importance. Diaphragm wasting appears to play an important role in compromising diaphragm contractile performance in patients with COPD. Because the etiology of diaphragm wasting in COPD is complex and largely unclear, identifying therapeutic targets is extremely difficult. However,

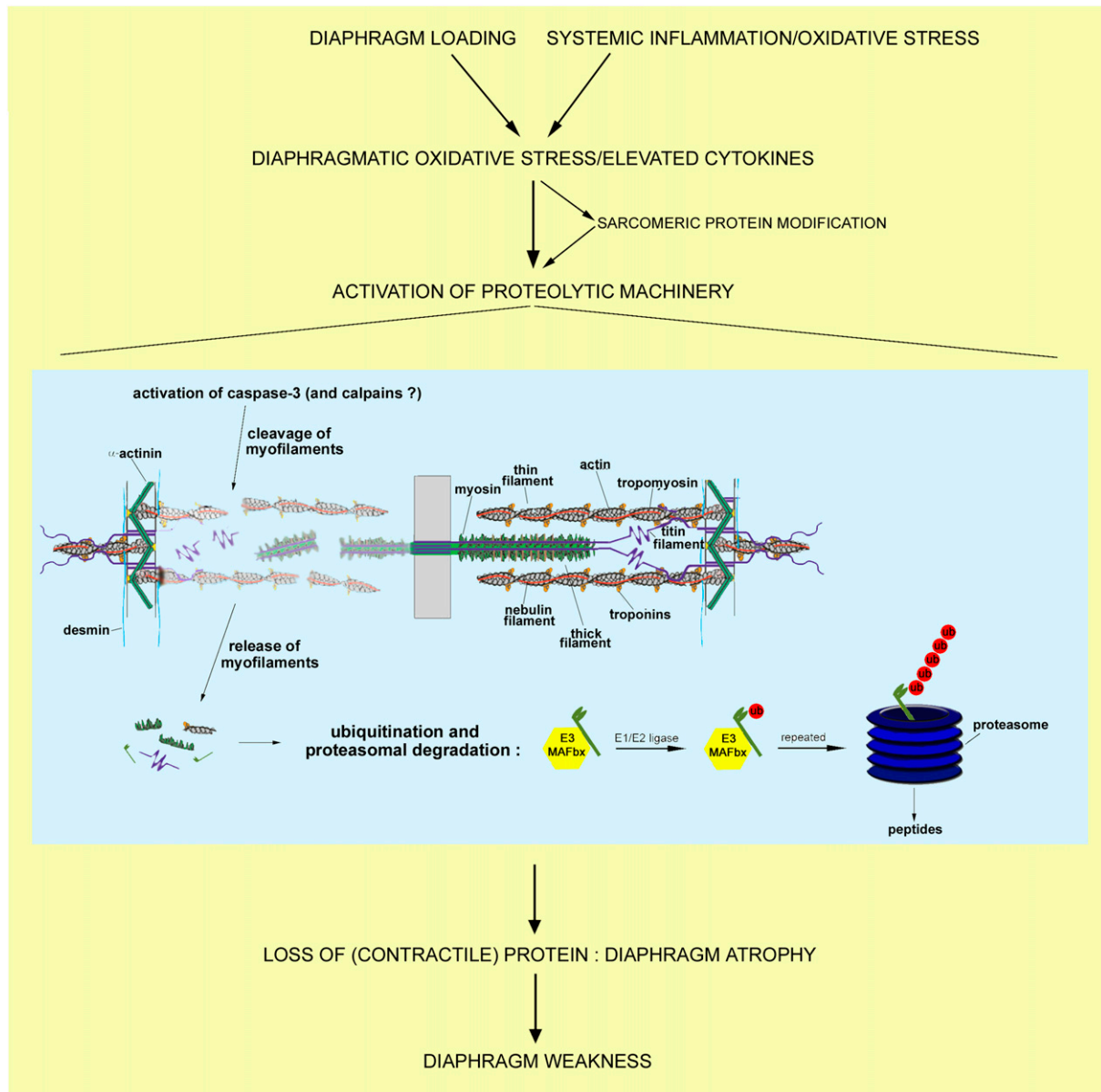


Figure 4. Schematic representation showing two proposed pathways toward diaphragm atrophy and weakness in chronic obstructive pulmonary disease (COPD). We hypothesize that patients with (mild to moderate) COPD are subject to increased diaphragm loading and/or systemic inflammation/oxidative stress leading to elevated levels of free radicals and/or cytokines in the diaphragm. These agents induce proteolysis by damaging sarcomeric proteins and stimulating caspase-3-mediated cleavage and release of myofilaments from the sarcomere. The released myofilaments are subsequently ubiquitinated through interaction of ubiquitin-ligases, such as the E3 ligase MAFbx, and degraded in the proteasome. The increased contractile protein degradation results in a net loss of contractile protein content and consequently to diaphragm atrophy and weakness. Ub = ubiquitin.

downstream in the line of events leading to wasting, increased proteolysis is likely to play a role, presumably through activation of the ubiquitin–proteasome pathway. Hence, agents inhibiting proteasomal activity are of potential therapeutic value. Recently, the proteasome inhibitor bortezomib has been approved for treatment of multiple myeloma in humans (58). It should be noted, however, that the ubiquitin–proteasome pathway is regarded only to degrade damaged or misfolded proteins. Consequently, inhibiting proteasomal activity might lead to accumulation of damaged proteins, ultimately leading to cell death. Nevertheless, *in vivo* administration of bortezomib in an animal model of muscle atrophy prevented muscle wasting by approximately 50% (59, 60), and in both studies, bortezomib was well

tolerated. These findings show promise for the use of proteasome inhibitors in syndromes associated with muscle wasting, such as the diaphragm in COPD. Theoretically, inhibition of E3-ligases rather than the proteasome provides an ideal drug target, because E3-ligases have very high substrate and tissue selectivity. Therefore, a specific inhibitor of, for example, MAFbx should be a highly specific drug, and might prove beneficial in preserving contractile protein content and preventing diaphragm atrophy in COPD diaphragm. To date, no ubiquitin–ligase inhibitor has reached the clinic.

Another strategy could be to tackle the muscle impairment itself rather than an upstream biochemical defect. A recent study (61) demonstrates the approach of reducing muscle weakness

in dystrophin-deficient mice, by blocking the action of myostatin. Myostatin is an important regulator of striated muscle growth, whose absence results in a marked increase of muscle mass in mice (62). Inhibitors of deacetylase attenuated the morphological and functional consequences of the primary genetic defect by inducing the expression of the myostatin antagonist follistatin in satellite cells (61). Satellite cells treated with this inhibitor showed increased expression of embryonic myosin heavy chains and formed myotubes earlier and with an increased size. On the whole-muscle level, treatment with this deacetylase inhibitor restored the muscle's force-generating capacity and morphology. These findings are of particular therapeutic interest, because deacetylase inhibitors are available for trials in humans.

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