Ventilator-induced diaphragm dysfunction: cause and effect

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Ventilator-induced diaphragm dysfunction: cause and effect. Am J Physiol Regul Integr Comp Physiol 305: R464–R477, 2013. First published July 10, 2013; doi:10.1152/ajpregu.00231.2013.—Mechanical ventilation (MV) is used clinically to maintain gas exchange in patients that require assistance in maintaining adequate alveolar ventilation. Common indications for MV include respiratory failure, heart failure, drug overdose, and surgery. Although MV can be a life-saving intervention for patients suffering from respiratory failure, prolonged MV can promote diaphragmatic atrophy and contractile dysfunction, which is referred to as ventilator-induced diaphragm dysfunction (VIDD). This is significant because VIDD is thought to contribute to problems in weaning patients from the ventilator. Extended time on the ventilator increases health care costs and greatly increases patient morbidity and mortality. Research reveals that only 18–24 h of MV is sufficient to develop VIDD in both laboratory animals and humans. Studies using animal models reveal that MV-induced diaphragmatic atrophy occurs due to increased diaphragmatic protein breakdown and decreased protein synthesis. Recent investigations have identified calpain, caspase-3, autophagy, and the ubiquitin-proteasome system as key proteases that participate in MV-induced diaphragmatic proteolysis. The challenge for the future is to define the MV-induced signaling pathways that promote the loss of diaphragm protein and depress diaphragm contractility. Indeed, forthcoming studies that delineate the signaling mechanisms responsible for VIDD will provide the knowledge necessary for the development of a pharmacological approach that can prevent VIDD and reduce the incidence of weaning problems.

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MECHANICAL VENTILATION (MV) is used clinically to achieve sufficient pulmonary gas exchange in patients unable to sustain adequate alveolar ventilation on their own. Common indications for MV include respiratory failure due to chronic obstructive pulmonary disease, status asthmaticus, and/or heart failure. In addition, MV is often an essential intervention in patients suffering from acute drug overdose, neuromuscular diseases, sepsis, and during surgery along with postsurgical recovery.

The number of patients receiving prolonged MV in the United States exceeds more than 300,000 each year in the intensive care unit (ICU) (20). Although MV can be a lifesaving measure, prolonged MV results in the rapid development of diaphragmatic weakness due to both atrophy and contractile dysfunction. This detrimental impact of prolonged MV on the diaphragm has been termed ventilator-induced diaphragmatic dysfunction (VIDD) and VIDD is predicted to be a major contributor to problems in weaning patients from the ventilator (26, 114). Although previous reports describing the impact of prolonged MV on the diaphragm have appeared in the literature, advances in our understanding of the mechanisms responsible for VIDD have grown rapidly during recent years. Therefore, this review will present our current knowledge about the events leading to VIDD along with a detailed discussion of the cellular mechanisms responsible for the rapid development of VIDD. We begin with a historical overview of the discovery of VIDD.

Ventilator-Induced Diaphragm Dysfunction: Historical Overview

Our understanding of the phenomenon of VIDD has evolved over the past 25 years as research has moved from descriptive milestones to a better understanding of the mechanisms responsible for VIDD (see Fig. IA). The first suggestion that prolonged MV results in diaphragmatic atrophy was published over 25 years ago. This retrospective study postulated that MV predisposes diaphragm myofibers to atrophy in infants and neonates that were provided long-term ventilator assistance (53). Although provocative, this investigation did not provide direct evidence that prolonged MV promotes diaphragmatic atrophy. The first prospective study to document the impact of MV on rodent diaphragm atrophy and contractile function appeared in 1994 (56). This landmark research revealed that 48 h of controlled MV (i.e., full ventilator support of breathing) resulted in significant loss of diaphragm mass and a large reduction in maximal diaphragmatic specific force production. This original account was quickly supported by another study depicting the influence of prolonged MV on in vivo diaphragm function in healthy baboons (6). This primate study concluded that prolonged MV results in significant impairment of diaphragmatic contractile performance as indicated by a decrease in both maximal transdiaphragmatic pressure and diaphragmatic endurance (6). Following these early investigations,
scientific interest in the effects of MV on diaphragm structure and function grew rapidly, and numerous animal studies published in 2002–2003 consistently concluded that prolonged MV results in the rapid development of both diaphragmatic atrophy and contractile dysfunction (16, 19, 33, 91, 93, 98, 100). Mechanistic studies into the cell signaling events responsible for VIDD began in 2003 when investigations revealed that prolonged MV results in diaphragmatic oxidative stress and that oxidant damage in the diaphragm is a requirement for VIDD (9, 119).

While animal studies consistently indicated that prolonged MV promotes VIDD, the question of whether long-term ventilator support produces VIDD in humans remained unknown until a milestone study published in 2008 revealed that prolonged MV results in rapid diaphragmatic atrophy in humans (58). These seminal findings have now been confirmed by other groups, and together, these studies clearly demonstrate that prolonged MV results in the rapid development of both diaphragmatic atrophy and contractile dysfunction in humans (39, 42, 47). The confirmation that VIDD occurs in humans has accelerated a widespread search for the mechanism(s) responsible for VIDD in hopes of identifying a biological target for drug intervention to prevent or delay VIDD in patients, and this effort continues to the present day as illustrated by the increase in publications in this field (Fig. 1B).

**MV-Induced Diaphragmatic Atrophy**

Both animal and human experiments consistently demonstrate that prolonged MV promotes diaphragmatic atrophy resulting in a reduction in diaphragm mass. In this section we discuss the temporal pattern of the MV-induced diaphragm atrophy that occurs in both animals and humans. Note that when published data exist, we will also highlight the impact of different modes of MV on diaphragm atrophy. Specifically, ventilator support to patients is classified into two general categories or modes: 1) full ventilator support (often called controlled MV); or 2) partial ventilator support (numerous
modes of partial support MV exist). When a patient is provided full ventilator support the ventilator provides all of the ventilation to the lungs and the patient’s respiratory muscles remain inactive. In contrast, during partial ventilator support, the patient’s respiratory muscles (i.e., diaphragm) provide a portion of the work of breathing and the ventilator provides the remainder.

**MV-Induced Diaphragm Atrophy**

**Animal studies.** Numerous animal studies using a variety of species (mice, rats, rabbits, and pigs) have reported that prolonged MV results in significant atrophy of diaphragm muscle fibers (16, 49, 73, 78, 100, 107). However, because the rat and human diaphragm are anatomically alike and contain a similar fiber type composition (76, 81), the rat has become the most commonly used animal model to study MV-induced changes in diaphragm fiber size and function. Several studies reveal that as few as 12 h of controlled MV results in a 10–15% reduction in the cross-sectional area of all rat diaphragm fiber types (i.e., type I, type IIa, and type IIx/b) (67, 69, 82, 103). This MV-induced rat diaphragmatic fiber atrophy increases as a function of time and approaches a 30% reduction in fiber cross-sectional area following 18–24 h of prolonged MV (33, 100, 117). It is important to note that both partial support and full support MV results in diaphragmatic atrophy albeit the MV-induced diaphragmatic atrophy that occurs during partial support ventilation occurs at a slower rate compared with the atrophy induced by full support ventilation (44, 49).

**Human studies.** To date, three independent studies have evaluated the impact of prolonged MV on diaphragm atrophy in humans. The first report demonstrated that 18–69 h of full support MV results in significant diaphragmatic atrophy (~50% reduction in fiber cross-sectional area) of both type I (slow) and type II (fast) muscle fibers in the costal diaphragm (58). This original finding was recently confirmed in another study indicating that diaphragmatic fiber atrophy occurs in humans exposed to full support MV for <24 h with the magnitude of the diaphragmatic atrophy being significantly correlated with the duration of MV (47). Additionally, a recent observational study using serial ultrasound measurements to assess diaphragmatic thickness revealed that diaphragm thinning occurs within 48 h after the initiation of partial support MV (39). This study confirmed that diaphragmatic atrophy occurs linearly as a function of time on the ventilator with the rate of atrophy averaging a 6% loss of diaphragm thickness per day of MV. Together, these human studies demonstrate that prolonged MV results in rapid atrophy of diaphragm muscle fibers in humans. Although it is difficult to make a direct comparison between the human and animal studies in the rates of MV-induced diaphragmatic atrophy, it is clear that the temporal patterns of MV-induced diaphragmatic atrophy are similar between rats and humans with diaphragmatic atrophy occurring in both species within 24–48 h after the initiation of MV (46) (Fig. 2, A–C). Furthermore, both human and animal studies reveal that MV-induced diaphragmatic atrophy occurs during both partial (44) and full support MV (33, 39, 47, 58).

**Mechanisms Responsible For Ventilator-Induced Diaphragmatic Atrophy**

Skeletal muscle fiber size is regulated by the balance between the rates of protein degradation and protein synthesis. It is now well established that proteolysis in the diaphragm is rapidly increased during full support MV (reviewed in Ref. 85). Furthermore, prolonged full support MV results in a depression of protein synthesis in the diaphragm (99). Collectively, this MV-induced increase in proteolysis coupled with depressed protein synthesis results in the net loss of protein and diaphragm fiber atrophy. The next sections will discuss the role that depressed protein synthesis and increased proteolysis play in MV-induced diaphragmatic atrophy. Importantly, we will also highlight the signaling pathways that regulate MV-induced decreases in diaphragm protein synthesis and increases in diaphragm protein degradation.

**MV-Induced Decreases in Protein Diaphragmatic Synthesis**

The impact of prolonged MV on protein synthesis in humans is currently unknown. However, animal studies show that full support MV promotes a rapid decline in protein synthesis in the rat diaphragm (32, 99). Specifically, measurement of rat diaphragmatic protein synthesis in vivo reveals that the rate of both mixed and myosin heavy chain protein synthesis declines

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**Fig. 2.** Illustration of the temporal pattern of MV-induced diaphragm atrophy in both rats and humans. **A:** time course of diaphragm type IIX/B fiber atrophy in the rat during 24 h of full support MV. Type IIX/B fibers were selected as a representative fiber to illustrate the temporal pattern of MV-induced diaphragm fiber atrophy. Data are from references 2, 30, 33, 35, 44, 62, 63, 67–69, 75, 82, 100, 101, 103, 108, 116, and 117. **B:** time course of diaphragmatic atrophy in humans during full support MV (data are from Refs. 47 and 58). Note that these data represent the average decline in diaphragm fiber cross-sectional area in both type I and II fibers. **C:** time-related changes in diaphragmatic thickness in humans receiving both full and partial support MV (data are from Ref. 39). It is important to note that none of these patients were septic during these measurements.
rapidly within the first 6 h of full support MV and remains depressed during the next 12 h of MV (99).

Only one investigation has explored the influence of partial support MV on rat diaphragm protein synthesis. This study reveals that compared with spontaneous breathing animals, partial support ventilation does not promote a significant decrease in diaphragm protein synthesis in vitro (32). How much ventilator support was provided in this study is unknown and whether or not these results can be replicated in vivo remains unclear and warrants additional work.

Regulation of diaphragmatic protein synthesis. Skeletal muscle contractile activity plays a key role in the control of protein synthesis. Specifically, increased muscle contractile activity promotes increased protein synthesis, whereas muscle inactivity results in decreased rates of protein synthesis (13, 54). The initial decrease in diaphragm protein synthesis that occurs during full support MV is likely due to decreased protein translation because myosin heavy chain mRNA levels remain unchanged (99, 120). Indeed, the rate of protein synthesis in muscle is largely controlled by the efficiency of translation, which is regulated at the level of initiation (52). In this regard, the Akt/mammalian target of rapamycin (mTOR) signaling pathway has been shown to play a key role in the regulation of translation (12). More specifically, the mTOR complex 1 (mTORC1) is a key regulatory protein in at least two rate-limiting steps in translation initiation. First, mTORC1 regulates mRNA binding to the ribosome by controlling availability of eukaryotic initiation factor 4E (eIF4E). When phosphorylated by Akt, mTORC1 can phosphorylate eIF4E-binding protein (4E-BP1), a competitive repressor of eIF4E (36). Phosphorylation of 4E-BP1 liberates eIF4E allowing progression in the formation of the functional ribosome. Furthermore, mTORC1 directly phosphorylates the 70-kDa ribosomal protein S6 kinase 1 (p70S6K1), which subsequently phosphorylates components required in the formation/maintenance of the ribosome (21, 31) (Fig. 3, left).

Emerging evidence from both human and animal experiments reveal that prolonged MV decreases Akt activation in the diaphragm due to hypophosphorylation (57, 68) resulting in decreased phosphorylation of p70S6K1 and 4E-BP1 (70) (Fig. 3, right). Complete details of the mechanisms regulating Akt remain unknown but possibilities include decreased IGF-1 or other growth factors and/or intrinsic factors (10, 33). Indeed, prolonged MV results in decreased levels of IGF-1 in the diaphragm (33). Together, these results suggest that disruption of Akt/mTOR signaling plays a key role in the MV-induced decrease in diaphragm protein synthesis.

Role of depressed protein synthesis in MV-induced diaphragm atrophy. Clearly, a decrease in protein synthesis can contribute to diaphragmatic atrophy during days to weeks of MV. Largest decrease in protein synthesis occurs during the initial 6 h of MV. Akt signaling is decreased during MV and contributes to decreased protein synthesis during this time period. mTOR signaling is also decreased during MV and contributes to decreased protein synthesis during this time period. Figure 3 illustrates the regulation of Akt and mTOR signaling in the diaphragm during both spontaneous breathing and prolonged MV.
prolonged MV. Nonetheless, because diaphragmatic atrophy appears in both the rat and human diaphragm within the first 12–24 h after the initiation of MV, it is predicted that this rapid onset of MV-induced diaphragmatic atrophy occurs due to increased proteolysis rather than decreased protein synthesis. Nonetheless, a sustained decrease in diaphragm protein synthesis would indeed contribute to diaphragmatic atrophy during long-term MV. A discussion of the role that proteolytic systems play in MV-induced diaphragm atrophy along with the signaling pathways that regulate proteolysis in the diaphragm are presented in the next segments.

**MV-Induced Proteolysis in the Diaphragm**

The finding that prolonged full support MV results in the rapid activation of proteases and increased proteolysis in the rat diaphragm was first reported by Shanel et al. (100). This original observation has been confirmed in numerous animal and human studies using full support MV. Indeed, abundant evidence demonstrates that prolonged full support MV increases the activity of all four major proteolytic systems in the diaphragm of both animals and humans exposed to 12 or more hours of MV (9, 22, 44, 45, 47, 57, 58, 63, 70, 97, 106, 117). A brief overview of these proteolytic pathways along with evidence for their activation in the diaphragm during prolonged MV follows.

Macroautophagy (hereafter referred to as autophagy) is a catabolic process that degrades cytosolic proteins and organelles using lysosomal proteases. Specifically, lysosomal proteases (i.e., cathepsins) are the cellular proteolytic system charged with the removal of both organelles and nonmyofibrillar cytosolic protein aggregates (72). During autophagy, targeted cytosolic constituents are encapsulated into a double-membrane vacuole called the autophagosome. When complete, the autophagosome moves through the cytosol to the lysosome where the contents of the autophagosome are degraded. A recent report reveals that prolonged MV increases the expression of key autophagy proteins (e.g., ATG5, ATG7, and beclin 1) and the number of autophagosomes in the human diaphragm (45). Furthermore, emerging evidence reveals an increase in autophagy biomarkers in rodent diaphragms following 8–18 h of full support MV (97, 107). Together, these results suggest that full support MV increases the rate of autophagy in both human and animal diaphragms.

Calpains are cysteine proteases that promote muscle atrophy by cleaving over 100 different cellular proteins (37). The calpain family of proteases contains 14 different members, but the two ubiquitous calpains located in skeletal muscle are calpains I and II (37). Numerous studies reveal that calpain cleavage of Z line-associated proteins (i.e., titin and nebulin) is responsible for the release of myofilament proteins that are then degraded by the ubiquitin-proteasome system and perhaps other proteases as well (37, 102). Importantly, it is established that prolonged MV activates calpain in the diaphragms of both humans and animals (47, 63, 75, 100).

Caspase-3 belongs to a large family of cysteine proteases and plays an important role in apoptosis and may also contribute to muscle protein degradation during a variety of muscle-wasting conditions. For example, caspase-3 is active in skeletal muscle during periods of unloading and active caspase-3 can degrade actin/myosin complexes (24, 67). Abundant evidence demonstrates that full support MV activates caspase-3 in both the rodent and human diaphragm (58, 67, 69, 75, 106). This is important because active caspase-3 is required for myonuclear apoptosis in the diaphragm (67).

The ubiquitin-proteasome system of proteolysis plays an important role in muscle protein degradation during a variety of wasting conditions (11, 38). The total proteasome complex (26S) is composed of a core proteasome subunit (20S) coupled with a regulatory complex (19S) connected to each end of the 20S core (84). The 20S proteasome can degrade oxidized proteins without ubiquitination, whereas the 26S proteasome degrades ubiquitinated proteins (40). Hence, the 26S proteasome degradation pathway is active after ubiquitin covalently binds to protein substrates and marks them for degradation. The binding of ubiquitin to protein substrates is often a three-step process that concludes with specialized ubiquitin ligases (E3s) that recognize specific protein substrates. Numerous specific E3 ligases exist in skeletal muscle (e.g., atrogin-1/muscle atrophy F-box and muscle ring finger-1), and these ligases play essential roles in skeletal muscle atrophy (11, 38). In this regard, full support MV increases the expression of muscle-specific E3 ligases (atrogin1/muscle atrophy F-box and muscle ring finger-1) along with an increase in ubiquitinated proteins in both human and rat diaphragms (22, 45, 47, 57). Furthermore, prolonged MV increases the activity of the 20S and 26S proteasome in both rodent and human diaphragms following MV (9, 22, 57). Collectively, these findings demonstrate that prolonged full support MV increases the activation of the ubiquitin-proteasome system in the diaphragm.

Currently, only two studies have investigated the impact of partial support MV on protease activation in the rat diaphragm with differing results. Specifically, Futier et al. (32) reported that 18 h of partial support MV did not increase proteolysis in the rat diaphragm. In contrast, Hudson et al. demonstrated that 18 h of partial support MV activates the 20S proteasome, calpain, and caspase-3 in the diaphragm (44). The reason(s) for these divergent results are unclear but could be due to differences in the levels of ventilator support between the two studies. Finally, to date, there are no published reports on the effects of partial support MV on protease activation in humans.

**Regulation of MV-induced protease activation in the diaphragm.** Recent studies provide new insight into the control of key signaling pathways responsible for MV-induced protease activation. First, growing evidence confirms that MV-induced reactive oxygen species (ROS) production in the diaphragm is required to activate key proteases and promote atrophy in the diaphragm. Indeed, prevention of MV-induced oxidative stress in the rat diaphragm using the antioxidants N-acetylcysteine or trolox has been shown to prevent MV-induced protease activation and diaphragmatic atrophy (1, 9, 68, 117).

The mechanisms responsible for MV-induced diaphragmatic oxidative stress have been investigated extensively. Specifically, MV-induced oxidative damage occurs due to a decrease in diaphragmatic antioxidant capacity along with a significant increase in oxidant production within diaphragm muscle fibers (29). Although it is feasible that the MV-induced increase in oxidant production in diaphragm fibers occurs via several oxidant producing systems (e.g., NADPH oxidase, xanthine oxidase, or mitochondria), the dominant site of oxidant production in the diaphragm during prolonged MV is the mito-
chondrion (30, 69, 82, 111, 116). For example, as few as 12 h of MV results in a large increase in ROS emission from rat diaphragm mitochondria during both state 3 and state 4 respiration (50, 82). This increase in mitochondrial ROS production is associated with increased lipid peroxidation and protein oxidation within mitochondria (50). Moreover, the activities of the electron transport chain complexes II, III, and IV are depressed in mitochondria isolated from diaphragms of rats exposed to only 12 h of MV and prolonged MV in rats also promotes diaphragmatic mitochondrial uncoupling as indicated by a decrease in the respiratory control ratio (50). Importantly, two independent studies have confirmed that prolonged MV also results in mitochondrial damage in the human diaphragm (80, 106). Finally, treatment of animals with a mitochondrial-targeted antioxidant prevents the MV-induced activation of several proteolytic systems and prevents VIDD (82). Together, these findings indicate that ventilator-induced mitochondrial ROS emission is a required upstream signal for protease activation in the diaphragm during prolonged MV.

In regard to ROS and diaphragm proteolysis, abundant evidence indicates that oxidative stress can accelerate proteolysis in skeletal muscle fibers in several ways (reviewed in Ref. 88) (Fig. 4). First, oxidative stress promotes increased gene expression of key proteins involved in both autophagy and the ubiquitin-proteasome system (reviewed in Ref. 88). Presumably this ROS-mediated gene expression occurs due to redox-sensitive transcriptional activating factors (e.g., NFkB, FoxO3a, etc.) that activate atrogenes (59, 101). Furthermore, MV-induced oxidative stress in the diaphragm is associated with increased 20S proteasome activity (9). Whether this increased 20S proteasome activity is due to allosteric upregulation of protease activity or increased expression of key subunits of the 20S proteasome is unknown. Also, MV-induced ROS production in skeletal muscle has been shown to activate calpain (82, 117). The specific mechanism(s) responsible for this ROS-mediated activation of calpain has not been studied but is potentially linked to ROS-mediated increases in cytosolic free calcium (37, 88). Finally, MV-induced oxidative stress has also been shown to activate caspase-3 in the diaphragm (82, 117). This oxidant-mediated activation of caspase-3 in the diaphragm during prolonged MV occurs via an intrinsic pathway of apoptosis (i.e., activation of caspase-9 or caspase-12) because caspase-8 is not activated in the diaphragm of animals or humans during prolonged MV (75, 106). Finally, oxidative modification of skeletal muscle proteins can enhance their susceptibility to proteolytic degradation (102). Specifically, oxidation of myofibrillar proteins increases their susceptibility to proteolytic degradation by the 20S proteasome, calpain, and caspase-3 (40, 41, 102). (For more details linking oxidative stress-induced signaling to skeletal muscle atrophy see references 79, 86, 88, 89, 105, and 110).

**Role of accelerated proteolysis in MV-induced diaphragm atrophy.** As stated previously, evidence from both human and animal studies reveal that all four major proteolytic systems are rapidly activated in the diaphragm following 12–24 h of full support MV (9, 22, 45, 47, 57, 58, 100, 106). However, the
relative contribution of each of these proteolytic systems in promoting VIDD remains a topic of debate. Existing evidence indicates that pharmacological inhibition of calpain activity decreases the rate of proteolysis in diaphragm muscle (in vitro) removed from animals exposed to prolonged MV (100). Furthermore, in vivo inhibition of calpain activity in rats during prolonged MV provides significant protection against MV-induced diaphragm atrophy (63, 75). Similarly, inhibition of caspase-3 activity in rats during prolonged MV also offers protection against MV-induced diaphragmatic atrophy (67, 75).

Interestingly, a recent study has uncovered that a regulatory cross-talk exists between calpain and caspase-3 activity in the diaphragm during prolonged MV whereby calpain activation promotes caspase-3 activation and vice versa (75). The mechanism(s) responsible for cross-talk regulation is unclear but appears to be linked to the signaling pathways that regulate the activation of both calpain and caspase-3 (75).

Currently, limited information exists regarding the role that the ubiquitin-proteasome system or autophagy plays in MV-induced proteolysis in the diaphragm. Nonetheless, a recent study delivered the protease inhibitor Bortezomib to rats in an effort to inhibit the ubiquitin-proteasome system in the diaphragm during prolonged MV (2). However, Bortezomib inhibited caspase-3 activation but did not inhibit the MV-induced increase in 20S proteasome activity in the diaphragm. Therefore, additional research is needed to clarify the contribution of this proteolytic pathway to MV-induced diaphragmatic atrophy.

In summary, it is clear that increased proteolysis plays a dominant role in MV-induced diaphragm atrophy during the first 12–24 h of MV. In this regard, both calpain and caspase-3 play important roles in the rapid development of MV-induced diaphragmatic atrophy. Indeed, inhibition of either calpain or caspase-3 activity provides significant protection against MV-induced diaphragmatic atrophy during the first 12–24 h of MV. The specific role that the ubiquitin-proteasome system and autophagy play in MV-induced atrophy is unknown and remains an important area for future research.

**MV-Induced Diaphragmatic Contractile Dysfunction**

During the past two decades, numerous experiments have reported that prolonged MV promotes diaphragm contractile dysfunction. In this segment we summarize the major findings of both animal and human experiments on the effects of prolonged MV on diaphragm contractile function. We also consider the impact of clinically relevant drugs on the severity of MV-induced diaphragm dysfunction and discuss potential mechanisms responsible for MV-induced diaphragm contractile dysfunction.

**Animal studies.** An early study revealed that 5 days of MV resulted in a depression of in vivo diaphragm contractility in piglets (91). Furthermore, this investigation demonstrated that phrenic nerve conduction and neuromuscular transmission were not affected by prolonged MV. Importantly, these results suggest that the MV-induced diaphragm contractile dysfunction occurs at the level of the peripheral muscle (91). Indeed, numerous animal studies consistently report that prolonged MV results in a rapid and time-dependent decrease in diaphragmatic specific force production measured in vitro using electrical stimulation of diaphragmatic muscle strips (9, 16, 19, 33, 35, 56, 87, 93, 98). A recent report suggests that as few as 6 h of MV results in diaphragmatic contractile dysfunction in mice (73). In a rat model of MV, 12 h of full ventilator support results in a ~15–20% reduction in maximal diaphragm-specific force production, whereas 48 h of MV can depress diaphragm maximal specific force by ~50% below the level of control animals (56, 87) (Fig. 5A). Moreover, prolonged MV also reduces rodent diaphragm-specific force production at submaximal stimulation frequencies (e.g., 10–40 Hz) (56, 73, 87). Investigations using both rodent (113) and porcine (78) models indicate that MV-induced contractile dysfunction occurs in all muscle fiber types. This ventilator-induced impairment in diaphragmatic contractile function in animals appears to be directly linked to diaphragm contractile inactivity because ventilator modes that provide partial ventilator support or short periods of intermittent spontaneous breathing during prolonged MV can reduce the magnitude of full ventilator support-induced contractile dysfunction (35, 44, 49, 94). Furthermore, a recent study reveals that short periods of bilateral phrenic nerve stimulation (10 min/h) protects the diaphragm against full support MV-induced diaphragm contractile dysfunction (118).

Only two studies have investigated the impact of prolonged MV on diaphragm fatigue in animals. The first study concluded...
that 11 days of full support MV combined with neuromuscular blockade resulted in impaired diaphragmatic fatigue in healthy baboons (6). In contrast, 18 h of full support MV in rats has been shown to improve diaphragmatic endurance measured in vitro (98). These divergent findings may be due to the fact that in vitro muscle endurance tests may not reflect in vivo muscle performance because these muscles are not perfused. Clearly, additional experiments are warranted to determine the impact of prolonged MV on diaphragmatic fatigue in vivo.

Human investigations of MV-induced diaphragm contractile dysfunction. To date, only two published reports exist regarding the impact of prolonged MV on diaphragmatic force production in humans. In both studies diaphragmatic force production was assessed in vivo by bilateral magnetic stimulation of the phrenic nerves followed by the measurement of either transdiaphragmatic pressure or changes in endotracheal tube pressure as the index of diaphragmatic force production. Both reports demonstrate that prolonged MV results in decreased diaphragmatic force production (42, 47). Indeed, human diaphragmatic force production decreases rapidly during the first 48 h of MV with the MV-induced decline of contractile dysfunction following a time-dependent pattern (42, 47). A comparison of the human and animal studies on the impact of prolonged MV on diaphragmatic force production suggests that the MV-induced decrease in diaphragmatic force production appears to be similar between humans and animal experimental models during the first 48 h of full support MV (46) (Fig. 5, A and B).

Impact of Age and Drugs on MV-Induced Diaphragmatic Contractile Dysfunction

In theory, several factors could interact with prolonged MV to negatively impact diaphragm contractile function. For example, old age or treatment of patients with neuromuscular blockers and/or glucocorticoids could exacerbate MV-induced diaphragm contractile dysfunction. Our understanding of the impact of these factors on MV-induced diaphragm contractile function in animals is highlighted in the next segments.

Impact of age on MV-induced diaphragm contractile dysfunction. An important factor that could influence the impact of prolonged MV on diaphragmatic function is age. In general, compared with young adult animals, aging results in a reduction in diaphragmatic maximal specific tension (18, 19). For example, compared with young adult rats, diaphragmatic maximal specific force production is \(-13\%\) lower in senescent animals (19). However, senescence does not appear to increase the percent decrease in MV-induced diaphragm-specific force production (19). That is, despite the similar relative MV-induced decline in diaphragmatic force production in young and old animals, the negative effects of MV are compounded to the age-related decrease in diaphragmatic contractile performance. It follows that this collective effect of both aging and MV-induced diaphragmatic contractile dysfunction could explain why patient age is an independent predictor of difficulties in patient weaning (23).

Influence of neuromuscular blockade on MV-induced diaphragm contractile dysfunction. Another clinically relevant issue related to MV-induced diaphragmatic contractile dysfunction is the impact of commonly used drugs that can impact muscle force production. Indeed, patients are commonly treated with drugs that could exacerbate the impact of MV on diaphragmatic function. For example, prolonged administration of nondepolarizing neuromuscular-blocking agents remains common in the ICU (7). These agents are used to minimize patient discomfort, prevent respiratory movements, improve arterial \(P_{O_2}\), and reduce oxygen consumption (7, 74). A possible complication of using neuromuscular-blocking agents in the ICU is skeletal muscle myopathy and contractile dysfunction. In this regard, two investigations reveal that use of the neuromuscular-blocking agent rocuronium increases the magnitude of MV-induced diaphragmatic contractile dysfunction in rats (108, 109). In contrast, another recent study using a porcine model of MV concluded that treatment of animals with rocuronium does not have an additive effect on MV-induced diaphragm dysfunction (78). It is unclear if these divergent results are due to species differences or result from the low dose of rocuronium used in the porcine experiments.

Impact of glucocorticoids on MV-induced diaphragm contractile dysfunction. Patients suffering from acute respiratory failure are often treated with corticosteroids for the underlying lung disease or other inflammatory conditions (62). This is significant because glucocorticoid treatment can promote steroid-induced myopathy of both respiratory and limb muscles (60, 96). Recent reports have investigated the combined effects of high-dose corticosteroid treatment on diaphragm function in animals using both partial and full support MV (61, 62). Unfortunately, these studies have yielded divergent results. For example, Maes et al. (61) reported that, during full support MV, the impact of corticosteroids on VIDD in rats is dose dependent with high doses of corticosteroids (i.e., 30 mg/kg) providing partial protection against VIDD, whereas a lower dose (i.e., 5 mg/kg) exacerbates VIDD. In contrast, Sassoon et al. (95) reports that high-dose glucocorticoid administration (i.e., 60 mg/kg) does not protect or exacerbate VIDD in rabbits during full support MV, but this dose of corticosteroids produces deleterious effects to the diaphragm during partial support MV. The reason(s) for the divergent findings in these studies are unclear but could be due to a species difference in the response to corticosteroids.

Mechanisms Responsible For MV-Induced Diaphragm Contractile Dysfunction

Identical to other skeletal muscles, the sarcomere is the basic functional element of diaphragm muscle fibers. Within the sarcomere, the interaction between the sarcomeric proteins (e.g., actin and myosin) provides the fundamental unit to generate force production. More specifically, the mechanical properties of diaphragm muscle fibers are characterized by the relationship between cytosolic free calcium, crossbridge attachment/cross-bridge cycling rate, and sarcomere length (25). It follows that any factor that influences one or more of these variables can impact diaphragm muscle force generation.

As highlighted earlier, MV results in a rapid and progressive decrease in diaphragmatic-specific force production. It can be estimated that the MV-induced impairment in diaphragm-specific force production is responsible for a significant portion of the total reduction in diaphragm force generation observed following prolonged MV. Although the impact of prolonged MV on diaphragm contractile proper-
ties is well characterized, the mechanism(s) responsible for these deficits remain largely unknown. Nonetheless, recent studies have provided clues about the potential mechanisms responsible for MV-induced diaphragm contractile deficits. In regard to the mechanism(s) responsible for MV-induced diaphragm contractile dysfunction, numerous potential contributors exist including oxidative stress-induced contractile dysfunction and/or sarcomere damage. A brief discussion of the potential role that each of these factors play in MV-induced diaphragm dysfunction follows.

Oxidative stress and MV-induced diaphragm dysfunction. The discovery that prolonged MV results in oxidative damage to the rat diaphragm was first reported in 2002 (100). This important finding has been confirmed by numerous animal and human studies (45, 48, 58, 82, 119). Moreover, MV-induced oxidative injury to the rat diaphragm is present following both full and partial ventilator support (44). This MV-induced oxidative stress occurs rapidly as biomarkers of protein oxidation accumulate in the rat diaphragm within the first 6 h of MV and major contractile proteins (e.g., actin and myosin) are targets for oxidative modification (119). The discovery that prolonged MV results in diaphragmatic oxidative stress is significant because it is well established that ROS and their derivatives have a significant impact on skeletal muscle contractile function (83, 92). Direct evidence linking oxidative stress to MV-induced diaphragm contractile dysfunction comes from studies demonstrating that systemic treatment of animals with antioxidants (e.g., trolox, N-acetylcysteine, or a mitochondrial-targeted antioxidant, SS-31) protects against MV-induced diaphragm contractile dysfunction (1, 9, 82). Unfortunately, the explanation for these observations remains largely unknown but could be linked to oxidative damage to myofilibrillar proteins leading to a decreased calcium sensitivity of diaphragm (4, 5, 83). Evidence to support this notion comes from a recent study using a porcine model of MV indicating that prolonged MV results in a decreased calcium sensitivity of diaphragm muscle fibers (77). That is, prolonged MV results in a less efficient calcium activation of diaphragm fibers (i.e., lower relative number of myosin cross-bridges attached in strong binding state at submaximal concentrations of calcium). The molecular mechanism(s) responsible for this MV-induced decline in calcium sensitivity in diaphragm muscle fibers remains unknown but could be linked to oxidative modification of diaphragm contractile proteins (5).

Another possible link between MV-induced oxidative stress and diaphragm contractile dysfunction is the previously discussed connection between ROS and calpain activation. Indeed, MV-induced ROS production in diaphragm muscle promotes calpain activation (82, 117). Active calpain can degrade key cytoskeletal proteins involved in maintaining sarcomere structure resulting in sarcomere disruption, which impairs the muscle’s ability to generate force (37). This topic will be addressed in more detail in the next segment.

MV-induced structural alterations in the diaphragm: impact on contractile dysfunction. Numerous animal studies consistently report that prolonged MV results in ultrastructural changes in diaphragm muscle fibers including abnormal myofilibrils (i.e., disorganized myofilibrillar pattern) and swollen mitochondria with atypical cristae (8, 90, 93, 95). In general, the magnitude of the MV-induced damage to sarcomeric structure ranges from 20% to 30% of the total fiber area. A recent study demonstrated that prolonged full support MV also results in ultrastructure damage in the human diaphragm (47). Identical to the animal studies, this investigation reported significant disorganization of the diaphragm sarcomere structure in humans ventilated >24 h, and the prevalence of this MV-induced ultrastructure damage increased as a linear function of time on the ventilator \( (\rho^2 = 0.80) \). Specifically, the average magnitude of sarcameric injury ranged from \( \sim 10\% \) of the total fiber area for patients ventilated 24 h to \( \sim 20\% \) sarcameric injury/total fiber area for patients ventilated \( \sim 50 \) h. In contrast, subjects ventilated for 100–249 h experienced diaphragmatic ultrastructure damage ranging from \( \sim 30\% \) to \( \sim 80\% \) of the total fiber area (47).

In theory, sarcomere disorganization could be another contributing factor to MV-induced diaphragm contractile dysfunction. Although direct evidence linking sarcamere damage to MV-induced diaphragm contractile dysfunction does not exist, it is logical that proteolytic disruption of sarcomere structure would contribute to MV-induced diaphragm contractile dysfunction.

In regard to the mechanism(s) responsible for MV-induced sarcomere disorganization, it seems likely that MV-induced activation of calpain plays a role. Specifically, prolonged MV results in activation of calpain in both human and rodent diaphragms (47, 63, 75, 100). Active calpain in skeletal muscle is associated with cleavage of the Z line-associated proteins (e.g., titin and nebulin) resulting in the release of myofilament proteins and sarcomere disorganization (37). Moreover, calpain can degrade numerous oxidized sarcomeric proteins including actin, myosin, and \( \alpha \)-actinin (102). Importantly, recent studies reveal that pharmacological inhibition of calpain activation can partially protect against MV-induced diaphragm contractile dysfunction (63, 75). Moreover, pharmacological inhibition of caspase-3 activation can also defend against MV-induced diaphragm contractile deficits (75). Note, however, that it is unclear as to whether the inhibition of caspase-3 is directly responsible for this protection or whether prevention of caspase-3 activation is protective against MV-induced diaphragm contractile dysfunction because active caspase-3 promotes calpain activation via synergistic cross-talk (75).

In reference to the specific diaphragm muscle proteins that are degraded during MV-induced sarcnromere damage, it is established that both titin and myosin play an essential role in skeletal muscle force production (25, 113). It follows that an MV-induced loss of titin or myosin from diaphragm muscle fibers could result in contractile dysfunction. A recent study reveals that 18 h of MV results in a significant reduction in rat diaphragm single muscle fiber force production, which is accompanied by a proportional loss of myosin heavy chain concentration (113). In contrast, prolonged full support MV does not reduce the titin content in diaphragm fibers (113). Therefore, a loss of titin does not contribute to MV-induced diaphragm contractile dysfunction, but it is feasible that the loss of myosin heavy chain is a contributing factor to the MV-induced diaphragm contractile dysfunction.
In summary, the precise mechanism(s) responsible for MV-induced diaphragm contractile dysfunction remains unclear. Nonetheless, it seems likely that the cause of MV-induced diaphragm contractile dysfunction is multiplicative and includes oxidative modifications to contractile proteins resulting in depressed fiber sensitivity to calcium, protease activation resulting in sarcomere disruption, and a loss of myosin heavy chain protein (Fig. 6).

**Contribution of VIDD to Weaning Failure**

One of the major complications associated with prolonged mechanical ventilation is the inability to separate the patient from the ventilator. Removing patients from ventilator support is termed “weaning,” and the incidence of difficult weaning varies between different ICUs but can reach the level of ~30% of patients exposed to prolonged MV (28, 71). The inability to wean patients from the ventilator results in extended hospital stays and increases morbidity and mortality (27). In patients experiencing weaning difficulty, weaning procedures can account for >40% of total ventilator time with even greater time (~60% of total ventilator time) invested in weaning of COPD patients (27).

The causes of weaning failure can be multifactorial and include aspects such as medications (e.g., neuromuscular blockers), confounding morbidities (e.g., sepsis), psychological factors (i.e., anxiety), neurological disorders, and an imbalance between respiratory load and the respiratory muscle capacity (26, 43, 71, 115). Although controversy exists, it has been predicted that depressed strength and endurance of respiratory muscles can play a key role in weaning difficulties (26, 34, 115). However, direct evidence demonstrating cause and effect that VIDD is primarily responsible for weaning problems is lacking due to the difficulty of performing these experiments in humans. Nevertheless, several lines of indirect evidence support the belief that impaired inspiratory muscle function contributes to weaning difficulties. First, although debate exists, several studies reveal that inspiratory muscle endurance is decreased in patients during prolonged MV and that maximal inspiratory pressure generation is lower in patients that experience difficult weaning compared with patients that are successfully weaned (14, 15, 17, 55). Furthermore, a recent study reveals that patients with diaphragmatic contractile dysfunction exhibit a high incidence of weaning failures compared with patients with normal diaphragm function (51). Finally, a growing number of studies suggest that inspiratory muscle training, designed to increase diaphragm strength and endurance, increases weaning success in patients who previously failed repeated weaning attempts by conventional methods (3, 65, 66, 104). Collectively, these studies support the concept that VIDD contributes to weaning failure and therefore the prevention of VIDD is a potential therapeutic target to prevent weaning difficulties.

**Summary and Future Directions**

It is clear that prolonged MV (both partial and full-support ventilation) results in diaphragmatic atrophy and contractile dysfunction (i.e., VIDD) in both animals and humans. Indeed, the onset of VIDD in both animals and humans is rapid as significant diaphragmatic atrophy occurs within the first 24–48 h of MV. Prolonged MV also results in damage to diaphragmatic fiber architecture (i.e., disrupted sarcomere structure) and impaired mitochondrial respiration. Moreover, MV promotes increases in mitochondrial production of ROS in diaphragm fibers resulting in oxidative damage to diaphragm.

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**Fig. 6. Potential mechanisms responsible for MV-induced diaphragm contractile dysfunction.**

1. Ca\(^{2+}\) desensitization due to oxidative modification of contractile proteins
2. Proteolytic degradation of myosin
3. Protease activation resulting in sarcomere disruption

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**MECHANICAL VENTILATION AND DIAPHRAGM**

**Review**

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matic proteins and lipids. Finally, prolonged MV is associated with a decrease in protein synthesis and activation of major proteolytic systems in the diaphragm that accelerates protein breakdown and fiber atrophy.

The explanation as to why prolonged inactivity in the diaphragm results in a more rapid onset of fiber atrophy compared with the time course of disuse atrophy in limb skeletal muscles remains an unsolved mystery. Nonetheless, growing evidence indicates that the MV-induced rapid increase in mitochondrial ROS emission plays a required role in VIDD and is essential for the swift activation of key proteolytic systems that accelerate protein turnover in the diaphragm.

The clinical significance of VIDD resides in the belief that diaphragmatic weakness contributes to the inability to wean patients from MV. Difficult weaning is an important clinical problem and occurs in ~30% of adult patients provided ventilator support for more than 3 days. Importantly, the inability to wean patients from the ventilator results in extended hospital stays and increased patient morbidity and mortality.

Although the phenomena associated with VIDD are well described, several unanswered questions linger related to why prolonged MV promotes the rapid development of diaphragm atrophy and contractile dysfunction. For example, it remains unclear why prolonged MV promotes diaphragm mitochondrial dysfunction and increased mitochondrial ROS emission. Furthermore, a more complete understanding of how MV-induced increases in diaphragm mitochondrial ROS emission is linked to protease activation and increased protein degradation will be required to identify biological targets for drug intervention to prevent VIDD and reduce the incidence of weaning problems. Clearly, there is much more to be learned about the cellular events leading to VIDD.

REFERENCES

Mechanical ventilation and diaphragm dysfunction


