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REVIEWS

The silenced diaphragm: The good and the bad[☆] Le diaphragme au repos : du bon et du mauvais

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Available online 22 April 2008

KEYWORDS

Positive-pressure ventilation;
Diaphragm muscle injury;
Diaphragm muscle atrophy;
Proteolysis

MOTS CLÉS

Ventilation mécanique ;
Lésion du muscle diaphragmatique ;
Atrophie musculaire diaphragmatique ;
Protéolyse

Summary With controlled mechanical ventilation (CMV) the diaphragm is inactive. The application of short-term CMV at the onset of cardiogenic shock or sepsis exerts protective effects on the diaphragm muscle. On the other hand, CMV can produce detrimental effects on healthy diaphragm muscle. The effects are rapid and progressive, and are associated with either muscle atrophy or myofibril damage; the mechanisms of both involve decreased protein synthesis and increased contractile protein degradation. Protein degradation is mediated via interactive molecular signaling, including oxidative stress, apoptosis, and proteasome-proteolysis. Auspiciously, strategies to maintain partial diaphragmatic contractions can mitigate ventilator-induced diaphragmatic dysfunction.

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Résumé En ventilation contrôlée (VC), le diaphragme est inactif. À la phase aiguë du choc cardiogénique ou septique, la mise au repos du diaphragme par la VC exerce un effet protecteur sur le muscle diaphragmatique. A contrario, la VC peut être à l'origine d'effets délétères sur le muscle diaphragmatique sain. Ces effets surviennent rapidement et de manière progressive et sont associés soit à une atrophie soit à des lésions des myofibrilles. Ces phénomènes sont liés à une diminution de la synthèse protéique et à une augmentation de la dégradation des protéines contractiles. Les stratégies ventilatoires permettant de maintenir une activité contractile diaphragmatique permettent d'atténuer cette dysfonction diaphragmatique induite par la ventilation.

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Introduction

Controlled mechanical ventilation (CMV) is a mode of ventilation in which the ventilator assumes the entire work of the respiratory muscles and, via chemo- and mechanoreceptor inhibition, silences the diaphragm. Patients with acute insults to the respiratory center, upper spinal cord, bilateral phrenic nerves, or neuromuscular junction require

[☆] Supported by the Department of Veterans Affairs Medical Research Service (C. Sassoon) and American Lung Association (E. Zhu).

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the application of CMV. Additionally, in acute respiratory distress syndrome, the administration of paralytics in order to improve oxygenation necessitates the application of CMV [1]. Because CMV remains an important supportive treatment for select critically ill patients, it is essential to understand its impact on respiratory muscle function.

Few studies have demonstrated the beneficial effects of CMV [2,3] on diaphragm muscle function. Le Bourdelles et al. [4] were the first to demonstrate in rats that CMV had deleterious effects in healthy diaphragm muscle function, and subsequent animal studies confirmed the harmful effects of CMV [5–9]. This article will review the contradictory influences of CMV on diaphragm muscle function and mechanisms, its clinical implications and potential approach to prevent its detrimental effects.

Protective effects of CMV

In animal models of both cardiogenic shock [2] and sepsis [3], CMV mitigates the loss of diaphragmatic force-generating capacity when compared to spontaneously breathing animals. In the cardiogenic shock canine model, Aubier et al. [2] demonstrated that within the same observation period (~140 min), all of the spontaneously breathing animals died of ventilatory pump failure preceding circulatory arrest. On the other hand, all of the CMV-plus-paralytic-treated animals survived to the termination of the experiment at 180 min. For a similar decrease in cardiac output, in the spontaneously breathing animals, blood flow to the diaphragm was greater than that in animals receiving CMV (21 versus 3% of the cardiac output, respectively). Yet the relatively increased regional blood flow in the diaphragm was associated with elevated blood lactate and consequent acidemia due to compromised systemic tissue perfusion [2,10]. The intracellular acidosis induced impairment in the diaphragm muscle contractile machinery, leading to pump failure [2]. These metabolic components were measured in the spontaneously breathing animals only. The prolonged survival of animals receiving CMV suggests that in acute circulatory failure, CMV is a rescue modality that can prevent pump failure. In addition, in the presence of adequate left-ventricular filling, intrathoracic positive pressure decreases left-ventricular afterload and likely prevents further deterioration of cardiac output.

CMV has also been demonstrated to mitigate the loss of diaphragmatic force-generating capacity in sepsis [3]. Ebihara et al. [3] studied rats with endotoxin-induced sepsis randomized into two groups, four hours of spontaneous breathing or CMV applied at the time of endotoxin infusion, and found that CMV partially prevented impaired diaphragm muscle contractility. Yet in both CMV and spontaneously breathing animals, markers of molecular signaling in sepsis, inducible nitric oxide synthase expression, and oxidative stress increased to the same extent. Both sepsis-induced oxidative stress and mechanical stress to the diaphragm muscle (i.e., from spontaneous breathing excursions) had synergistic effects on sarcolemmal fragility and injury. CMV preserved diaphragmatic force by reducing the level of mechanical stress imposed on the fragile sarcolemma. Thus, in sepsis, the early CMV application protects diaphragm muscle function.

In both animal models described above, CMV was applied concomitant to the insult and for a remarkably short duration. In clinical practice, concurrent timing of CMV application with insult inception is rarely possible, since the inception is not always predictable or obvious. However, it is more important to ask whether the application of CMV will produce beneficial effects following the insult, or whether the protective effect of CMV will continue with prolonged application as the underlying disease evolves with treatment. The answers to those questions will require further study.

Harmful effects of CMV

Several animal studies have demonstrated that CMV impairs the contractile function [4–9] of previously healthy diaphragm muscle with intact neural outflow tract and neurotrophic influences, a condition referred as ventilator-induced diaphragmatic dysfunction (VIDD). The impairment occurs rapidly — as early as 12 hours in rats [7] — and is progressive [7,8,11]. The reason for this phenomenon is unclear. It is possible that the diaphragm's constant rhythmic action makes it susceptible to inactivity, even for a short duration. Diaphragmatic force decline is profound, by 46% after 24 h in rats [7], and by ~50% after three days in rabbits [8,11], whereas in non-rodents, diaphragmatic force decline is less: by 20% after five days in piglets [6] and by 25% after 11 days in baboons [5].

Function-structure relationships

The mechanisms of reduced diaphragmatic force are multifactorial and seem to reside within the myofibrils, with sarcomeric contractile proteins as the ultimate target, although to date impairment in excitation-contraction coupling (a decrease in sarcolemma resting membrane action potential and/or sarcoplasmic reticulum Ca⁺⁺ release capacity) has not been investigated systematically. Several studies have shown that myofibril protein synthesis decreased [12] and protein degradation increased [11,13,14], resulting in myofibrillar disruptions [8,15,16] and/or reduced size (atrophy) [17]. Concomitant with diaphragm muscle atrophy was a loss of myofibrillar protein that may have contributed to diaphragmatic dysfunction [17]. The reduction in myofibrillar protein concentration would result in fewer myosin cross-bridges per cross-sectional area (CSA) and decreased force production. Diaphragm muscle fiber atrophy has been observed primarily in rats, and has involved all fiber types, with fast fibers atrophying to a greater extent than slow fibers, while limb muscles have remained normal despite inactivity with CMV application [17,18]. In rabbits, two days of CMV with positive-end expiratory airway pressure (PEEP) of 2 cm H₂O induced diaphragm muscle fiber atrophy [9], whereas three days of CMV without PEEP maintained fiber CSA intact [8]. PEEP induced tonic diaphragm muscle fiber shortening, which may have accelerated the atrophic process, a phenomenon similar to that observed in limb muscle that has been immobilized in a shortened position [19]. However, the PEEP level and its interactive effects with the degree of diaphragm neural activation remain to be investigated, particularly in light of the protective effects of PEEP in acute lung injury.

In the absence of myofibril atrophy, how does one explain the decline in diaphragmatic force with CMV? In rabbits [8,15] and piglets [16], ultrastructural myofibril disruptions have been observed. Zhu et al. [11] demonstrated the significant relationship between abnormal myofibril volume density and maximum tetanic force. The point-counting method can be used to quantify the volume density of abnormal myofibrils from electron microscopic images. The increased volume density of abnormal myofibrils accounted for ~40% of the loss of maximum tetanic force. Although cause and effect have not been demonstrated, in theory the reduced number and integrity of myofibrils should reduce the number of cross-bridge attachments for force production, and therefore, lead to less force. In support of this hypothesis, after three days of assist-control mechanical ventilation (AMV) that partially maintained diaphragmatic neural activation, the volume density of abnormal myofibrils was less than that with CMV, and also was commensurate with the preserved force [13] (Fig. 1).

Mechanisms of myofibrillar atrophy/damage

Three processes are involved in the mechanisms of CMV-induced diaphragm muscle atrophy:

- oxidative stress;
- programmed cell death (apoptosis);
- proteolytic systems (see reference [20] for an excellent review).

These processes may act not independently, but interactively: for example, oxidative stress-induced sarcoplasmic reticulum (SR) stress results in Ca^{++} release in the cytosol, which in turn activates calpain and caspases. Activated calpain, a Ca^{++} -dependent protease, can cause proteolysis of sarcomeric cytoskeletal proteins (i.e., titin, nebulin, and desmin) [20]. Caspases (endoproteases responsible for the final execution of cell death) including caspase-3, regulate cellular DNA fragmentation and promote protein degradation. Translocation of activated caspase-3 into the nucleus results in DNA fragmentation and apoptosis [18].

Oxidative stress

CMV-induced diaphragm inactivity is associated with both compromised antioxidant defenses [21,22] and increased oxidative stress [17,23]. Total antioxidant capacity and glutathione concentrations, a non-enzymatic antioxidant, diminish with CMV [22], whereas CMV exerts contradictory effects on enzymatic antioxidant (e.g., glutathione-peroxidase). In rats, glutathione-peroxidase activity decreases after 12 h of CMV [22], while in piglets it remains unchanged after three days of CMV [21].

Decreased antioxidant buffering capacity with overproduction of reactive oxygen species results in oxidative stress, which occurs as early as six hours [23], and is associated with atrophy of all fiber types and diaphragmatic force loss at 12 to 18 h [17,24]. The trigger for the increased oxidative stress, and the specific pathway through which it occurs, remain unclear, although nitric-oxide synthase is not involved in oxidative damage of the diaphragm muscle [25]. CMV induces elevated markers of lipid oxidation (i.e.,

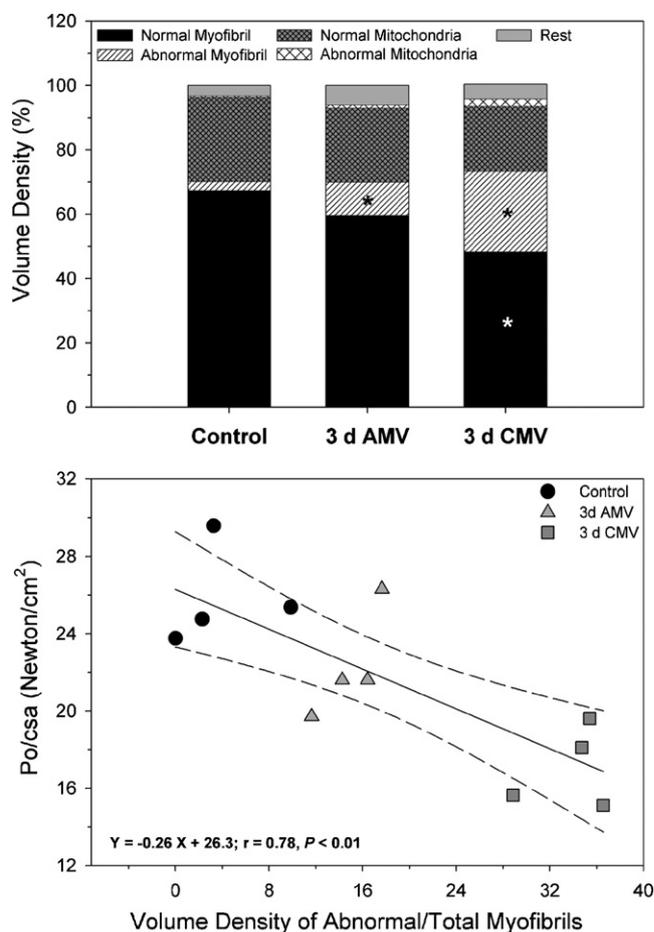


Figure 1 Mean volume density of normal and abnormal myofibrils, normal and abnormal mitochondria and rest in control, assist-control mechanical ventilation (AMV) and controlled mechanical ventilation (CMV). Rest is comprised of lipid droplets and vacuoles (Upper panel). The relationship between maximum tetanic force (P_o , normalized for cross-sectional area (CSA)) and the ratio of volume density of abnormal to total myofibrils, $n=4$, is shown in lower panel.

* $P < 0.05$: three days CMV versus control for normal myofibrils; three days CMV versus three days AMV and control; and three days AMV versus control for abnormal myofibrils.

8-isoprostane, lipid hydroperoxides, and thiobarbituric acid) and protein oxidation (i.e., protein carbonyls). Lipid oxidation may result in cellular membrane dysfunction (i.e., decreased Ca^{++} ATPase activity), retard Ca^{++} removal from and cause its accumulation in the cytosol [26]. The elevated Ca^{++} concentration then activates calpain (see above). Elevated intracellular oxidant production provides direct evidence of oxidative stress as demonstrated by the increased emission of dichlorodihydrofluorescein dye, a chemical that fluoresces upon reaction with oxidative species when diaphragm muscle strips of animals receiving CMV are incubated in vitro with the dye [22].

With respect to protein oxidation, reactive oxygen species have as a preferential target myofibrillar proteins with molecular masses of 200, 128, 85, and 40 kDa, with the highest value identified as myosin (200 kDa) and the lowest as actin (40 kDa) [23]. It is conceivable that con-

tractile proteins that are damaged by protein oxidation render them susceptible to degradation by proteases, resulting in both diaphragm muscle atrophy and decreased diaphragmatic force-generating capacity. Indeed, CMV has been found to augment both calpain and 20S proteasome activities [17]. The elevated calpain activity in turn cleaves cytoskeletal proteins and/or promotes myonuclear apoptosis [20]. The 20S proteasome is the core structure of the 26S proteasome complex in the ubiquitin-proteasome pathway (see reference [27] for review), and the unbound form can independently degrade oxidized proteins without requiring ubiquitin conjugation. The hypothesis that oxidative stress contributes to diaphragm muscle fiber atrophy is further supported by the fact that administering the antioxidant Trolox, a soluble vitamin E analog, prevents atrophy and preserves diaphragmatic force-generating capacity [24]. Trolox reduces protein carbonyls formation [28] and 20S proteasome activity [24] to control levels, but does not alter the suppressed antioxidant glutathione concentrations. Recently, McClung et al. [29] demonstrated that Trolox renders its protective effects by attenuating myofilament protein substrate availability to degradation by the proteasome. Trolox mitigates diaphragm muscle atrophy independent of the IGF-1/PI3K/Akt/Foxo pathway, which is involved in regulating atrophic factors in the ubiquitin-proteasome system [28] (see below). Antioxidant may also exert its protective effects by suppressing calpain and caspase protease activity [20,28].

Programmed cell death (apoptosis)

Caspase-3-mediated myonuclear apoptosis contributes to diaphragm muscle atrophy [20]. The timing for apoptosis

induction coincides with that of oxidative stress, as early as six hours after CMV and prior to changes in fiber CSA [18,23]. After 12 h of CMV, the number of myofiber nuclei is reduced whereas fluorometric TUNEL (Tdt-mediated dUTP nick-end labeling) showed that the number of nuclei with DNA strand fragmentation increased, and is associated with reduced Type I and IIa fiber CSA (by 17 and 23%, respectively). With early diaphragm muscle inactivity, myonuclear loss seems to be associated with a proportional decrease in cytoplasm, so that myonuclear domain size, — that is, the amount of cytoplasm per myonucleus — remains unchanged. The myonuclear domain size reflects a muscle fiber's transcriptional capacity [30]. A small myonuclear domain size indicates a relatively large amount of transcriptional capacity and the potential for rapid fiber CSA recovery if appropriate stimuli are present [30]. The administration of caspase inhibitor prevents myonuclei loss and increases DNA-fragmented nuclei and myofiber atrophy [18]. The effect of prolonged CMV on myonuclear domain size remains unclear. Nonetheless, caspase-3-mediated apoptosis appears to play an important role in the development of CMV-induced diaphragm muscle fiber atrophy.

Proteolytic systems

The proteolytic systems in skeletal muscle are the lysosomal protease, Ca⁺⁺-dependent calpain, caspase-3, and ATP-dependent ubiquitin proteasome [20]. Although the ubiquitin-proteasome system appears responsible for most muscle protein degradation, the proteasome does not break down complexes of proteins contained in actomyosin or myofibrils. One or more proteases are required in the initial process to release constituents of actomyosin before the

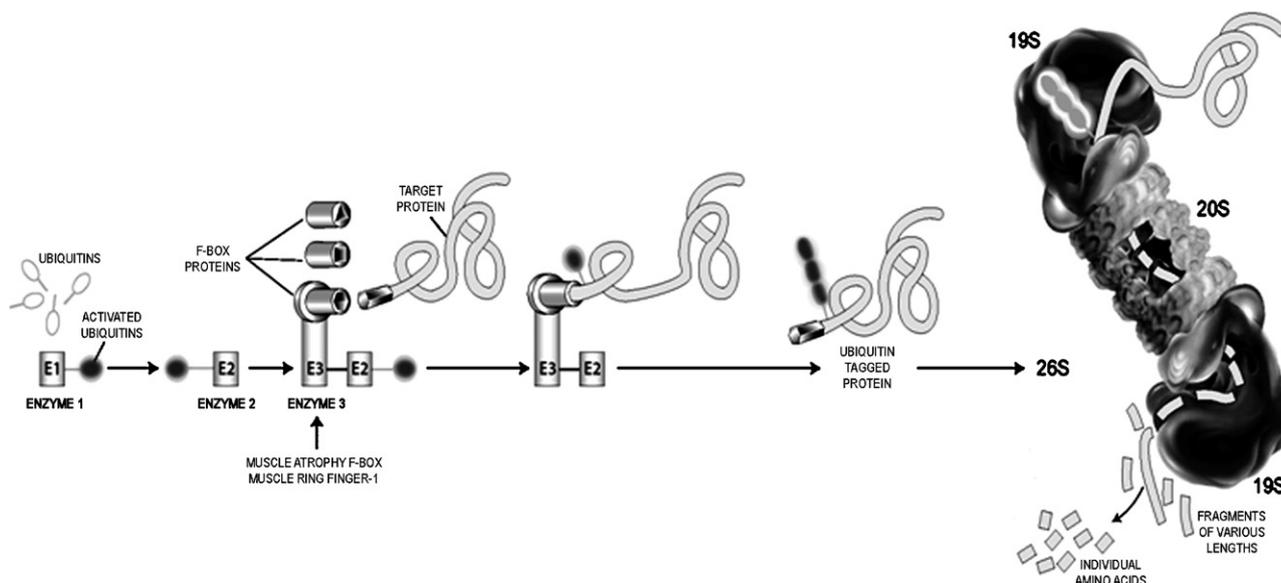


Figure 2 Ubiquitin-proteasome pathway for protein degradation. The substrate proteins are designated for degradation by conjugation to ubiquitin in an ATP-dependent reaction. The ubiquitin-activating enzyme (E1), ubiquitin-carrier protein (E2), and ubiquitin-protein ligase (E3) are required for the formation of ubiquitin-protein conjugates and transport to the proteolytic complex, the 26S proteasome, where the protein is unfolded. Then, the ubiquitin is released and the protein is degraded into small peptides and amino acids. The 26S proteasome consisted of the core 20S and two 19S regulators proteins. Muscle atrophy F-box (MAF-box, atrogin1) and muscle ring finger-1 (MuRF1) are E3 ligases, whose expression is upregulated with CMV. Adapted from reference [27] with permission.

ubiquitin-proteasome system can degrade the contractile proteins [20]. Lysosomal proteases are primarily responsible for proteolysis of extracellular proteins and cell surface receptors. Calpain proteases are involved in the cleavage of cytoskeletal proteins (e.g. titin, nebulin, and desmin) that anchor contractile elements. Caspase-3 proteases cleave actomyosin complexes [18,31], whereas the ubiquitin-proteasome pathway degrades the monomeric actin and myosin. All of the proteolytic systems appear to be involved in CMV-induced diaphragm muscle inactivity [18,28,32]. Compared to controls, leupeptin (an inhibitor of lysosomal proteases) given at the onset of CMV completely prevented CMV-induced reduction in diaphragmatic force and atrophy [32]. The augmented levels of both cathepsin B and calpain activity with CMV were restored to control levels.

In the ubiquitin-proteasome system, the binding of ubiquitin to protein substrates requires ubiquitin-activating enzyme (E1), ubiquitin-carrier enzyme (E2), and ubiquitin ligases (E3) [27]. The ubiquitin-conjugated proteins then are transported to a proteolytic complex, the 26S proteasome, which consists of the core 20S proteasome and the 19S regulators attached to both ends (Fig. 2). In the 26S proteasome, the protein is degraded into small peptides and amino acids. As mentioned above, the 20S proteasome can be free and degrade oxidized proteins without the need for ubiquitin conjugation [17]. Two of the E3 ligases, the muscle atrophy F-box (MAF-box, atrogin1, atrogenes) and muscle ring finger-1 (MuRF1) genes, are overexpressed in various mod-

els of skeletal muscle atrophy [33]. Similarly, MAF-box and MuRF1 are upregulated during CMV-induced diaphragm muscle inactivity [11,13,28]. What upstream pathway regulates atrogenes expression? In myotubes culture, IGF-1/PI3K/Akt (insulin-like growth factor-1 — phosphatidylinositol 3-kinase — protein kinase B serine threonine kinase) signaling plays an important role in atrogenes expression [34]. IGF-1/PI3K/Akt suppresses MAF-box by inactivating the expression of Foxo (forkhead box-O) expression and preventing its nuclear translocation (Fig. 3). Following six and 18 h of CMV, diaphragmatic Akt activation decreased in parallel with increased Foxo nuclear translocation and increased MAF-box and MuRF1 expression [28]. Thus, IGF-1/PI3K/Akt signaling seems to play an important role in regulating the E3 ligase in the ubiquitin-proteasome pathway.

Interactive effects of CMV with pharmacological agents

Both neuromuscular junction blocking agents (NMBA) and corticosteroid have been implicated in the development of critical illness myopathy with consequent difficulty in weaning or prolonged mechanical ventilation. Nondepolarizing NMBA is used for sustained paralysis during mechanical ventilation, with the intention to improve oxygenation and assist in managing increased intracranial pressure. Nondepolarizing NMBA consists of two classes:

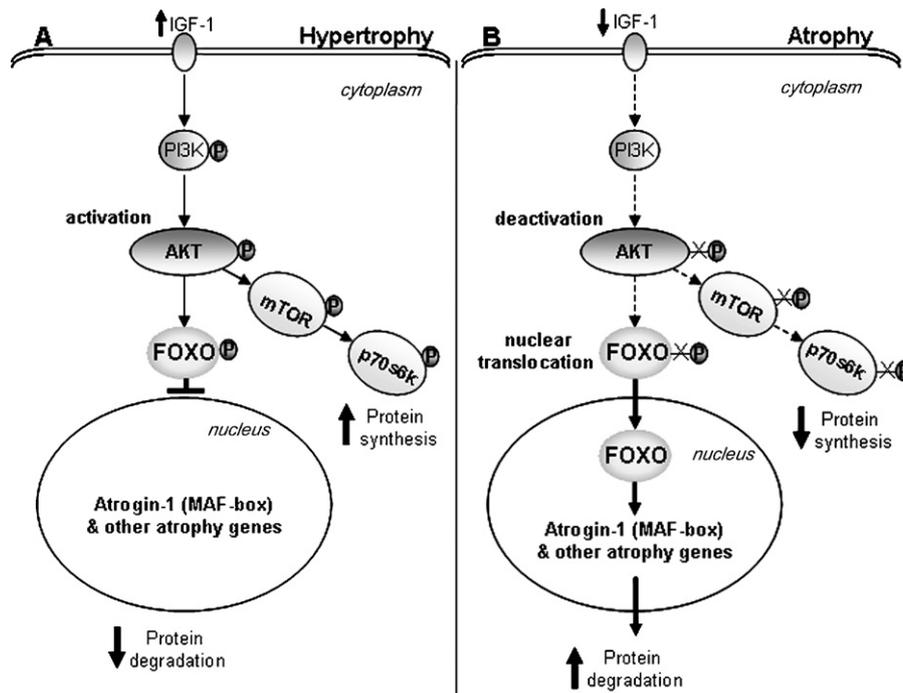


Figure 3 The IGF-1/PI3K/Akt (insulin-like growth factor-1 — phosphatidylinositol 3-kinase — protein kinase B serine threonine kinase) signaling pathway in the mechanisms of muscle hypertrophy and atrophy.

A: increased IGF-1 activates PI3K, leading to phosphorylation of Akt and Foxo. Phosphorylated Foxo is sequestered within the cytoplasm, preventing both its nuclear translocation and atrogin-1 (MAF-box) activation. Phosphorylated Akt also activates mTOR (mammalian target of rapamycin) and p70S6k, resulting in increased protein synthesis.

B: suppression of IGF-1, as with CMV, deactivates Akt, leading to nuclear translocation of Foxo, which then activates atrogin-1 and other atrogenes, resulting in increased proteolysis. The solid line indicates activation and/or phosphorylation; the dashed line indicates deactivation and/or dephosphorylation. Adapted from reference [34] with permission.

aminosteroids and benzylisoquinolines. Both types of NMBAs have been associated with the development of myopathy. In rats, a 24h infusion of rocuronium (an aminosteroidal NMA) augmented the detrimental effects of CMV alone on diaphragm force-generating capacity, atrophy of Type IIx/b fibers, and upregulation of MuRF1 expression, but did not alter MAF-box expression [35]. Conversely, a low or high dosage of cisatracurium (a benzylisoquinoline) infusion did not aggravate the deleterious effects of CMV. Because corticosteroid increases intracellular calcium and rocuronium possesses a similar molecular structure as corticosteroid, the aggravated decline in diaphragmatic force observed with rocuronium was postulated to be caused by an increase in intracellular calcium beyond that of CMV with placebo or with cisatracurium, with subsequent enhanced calpain activity and protein degradation (see above) [36].

Preliminary observations in our laboratory have demonstrated that the administration of high-dosage intravenous corticosteroid (methylprednisolone 250 mg/d) did not exert synergistic detrimental effects with CMV on diaphragm muscle function, suggesting that the mechanisms of corticosteroid's negative effects on the diaphragm are similar to that of CMV [37]. Short-term, high-dosage corticosteroid has been used in patients with acute spinal cord injury [38].

Clinical implications and prevention

In critically ill patients, it is extremely difficult to substantiate that CMV is the cause of diaphragmatic dysfunction because multiple confounding factors (e.g. sepsis, malnutrition) contribute to diaphragm muscle weakness and atrophy. Most recently, preliminary observations in 14 adult brain-dead organ donors with intact circulation and CMV application for 18–69h showed atrophy of diaphragm slow and fast fiber types, in comparison with those of control subjects ($n=8$) who underwent surgical resection of pulmonary solitary nodules and receiving mechanical ventilation for two to three hours [39]. Slow fiber-type CSA decreased by 57% and fast-fiber CSA by 53%. Interestingly, biopsy of pectoralis major muscle did not show any fiber atrophy. Diaphragm muscle atrophy was associated with decreased antioxidant glutathione concentration (by 23%), increased active caspase-3 expression (by twofold), and with elevated mRNA levels of MAF-box (by threefold) and MuRF1 (by sevenfold) [39]. Thus, in humans whose diaphragms are presumed to have been previously healthy, CMV-induced diaphragm muscle inactivity also induces atrophy fairly rapidly.

In contrast to complete inactivity, maintaining partial neural activation of the diaphragm seems to mitigate diaphragm muscle functional loss. Compared to the controls, diaphragmatic force-generating capacity decreased by 14% after three days of AMV, and by 48% after three days of CMV. The latter was associated with an approximately threefold increase in MAF-box mRNA expression, but with AMV these expression levels showed no significant changes [13]. However, it remains unclear whether AMV can maintain diaphragmatic force-generating capacity under prolonged mechanical ventilation (greater than three days). In another study, when spontaneous breathing for five minutes or 60 min

was interposed during 24h of CMV four times a day, diaphragmatic force-generating capacity decreased by an average of 19%, and decreased by 28% with continuous CMV. Intermittent spontaneous breathing also prevented diaphragm muscle atrophy. Although intermittent spontaneous breathing for short durations attenuated diaphragmatic force loss, its protective effects were modest — ~9% [40] — and it is unclear whether these benefits are sustainable with prolonged mechanical ventilation.

Experimental results from animal studies suggest that if at all possible, it is prudent to apply forms of mechanical ventilation that permit diaphragmatic contractions to be maintained, although it is not yet known what degree of diaphragmatic contractions prevents atrophy or force loss. Similarly, it remains unknown whether applying intermittent diaphragm muscle training from the onset of mechanical ventilation will preserve diaphragmatic muscle function. In a prospective trial, critically ill patients who were predicted to receive mechanical ventilation for longer than 72h were randomized into control ($n=13$) and those receiving inspiratory muscle training (IMT, $n=12$) from the onset of mechanical ventilation [41]. The study excluded patients with flail chest, coronary artery disease, alveolar hemorrhage, hemodynamic instability and neuromuscular disease. The reasons for applying mechanical ventilation were acute respiratory failure, decreased consciousness, corticosteroids, and vasoactive drugs. It is unclear whether patient-triggered or time-triggered ventilation was applied. A threshold load was used for the IMT by setting the ventilator pressure-triggering sensitivity at 10 or 20% of initial maximum inspiratory pressure (PI_{max}), whichever was tolerated, and was applied twice daily for five minutes. When the patient tolerated the initial load, the next training duration was increased by five minutes up to a maximum of 30 min. Afterwards, the load was increased by 10% increments until 40% of the initial PI_{max} was attained. Sedation and analgesia with intravenous midazolam and fentanyl, respectively were administered. The IMT session was aborted according to specified criteria. Weaning with decreasing pressure support was initiated once specified weaning criteria were met. The initial and final PI_{max} in the training group were similar to those of the controls (initial –51 versus –48 cm H₂O, and final –56 versus –55 cm H₂O, respectively). The duration of mechanical ventilation or weaning trial was similar for both groups, with a trend toward a short duration for the IMT group compared with the controls (mean duration of mechanical ventilation was 8.6 versus 9.8 days, and weaning trial was 23 versus 31 h, respectively). Thus, IMT sessions began at the onset of mechanical ventilation did not provide any benefits that improved diaphragmatic function. The lack of IMT benefits may be due to the small sample size. It is also conceivable that the magnitude of the stimulus for IMT in the critically ill patients (i.e., the threshold load applied, and/or session frequency and duration) was inadequate to elicit a physiological training effect. Alternatively, maintaining diaphragmatic contractions with patient-triggered ventilation, a form of diaphragmatic conditioning in acutely ill patients, may have been adequate such that additional effects of low intensity IMT were not discerned. The results from the study above contrast with the beneficial effects of threshold-load IMT in chronic ventilator-dependent patients who have difficulty weaning [42].

Future considerations

The mechanisms of ventilator-induced dysfunction are multifactorial and continue to be explored. The phenomenon of ventilator-induced diaphragmatic dysfunction has been clearly established only in experimental animals with healthy diaphragm muscle. As mentioned above, the many confounding factors in the critically ill patients would make similar studies in humans difficult to interpret. However, findings obtained from animal studies do not preclude their application to critically ill patients. Current unknowns included the degree of diaphragmatic neural activation required to prevent diaphragmatic force-loss with prolonged mechanical ventilation of greater than three days. Perhaps the benefits of assist-control mechanical ventilation do not depend on the level of diaphragmatic activity [43]. It is unclear whether the ventilatory modes or settings that can alter the magnitude of respiratory muscle load have any effects on diaphragmatic function. It also is unknown whether relatively high levels of positive end-expiratory airway pressure (PEEP) that shorten diaphragm muscle fiber without causing lung overdistention have any effect on diaphragm muscle function. The only study in humans on respiratory muscle conditioning from the onset of mechanical ventilation with intermittent muscle training yielded a negative result [41], likely due to the small sample size and other reasons mentioned above. Not until pharmacological agents for preventing ventilator-induced diaphragm muscle dysfunction become available, it may be worthwhile to revisit the question whether diaphragm muscle conditioning improves diaphragmatic function. In animal studies it is attractive to completely reverse diaphragmatic dysfunction by applying a specific inhibitor to the signaling cascade involved in proteolysis [18,24,32]; however, as in sepsis, its application in humans is complex and remains to be tested.

Conclusion

In animal experiments, CMV-induced diaphragm inactivity decreases protein synthesis and degradation of key contractile proteins, resulting in diaphragmatic force loss. Protein degradation is mediated via oxidative stress, apoptosis, and proteasome-proteolysis. On the other hand, maintaining diaphragm muscle contractions during mechanical ventilation attenuates the decline in diaphragmatic force. For this reason, if at all possible it is prudent to apply mechanical ventilation in which the patient triggers the ventilator.

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