Mechanical ventilation is a life-saving form of supportive therapy for respiratory failure. In addition to support of gas exchange, other potential benefits of mechanical ventilation include reversal of respiratory muscle fatigue, prevention of muscle fiber injury during sepsis, and restoration of blood flow to vital organs in shock states by preventing a "respiratory steal" phenomenon by intensely working respiratory muscles (1–3). However, mechanical ventilation is clearly a two-edged sword. It is also associated with major complications such as infection, barotrauma, cardiovascular compromise, tracheal injuries, oxygen toxicity, and ventilator-induced lung injury (4).

In addition to the above well known complications of ventilatory support, a rapidly accumulating body of evidence suggests that mechanical ventilation, with its attendant diaphragm muscle inactivity and unloading, is an important cause of diaphragmatic dysfunction. For the purposes of this Critical Care Perspective, we define this phenomenon, henceforth referred to as ventilator-induced diaphragmatic dysfunction (VIDD), as a loss of diaphragmatic force-generating capacity that is specifically related to the use of mechanical ventilation.

The objectives of this Critical Care Perspective are as follows: (1) to review the existing evidence for VIDD, (2) to summarize the cellular changes in the diaphragm associated with the VIDD phenomenon, (3) to interpret these new findings in light of known effects of other forms of skeletal muscle disuse, and (4) to suggest future areas of research as well as potential therapeutic avenues.

WHEN TO SUSPECT VIDD

Difficulties in discontinuing ventilatory support are encountered in 20–25% of mechanically ventilated patients (5), with a staggering 40% of time spent in the intensive care unit being devoted to weaning (6). Because the respiratory muscles play a pivotal role in determining the weaning outcome (1, 7), VIDD will have a major impact on clinical intensive care unit practice. In the clinical setting, VIDD is a diagnosis of exclusion based on (1) an appropriate clinical history of having undergone a period of controlled mechanical ventilation (CMV), and (2) other possible causes of diaphragmatic weakness having been sought and ruled out. Hence the "typical" clinical scenario in which to suspect VIDD is a patient who fails to wean after a period of CMV. The weaning failure is related to respiratory muscle dysfunction. Other known causes of respiratory muscle weakness such as shock, ongoing sepsis, major malnutrition, electrolyte disturbances (8), and neuromuscular disorders acquired in the intensive care unit (9, 10) are ruled out. For example, prolonged neuromuscular blockade can be excluded by the lack of an abnormal response to train-of-four stimulation; critical illness polyneuropathy by the absence of neuropathic changes on electrophysiological testing; and acute quadriplegic myopathy by the lack of corticosteroid exposure history (or by muscle biopsy in indeterminate cases) (9, 11). Under the scenario outlined above, it has long been suspected that mechanical ventilation itself could contribute to weaning difficulties due to atrophy and other disuse effects on the respiratory muscles, but only more recently have experimental observations come to light that strongly support this hypothesis (12).

WHAT IS THE EVIDENCE FOR VIDD?

Animal studies have consistently found that CMV leads to decreased force-generating capacity of the diaphragm (see Table 1). In the intact diaphragm of various animal species studied in vivo, transdiaphragmatic pressure generation during phrenic nerve stimulation is significantly reduced at both submaximal and maximal stimulation frequencies (13–15). This occurs in a time-dependent manner, with the force decline becoming evident quite early (1 day in rabbits [15], 3 days in piglets [14]), and worsening as mechanical ventilation is prolonged. Within a few days of instituting CMV (3 days in rabbits [15], 5 days in piglets [14], 11 days in baboons [13]), the pressure-generating capacity of the diaphragm has declined by 40–50%. Endurance of the diaphragm is also adversely affected, as indicated by a reduced ability to sustain diaphragmatic force in the face of an inspiratory resistive load (13). The decrease in diaphragmatic force-generating capacity is not due to changes in lung volume or abdominal compliance (13, 14). Nervous impulse transmission at the level of the phrenic nerve and the neuromuscular junction also remains intact (14). However, the compound muscle action potential is decreased after CMV, suggesting that impaired muscle fiber membrane excitability and/or excitation–contraction coupling are involved (14).

In keeping with studies of the intact diaphragm in vivo, contractility measurements performed on isolated diaphragm strips (15–20) also indicate a rapid (as early as 12 hours), progressive, and severe decline in contractile function during CMV. The reduction in maximal isometric force production is on the order of 30–50% after 1–3 days of CMV (15–18, 20). Effects on in vitro fatigue resistance are less consistent, with reports of fatigability being increased (21), decreased (22), and unchanged (15, 20). It is important to recognize that because diaphragmatic force is generally normalized to account for differences in muscle cross-sectional area in these studies, the loss of force-generating capacity cannot be ascribed to atrophy alone. Similarly, the fact that the diaphragm is routinely placed at its optimal length during such experiments effectively rules out altered muscle operating...
length as the major explanation for the force decline. Therefore, these isolated diaphragm strip studies indicate that pathophysiological changes in VIDD are located at the cellular level within the diaphragm muscle fibers themselves. Finally, it should be noted that although anesthetic medications and in some cases neuromuscular blocking agents have been used in these studies, adverse effects of mechanical ventilation on diaphragmatic function were similarly observed when these medications were either controlled for (to the extent that this is possible in the case of anesthetics) or completely omitted (in the case of neuromuscular blockers).

Although the evidence for VIDD in animal models is convincing, it is considerably more difficult to obtain conclusive proof for the existence of VIDD in humans. This is due in large part to the presence of multiple confounding factors (e.g., underlying disease state, drug therapy, different modes of mechanical ventilation) as well as technical limitations in accurately assessing diaphragmatic function in critically ill patients. Nonetheless, the few studies available suggest that VIDD may indeed be present in many such individuals. Histopathologic analysis of 13 neonates who received ventilatory assistance for 12 days or more immediately before death revealed diffuse diaphragmatic muscle fiber atrophy, whereas such changes were not present in either extradiaphragmatic muscles or diaphragms of 26 infants ventilated for 7 days or less (23). Furthermore, in 33 mechanically ventilated but clinically stable patients with a variety of underlying diseases, the twitch transdiaphragmatic pressure elicited by supramaximal magnetic stimulation of the phrenic nerves was found to be reduced by about 50% on average compared with normal subjects (24). Although the mode of mechanical ventilation was not reported, it is tempting to speculate (based on characteristics of the patients) that at least some were managed with CMV.

DIAPHRAGM MUSCLE FIBER CHANGES ASSOCIATED WITH VIDD

Muscle Atrophy

Although the reductions in diaphragmatic force-generating capacity found after CMV cannot be attributed solely to muscle atrophy, diaphragmatic wasting further impairs maximal pressure generation in vivo. Moreover, because the susceptibility to diaphragmatic fatigue in vivo is inversely related to maximal strength (25), diaphragm atrophy will also increase the risk of diaphragmatic fatigue once spontaneous breathing is resumed (e.g., during attempts at weaning from mechanical ventilation [1]). Reduced diaphragm muscle mass and/or muscle fiber atrophy after CMV has been observed in most experimental studies in which these parameters were evaluated (13, 16, 18–20). Atrophy develops more rapidly (as early as 18 hours [26]) and to a significantly greater extent in the diaphragm during CMV than in peripheral skeletal muscles (16, 18), despite the fact that the latter are also inactive. In general terms, disuse atrophy can result from decreased protein synthesis (27), increased proteolysis (28), or both. Decreased protein synthesis in the diaphragm has not been documented but can be anticipated on the basis of limb muscle disuse models. In addition, mRNA levels for an anabolic protein, insulin-like growth factor-I, were decreased in the rat diaphragm after 24 hours of CMV (20). Increased proteolysis has been documented in rat diaphragms after 18 hours of CMV (26). Among the three primary proteolytic systems employed by mammalian cells (lysosomal proteases, calpains, and the proteasome), both calpains and the proteasome are activated in the diaphragm during CMV (26). Calpains do not fully degrade, but only partially cleave proteins in vivo, which renders them amenable to the proteasome. The proteasome exists in two major forms. The 26S proteasome degrades proteins in an energy-dependent, ubiquitin-mediated process, and is primarily responsible for muscular atrophy in several disease states (e.g., cancer, acquired immunodeficiency syndrome, trauma). The 20S proteasome is specialized in degrading proteins oxidized by reactive oxygen species, without the need for ubiquitin conjugation or ATP hydrolysis (29). Rats exposed to CMV demonstrate a 500% increase in 20S proteasome activity (25). This increase in 20S proteasome activity, together with evidence for oxidative stress-induced protein modification in the diaphragms of animals exposed to CMV (see below; and References 26 and 30), suggests that oxidative stress has a central role in the pathogenesis of diaphragmatic atrophy in VIDD.

Oxidative Stress

CMV is associated with augmented oxidative stress in the diaphragm, as indicated by increased protein oxidation and lipid peroxidation by-products (26). The onset of oxidative modifications is rapid, occurring within 6 hours of the institution of CMV (30). Activity of the antioxidant enzyme superoxide dismutase is also augmented (22), suggesting that antioxidant defenses are induced in an attempt to limit oxidative stress-mediated cellular injury. Interestingly, increased oxidative stress has also been found after limb muscle disuse, although the immobilization

### TABLE 1. EXPERIMENTAL STUDIES OF VENTILATOR-INDUCED DIAPHRAGMATIC DYSFUNCTION

<table>
<thead>
<tr>
<th>First Author (Ref.)</th>
<th>Year</th>
<th>Animal</th>
<th>n (CMV)</th>
<th>Duration</th>
<th>Control Vt (ml/kg)</th>
<th>RR</th>
<th>PEEP</th>
<th>Force Decline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le Bourdelles (16)</td>
<td>1994</td>
<td>Rats</td>
<td>18 (9)</td>
<td>48 h</td>
<td>Yes</td>
<td>8</td>
<td>0</td>
<td>49</td>
</tr>
<tr>
<td>Anzueto (13)</td>
<td>1997</td>
<td>Baboons</td>
<td>7</td>
<td>11 d</td>
<td>No</td>
<td>15</td>
<td>0</td>
<td>25*</td>
</tr>
<tr>
<td>Radell (14)</td>
<td>2002</td>
<td>Piglets</td>
<td>7</td>
<td>5 d</td>
<td>No</td>
<td>12-15</td>
<td>16-19</td>
<td>3.0-5.0</td>
</tr>
<tr>
<td>Sassoon (15)</td>
<td>2002</td>
<td>Rabbis</td>
<td>30 (12)</td>
<td>1–3 d</td>
<td>Yes</td>
<td>6-8</td>
<td>40-50</td>
<td>0</td>
</tr>
<tr>
<td>Yang (18)</td>
<td>2002</td>
<td>Rats</td>
<td>9 (5)</td>
<td>44-93 h</td>
<td>Yes</td>
<td>5</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td>Shanely (26)</td>
<td>2002</td>
<td>Rats</td>
<td>38 (16)</td>
<td>18 h</td>
<td>Yes</td>
<td>10</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>Powers (17)</td>
<td>2002</td>
<td>Rats</td>
<td>39 (15)</td>
<td>12-24 h</td>
<td>Yes</td>
<td>10</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>Shanely (22)</td>
<td>2003</td>
<td>Rats</td>
<td>14 (6)</td>
<td>18 h</td>
<td>Yes</td>
<td>10</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>Bernard (19)</td>
<td>2003</td>
<td>Rabbis</td>
<td>17 (7)</td>
<td>49 h</td>
<td>Yes</td>
<td>8</td>
<td>60</td>
<td>NA</td>
</tr>
<tr>
<td>Capdevila (21)</td>
<td>2003</td>
<td>Rabbis</td>
<td>19 (9)</td>
<td>51 h</td>
<td>Yes</td>
<td>8</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>Racz (39)</td>
<td>2003</td>
<td>Rats</td>
<td>52 (16)</td>
<td>24 h</td>
<td>Yes</td>
<td>10</td>
<td>55-60</td>
<td>1</td>
</tr>
<tr>
<td>Cayan-Ramirez (20)</td>
<td>2003</td>
<td>Rats</td>
<td>31 (12)</td>
<td>24 h</td>
<td>Yes</td>
<td>10</td>
<td>55-60</td>
<td>1</td>
</tr>
<tr>
<td>Zeigeroglu (30)</td>
<td>2003</td>
<td>Rats</td>
<td>52 (22)</td>
<td>3-18 h</td>
<td>Yes</td>
<td>10</td>
<td>80</td>
<td>1</td>
</tr>
</tbody>
</table>

**Definition of abbreviations:** CMV = controlled mechanical ventilation; control = presence of control group; force decline = percent decline in diaphragmatic force production in mechanically ventilated animals versus control animals or baseline; NA = not available; PEEP = positive end-expiratory pressure; RR = respiratory rate.

* In vivo transdiaphragmatic pressure development in response to phrenic nerve stimulation.
period required to show this change was significantly longer (31). Immobilization models in limb muscles have shown upregulation of the superoxide-generating enzyme xanthine oxidase and elevated levels of transition metals such as iron, calcium, copper, and manganese (31). An increase in \([Fe^{2+}\)] may facilitate the generation of hydroxyl radicals from superoxide and hydrogen peroxide. In addition, both copper and manganese can catalyze the oxidation of glutathione, thereby reducing the overall antioxidant capacity of the cell. Whether such mechanisms are actually triggered in the diaphragm during CMV remains to be determined. Finally, it is noteworthy that increased oxidative stress has been strongly linked to diaphragmatic dysfunction and weakness under other conditions (32–34). This is consistent with the fact that a number of critical proteins involved in energetics, excitation–contraction coupling, and force generation demonstrate a propensity for oxidative modification (34, 35). In one study (30), mechanical ventilation-induced diaphragmatic protein oxidation was evident in insoluble (but not soluble) proteins with molecular masses of about 200, 128, 85, and 40 kD. These findings raise the possibility that actin (40 kD) and/or myosin (200 kD) undergo oxidative modification during CMV. This intriguing possibility awaits confirmation by more specific identification of the modified proteins.

**Structural Injury**

Structural abnormalities of different subcellular components of diaphragm muscle fibers have been found after 2–3 days of CMV in rabbits (15, 19). The changes consisted of disrupted myofibrils (15, 19), increased numbers of lipid vacuoles in the sarcoplasm, and abnormally small mitochondria containing focal membrane disruptions (19). Similar alterations were observed in the external intercostal muscles of ventilated animals (19) but not in the hindlimb muscle (15). In addition, structurally abnormal myofibrils were significantly correlated with the detrimental effects of CMV on force output by the diaphragm (15). The precise mechanisms of injury have not been clearly identified, but there are at least three plausible explanations: (1) activation of calpains (26), which have the ability to degrade several sarcomeric proteins (36), (2) direct cellular injury secondary to augmented oxidative stress (26), and (3) episodes of so-called breakthrough diaphragmatic activity during mechanical ventilation (15). With regard to the latter, it is known that after a sustained period of muscle unloading, resumption of muscle activity is associated with an increased vulnerability to contraction-induced muscle fiber injury (37). Muscles exposed to conditions of zero gravity also experience significant muscle fiber injury on resuming normal activity on earth (38). Therefore, these findings suggest that another important manifestation of VIDD may be an increased susceptibility of the diaphragm to contraction-induced injury when respiratory efforts are resumed, such as during attempts at weaning from the ventilator.

**Muscle Fiber Remodeling**

At the molecular level, the myosin heavy chain component of the myosin molecule constitutes the primary basis for the traditional classification of muscle fibers as being either slow-twitch (Type I) or fast-twitch (Type II). Muscles can modify their overall myosin heavy chain profile by (1) preferential atrophy/hypertrophy of fibers containing a specific myosin heavy chain isoform and (2) actual transformation from one fiber type to another. Both short-term (less than 48 hours) and longer term CMV result in significant modifications of myosin heavy chains within the diaphragm. Hence, after 18 hours of CMV in rats both Type I and II fibers are reduced in size, but with the Type II fiber population exhibiting a greater degree of atrophy (26). Consistent with the above, 24 hours of mechanical ventilation reduced the transcript levels of Type IIA and IIB myosin isoforms by about 20% (39). In rabbits, 2 days of CMV also resulted in atrophy of the respiratory muscles and a preferential decrease in the cross-sectional area of Type IIA and IIB fibers (19). Because the force produced by slow Type I fibers is less than that generated by fast Type II fibers (40), a fast-to-slow modification of myosin phenotype of this type could potentially contribute to the decline of maximal force production by the diaphragm after short-term CMV. However, more prolonged CMV appears to result in a different pattern (15, 18). Thus, after 2–4 days of CMV, rat diaphragms demonstrate an increased percentage of so-called hybrid fibers with coexpression of both slow and fast myosin heavy chain isoforms within the same fiber, which occurs at the expense of the pure Type I fiber population (18). This indicates an actual transformation of the myosin heavy chain profile within individual Type I fibers toward the Type II myosin phenotype. Rabbits ventilated for 3 days also show an increase in Type IIA myosin heavy chain expression in the diaphragm, which appears to occur at the expense of Type IIX isoform (15).

In limb muscles, short-term inactivity may also result in a fast-to-slow transformation (41), whereas longer periods of inactivity generally cause a shift toward a faster myosin profile (42). The duration of inactivity required to elicit these changes is much longer in limb muscles, which once again suggests that the diaphragm may be particularly vulnerable to disuse effects. It has also been speculated that changes in the expression of myogenic regulatory transcription factor proteins, such as myogenic determination factor (MyoD) and myogenin, may play a role in the above-described remodeling process. Interestingly, 24 hours of CMV causes reciprocal changes in transcript levels for MyoD (decreased) and myogenin (increased) in the rat diaphragm (36). On the basis of analogous experiments in the gastrocnemius, these changes were attributed to the effects of unloading rather than cyclic passive shortening (39). Although the main function of MyoD and myogenin is to drive myogenesis during embryonic development, these transcription factors are also important in guiding the process of muscle fiber repair after injury (43), and could influence slow versus fast fiber type specialization in adult muscle (42).

**Metabolic Enzymes**

It is unclear whether significant changes in metabolic enzyme activity within diaphragm muscle fibers, which could contribute to VIDD, occur during CMV. Although one study reported increased citrate synthase activity after 18 hours of CMV (22), longer periods of mechanical ventilation were not associated with significant changes in enzymes of the Krebs cycle (citrate synthase, succinate dehydrogenase) or anaerobic glycolysis (lactate dehydrogenase) in the diaphragm (15, 16). On the other hand, decreased efficiency of mitochondrial oxidative phosphorylation coupling was suggested in a study of rabbits subjected to CMV for 2 days (19).

**CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS**

The major clinical implication of the above-described findings is that even after relatively short periods of mechanical ventilation, substantial diaphragmatic weakness and wasting may occur, with consequent adverse effects on the process of weaning from mechanical ventilation. Data suggest that the intercostals are also affected by CMV in a similar fashion (19, 21). Among several important and clinically relevant questions which need to be addressed by future research are the following.

What is the optimal degree of respiratory muscle effort that should be targeted during mechanical ventilation, to either prevent or reverse VIDD once it is established? In particular, are partial...
support modes of mechanical ventilation less harmful than CMV? Can partial support modes reverse VIDD after a period of CMV? The answers are currently unknown. Although intuitively, the effects of inactivity and unloading (i.e., VIDD induced by CMV) should be reversible by respiratory muscle activity, the time course of this recovery is not known. Indeed, studies of peripheral skeletal muscles suggest this is a slow process, and that after a sustained period of muscle unloading, resumption of muscle activity is associated with an increased vulnerability to contraction-induced muscle fiber injury (37). In the special case of prolonged CMV in tetraplegic patients (who have interrupted neural trophic impulses), gradual diaphragmatic pacing was sufficient to reverse diaphragm muscle fiber atrophy in comparison with the nonpaced situation (44, 45).

To what extent is the development of VIDD determined by other aspects of the ventilatory strategy such as respiratory rate, tidal volume, and the use of positive end-expiratory pressure (PEEP)? During mechanical ventilation, the diaphragm is intermittently and repetitively shortened by cyclical lung inflation (46). Therefore, changes in the respiratory rate and tidal volume applied during mechanical ventilation will necessarily alter the speed and extent of diaphragmatic shortening. The use of PEEP, by contrast, will lead to baseline shortening of the diaphragm at functional residual capacity. It has long been known that the adverse effects of disuse on limb muscle structure are exacerbated by muscle shortening (47). In addition, maintaining skeletal muscles (including the diaphragm) in a shortened position causes a loss of sarcomeres in series (48, 49). Interestingly, two studies that employed PEEP (16, 18) found that CMV (48 hours or more) resulted in significantly decreased optimal length of the diaphragm, a finding that strongly suggests the presence of such sarcomere loss. Differences in myosin isoform expression patterns and the degree of atrophy observed among certain studies may also be at least partly related to the levels of diaphragmatic shortening imposed by the specific ventilator settings applied in these investigations.

How is the susceptibility to VIDD influenced by alterations in the baseline state of the diaphragm? It is important to recognize that all the studies demonstrating VIDD have been performed in animals with a previously healthy diaphragm. Therefore, we do not know to what extent the response to mechanical ventilation might be modified by various pathologic conditions. For instance, oxidative stress is implicated in the loss of diaphragmatic force-generating capacity associated with sepsis (32) as well as mechanical ventilation (26). However, short-term CMV actually improves force-generating capacity of the diaphragm in sepsis and does not appear to alter the level of oxidative stress under these conditions (2). Along these same lines, the response to mechanical ventilation could conceivably be quite different in a diaphragm previously loaded to the point of injury and/or fatigue, both of which are also associated with increased oxidative stress (33, 50). Under these specific circumstances, does mechanical ventilation favor or prevent the development of further oxidative stress, injury, and contractile dysfunction? Moreover, once diaphragmatic injury has occurred, does mechanical ventilation facilitate or impair the subsequent muscle repair process, particularly if it alters the expression of myogenic transcription factors involved in muscle regeneration (36)? The answers to these important questions await further study.

What are the precise mechanisms of diaphragmatic weakness in VIDD? Although it has been shown that VIDD is associated with atrophy, oxidative stress, myofibrillar disruption, and various remodeling responses within diaphragm muscle fibers, the precise involvement of each of these factors in the loss of diaphragmatic force-generating capacity is unclear. Additional mechanisms such as apoptosis might also be involved. Further-
VIDD, with the ultimate goal of translating this knowledge into the clinical arena. The diaphragm is not a biologically inert organ that can be light-heartedly substituted by the ventilator; the vital pump is both malleable and vulnerable.

Conflict of Interest Statement: T.V. has no declared conflict of interest; B.J.P. has no declared conflict of interest.

References


