Assist–Control Mechanical Ventilation Attenuates Ventilator-induced Diaphragmatic Dysfunction

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Controlled mechanical ventilation induced a profound diaphragm muscle dysfunction and atrophy. The effects of diaphragmatic contractions with assisted mechanical ventilation on diaphragmatic isometric, isotonic contractile properties, or the expression of muscle atrophy factor-box (MAF-box), the gene responsible for muscle atrophy, are unknown. We hypothesize that assisted mechanical ventilation will preserve diaphragmatic force and prevent overexpression of MAF-box. Studying sedated rabbits randomized equally into control animals, those with 3 days of assisted ventilation, and those with controlled ventilation, we assessed in vitro diaphragmatic isometric and isotonic contractile function. The concentrations of contractile proteins, myosin heavy chain isoform, and MAF-box mRNA were measured. Tetanic force decreased by 14% with assisted ventilation and 48% with controlled ventilation. Maximum shortening velocity tended to increase with controlled compared with assisted ventilation and control. Peak power output decreased 20% with assisted ventilation and 41% with controlled ventilation. Contractile proteins were unchanged with either modes of ventilation; myosin heavy chain 2X mRNA tended to increase and that of 2A to decrease with controlled ventilation. MAF-box gene was overexpressed with controlled ventilation. We conclude that preserving diaphragmatic contractions during mechanical ventilation attenuates the force loss induced by complete inactivity and maintains MAF-box gene expression in control.

Keywords: artificial respiration; diaphragm; isometric contractions; isotonic contractions; muscle atrophy

Mechanical ventilation can be a lifesaving supportive therapy for patients with acute respiratory failure. However, prolonged mechanical ventilation is thought to produce a condition known as ventilator-induced diaphragmatic dysfunction, making it difficult to wean the patient from the ventilator (1). Currently, little is known about the underlying mechanisms responsible for such phenomenon, but in experimental animals, short periods (i.e., 2–3 days) of controlled mechanical ventilation (CMV) produce a profound loss in the ability of the diaphragm to generate force (2–4). CMV is unique in that it results in the complete absence of neural activation and mechanical activity of the diaphragm muscle. Presumably, one or both of these factors act to produce large and rapid losses in diaphragmatic function observed in earlier studies (2–4).

Unlike CMV, other forms of mechanical ventilation, such as assisted mechanical ventilation (AMV), are associated with partial neural activation and mechanical activity of the diaphragm, and on this basis, it seems reasonable to hypothesize that AMV may not produce as large a loss in diaphragmatic function as is observed with CMV. Hence, the objective of this study was to test this hypothesis by contrasting the effects of both CMV and AMV on the force–velocity relationship, which represents one of the most important contractile properties of skeletal muscle. Notably, the force–velocity relationship describes the maximal force that a muscle can generate at any given shortening velocity and includes important measures of muscle function such as maximal isometric tension (Pm) and maximal shortening velocity (Vmax). We complemented these mechanical measurements with analyses of myosin heavy chain (MyHC) and myosin light chain (MyLC) isoforms, as it has been shown that these proteins play a key role in determining the shape of the force–velocity relationship (5, 6). Additionally, we examined the effects of mechanical ventilation on the expression of muscle atrophy factor-box (MAF-box), a gene that has been shown to be associated with muscle atrophy across a wide array of altered physiological conditions (7). Some of the results of this study have been reported in the form of an abstract (8).

METHODS

Animal Preparation and Surgical Procedures

The Research and Development Subcommittee on animal studies of the Veterans Affairs Long Beach Healthcare System approved the study. We studied 18 adult male pathogen-free New Zealand White rabbits. The animals were assigned randomly in equal numbers (n = 6) into 3 days of AMV, CMV, and surgical control groups. Animals in the AMV group received 3 days of flow-triggered, pressure-limited ventilation (model 840 Nellcor-Puritan Bennett; Mallinckrodt, Carlisle, PA) with a backup rate of four breaths per minute. Animals in the CMV group received 3 days of time-triggered, pressure-limited ventilation (model 7200ae Nellcor-Puritan Bennett), with the inspiratory pressure and inspiratory time set similar to the AMV group and with the ventilator rate set sufficiently high to suppress inspiratory efforts as detected from the inspiratory flow waveform. This ventilator rate was within a range of 40–50 breaths per minute and has previously been shown to suppress diaphragmatic electrical activity (3) (see the online supplement). The animals were killed after 3 days of either AMV or CMV. The surgical control group was killed after the surgical procedures. Because we have previously shown that anesthetic or sedative drugs did not have any influence on the effect of mechanical ventilation on diaphragmatic function (3), in this study, a sham control group consisting of sedated animals breathing spontaneously for 3 days was not included.

The surgery was performed under general anesthesia using aseptic techniques as previously described (3) (see the online supplement). Arterial blood gas tensions and electrolytes were measured as in our previous study (3). In addition, in three animals of each group, prealbumin was also measured on the first and last days of the experiments.

Animal Monitoring during Mechanical Ventilation

During AMV and CMV, continuous intravenous sedation was maintained with diazepam at a loading dose of 4 mg/kg intramuscularly,
followed by a continuous intravenous infusion of 2–5 mg/hour titrated to limb movement. This diazepam dose was insufficient to suppress the respiratory center. In the CMV group, suppression of the diaphragm was maintained by adjusting the ventilator settings and if necessary by administration of additional maintenance doses of a ketamine–xylazine mixture (see the online supplement). A physician or a research scientist provided round-the-clock coverage and animal care for the duration of the study.

Assessment of Diaphragm Muscle Activity during Mechanical Ventilation

On the last day of the experiment, under general anesthesia while the animals were receiving AMV or CMV, a laparotomy was performed for insertion of a pair of wire electrodes into the ventral portion of the diaphragm for diaphragmatic electrical activity measurements. Diaphragmatic electrical activity was detectable during AMV and was completely suppressed during CMV (see the online supplement).

In Vitro Measurements of Diaphragm Contractile Properties

Isometric contractile properties. After the diaphragmatic electrical activity measurements, the animals were killed with an overdose of pentobarbital sodium (100 mg/kg intravenously). The diaphragm muscle was rapidly excised from the midcostal region, with the insertion of fibers at the rib and central tendon intact. A diaphragm muscle strip (approximately 5 mm wide) was obtained and mounted vertically between two platinum plate electrodes that cover the entire length of the muscle strip in a 26°C bath containing Rees-Simpson solution (9) through which 95% O2–5% CO2 was continuously aerated, maintaining a pH of 7.40. The dependency of muscle force generation on the frequency of stimulation was determined using the Cambridge system, controlled using customized routine software (LabTech Notebook, Andover, MA), and implemented on a computer. Force and length were independently controlled, allowing the Cambridge system to operate either in isometric or isotonic modes (discussed later here). For the isotonic mode, 1-second duration trains of stimuli at 20, 40, 50, 75, and 100 Hz were set with at least a 2-minute interval between each stimulus frequency. The P₀ was then determined. Forces were normalized for the muscle cross-sectional area (see the online supplement).

Isotonic contractile properties. After isometric force measurements, the muscle strip was allowed to equilibrate for 15 minutes before measurement of isotonic contractile properties. The stability of the in vitro preparation was evidenced by the relatively constant P₀, measured just before the isotonic contractile measurements as compared with that measured during the isometric trials. Average tetanic force measured before the force−velocity trials in control, AMV, and CMV were 25.2, 21.1, and 18.9 N/cm², respectively, as compared with 25.9, 22.2, and 13.4 N/cm² during the isometric contractile trials.

The force–velocity relationship of the diaphragm was determined using the isotonic mode of the Cambridge system with the computer controls afterload. The muscle was tested at a minimum of 15 different after-load conditions (3–100% P₀). The muscle began each contraction at length at which diaphragm muscle strip produced maximal isometric tension. Velocity of contraction, expressed as muscle length (normalized for the length at which diaphragm muscle strip produced maximal isometric tension) per second (ML/second), was plotted against force. Power, the product of force and velocity of contraction, expressed as watts per kilogram muscle wet weight, was calculated and plotted against force. In addition, the maximum power was determined (see the online supplement).

Contractile Protein and Molecular Analysis

Quantification of contractile protein fractions. Diaphragm muscle homogenates were loaded into the wells of a 4−15% gradient Tris-HCl polyacrylamide gels (Bio Rad, Hercules, CA) at a protein concentration of 2 μg/well (see the online supplement). The relative proportions of myosin and actin were obtained by measuring the density of each contractile protein band and expressing it as a percentage of the total density of all proteins in that sample.

Discontinuous polyacrylamide gel electrophoretic separation of MyHC isoforms. MyHC protein isoforms were separated using techniques described by us previously (10, 11) (see the online supplement). This method separated the fast MyHCα, MyHCβ, MyHCγ, and slow MyHC-β isoforms (order of migration). The MyHC isoform bands were quantified using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Determination of MyLC isoforms. Analyses of MyLC protein isoform composition were performed as described previously (5). Purified myofibrillar protein from each sample was denatured and loaded (5–10 μg) into the well of a 5−20% gradient gel (see online supplement). Data for a given essential MyLC isoform were expressed relative to the total area for all the essential light chains, that is, the slow MyLC₁, fast MyLC₂, and fast MyLC₃. A similar approach was used for the regulatory MyLC isoforms, that is, the slow MyLC₂ and fast MyLC₃.

Determination of MyHC isoform and MAF-box mRNA using reverse transcription coupled with polymerase chain reaction. One microgram of total RNA was reverse transcribed for each muscle sample using the Superscript II RT and a mix of oligo dT (100 ng/reaction) and random primers (200 ng/reaction) (Invitrogen; Life Technologies, Carlsbad, CA) in a 20-μl total reaction volume at 42°C for 50 minutes, according to the provided protocol (see the online supplement). In each polymerase chain reaction, 18S ribosomal RNA was co-amplified with the target cDNA (mRNA) to serve as an internal standard and to allow correction for differences in starting amounts of total RNA. The Classic 18S and Alternate 18S were used for the polymerase chain reaction with MyHC and MAF-box, respectively.

Statistics and Data Analyses

Values are means ± SE unless specifically indicated. A two-way analysis of variance (SigmaStat, version 2.03; SPSS Science, Chicago, IL) was used to compare diaphragm tetanic force using the grouping variables of frequency of stimulation and mode of ventilation. Where appropriate, a one-way analysis of variance was used for comparison of other variables among groups. When the F value was significant, a post hoc analysis was performed using Tukey’s test for pairwise multiple comparisons. A linear regression was used to determine the contribution of MAF-box mRNA levels to diaphragm maximal tetanic force. Group differences and linear regression were considered significant when p ≤ 0.05.

RESULTS

The average body weights were not significantly different among the groups: 3.6 ± 0.3, 3.9 ± 0.2, and 3.8 ± 0.3 (± SD) kg for the control, AMV, and CMV groups, respectively. Because changes in body weight did not reflect acute changes in nutritional state, body weight was not recorded at the end of the experiment. Instead, we measured serum prealbumin at the beginning and the end of the experiment. Plasma prealbumin half-life is 48 hours (12); hence, any changes can be detected within the duration of the study. Average baseline prealbumin values in the control, AMV, and CMV groups were 10.1 ± 0.4, 9.3 ± 0.3, and 10.9 ± 0.8 (± SE) g/dl, respectively. After 3 days of AMV or CMV, prealbumin values were essentially unchanged: 8.4 ± 0.8 and 10.1 ± 0.3 g/dl, respectively.

Figure 1 shows the PaO₂/FiO₂ ratio, pH, PaCO₂, and bicarbonate of control and the trend during 3 days of AMV or CMV. At a given time, PaO₂/FiO₂, pH, PaCO₂, and bicarbonate were similar among or between groups. At time 0, arterial blood was obtained during spontaneous breathing, hence the elevated PaCO₂.

Diaphragmatic Isometric Contractile Properties

In comparison to the control, CMV profoundly decreased both P₀ and peak twitch force (Pₜ) (by 48%, p < 0.01; Table 1). This observation was similar to our previous study (3). P₀ and peak twitch force tended to decrease with AMV, but the decreases did not achieve statistical significance. The length at which diaphragm muscle strip produced maximal isometric tension was comparable among groups. This finding is consistent with that from our earlier study (3) where we also observed that the length at which diaphragm muscle strip produced maximal isometric tension was unaffected by CMV. Twitch time from onset of
muscle contraction to \( P_t \) and time for \( P_t \) to relax to one-half of \( P_t \) tended to increase, particularly the time for \( P_t \) to relax to one-half of \( P_t \) in the CMV group, but they statistically were not significant. Figure 2 shows the force–frequency relationship of all groups. At all stimulation frequencies, force was markedly reduced with CMV and was relatively well maintained with AMV.

**Diaphragm Isotonic Contractile Properties**

The force–velocity relationships of the three groups are shown in Figure 3. In the low velocity–high force region, as noted previously here, \( P_o \) of the CMV group was significantly less than that in both the control and AMV groups (Figure 3, middle panel). At the other end of the force–velocity relationship, in the CMV group, \( V_{\text{max}} \) tended to be greater than that of the AMV and the control; however, it did not achieve statistical significance. In all groups, peak power output occurred at approximately 21% of \( P_t \) (Figure 4, top panel). Power output and its peak value were profoundly reduced with CMV (\( p < 0.02 \)) and tended to decrease with AMV, but the latter was not significantly different from control (Figure 4, top to bottom panels; Table 1).

**TABLE 1. IN VITRO DIAPHRAGMATIC CONTRACTILE PROPERTIES**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AMV</th>
<th>CMV</th>
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<tbody>
<tr>
<td>( L_o ), cm</td>
<td>3.2 ± 0.1</td>
<td>3.0 ± 0.2</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>TPT, ms</td>
<td>68.9 ± 1.4</td>
<td>78.5 ± 2.1</td>
<td>75.9 ± 8.1</td>
</tr>
<tr>
<td>RT1/2, ms</td>
<td>95.3 ± 2.6</td>
<td>102.7 ± 2.5</td>
<td>155.3 ± 26.5</td>
</tr>
<tr>
<td>( P/CSA ), N/cm²</td>
<td>8.2 ± 0.8</td>
<td>7.5 ± 0.6</td>
<td>4.3 ± 0.6*</td>
</tr>
<tr>
<td>( P_t/CSA ), N/cm²</td>
<td>25.7 ± 0.9</td>
<td>22.2 ± 1.1</td>
<td>13.4 ± 0.8*</td>
</tr>
<tr>
<td>( V_{\text{max}} ), ML/s</td>
<td>6.4 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>7.1 ± 1.1</td>
</tr>
<tr>
<td>Power(_{\text{max}}), W/kg</td>
<td>71.2 ± 5.9</td>
<td>56.6 ± 5.5</td>
<td>42.1 ± 7.1*</td>
</tr>
</tbody>
</table>

*Definition of abbreviations: AMV = assisted mechanical ventilation; CMV = controlled mechanical ventilation; CSA = cross-sectional area (see online supplement for calculations); \( L_o \) = length at which diaphragm muscle strip produced maximal isometric tension; \( P_o \) = maximum tetanic force; \( P_t \) = peak twitch force; RT1/2 = time for \( P_t \) to relax to one-half of \( P_t \); TPT = time from onset of muscle contraction to peak twitch force.

Values are mean ± SE; \( n = 6 \) animals in each group, except for \( V_{\text{max}} \) and Power\(_{\text{max}}\) in the CMV group, \( n = 4 \).

* \( p < 0.05 \), CMV vs. control and AMV (\( P_t \) and \( P_o \)); CMV vs. control (Power\(_{\text{max}}\)).

**Contractile Protein Isoform Analyses: Myosin and Actin**

In a previous study (3), we observed that CMV produced a profound reduction in \( P_o \). Some investigators (13, 14) have suggested that unloading of skeletal muscle produces a selective loss of actin and that this accounts for both a reduction in \( P_o \) and an increase in \( V_{\text{max}} \). This caused us to examine the relative proportions of myosin and actin. As shown in Table 2, the relative proportions of both of these proteins (expressed as relative to the total pool of myofibrillar proteins) were similar among the three groups.

The maximal shortening velocity of skeletal muscle is known to be dependent primarily on the MyHC isoform composition of the muscle/muscle fiber. As shown in Table 2, the control diaphragm muscles expressed the MyHC\(_{\text{slow}}\) (approximately 35%), fast MyHC\(_{2A}\) (approximately 55%), and fast MyHC\(_{2X}\) (approximately 10%) protein isoforms. The absence of the fast MyHC\(_{2B}\) isoform is consistent with our previous study (3). Neither AMV nor CMV produced significant alterations in the MyHC isoform composition of the diaphragm muscle. Consistent with this observation, the mRNA levels of each MyHC isoform were unaffected, although with CMV, the MyHC\(_{2X}\) tended to increase and MyHC\(_{2A}\) to decrease (Figure 5).

**Figure 1.** \( \text{PaO}_2/\text{FiO}_2 \), pH, \( \text{PaCO}_2 \), and bicarbonate (HCO\(_3^-\)) in control animals, and the trend in animals receiving 3 days of assisted (AMV) and controlled mechanical ventilation (CMV). At time 0, arterial blood was withdrawn during spontaneous breathing. Values are mean ± SE.

**Figure 2.** Diaphragmatic tetanic force at various stimulation frequencies in control, AMV, and CMV. Values are mean ± SE. *\( p < 0.01 \), CMV versus control and AMV. CSA = cross-sectional area.
Some studies have shown that essential MyLC isoforms (especially fast MyLC3) may also influence Vmax (6). We observed that the control diaphragm muscle expressed all three essential MyLC isoforms, with fast MyLC1 representing approximately 50 to 60% of the total pool of essential MyLCs (Table 2). Neither AMV nor CMV affected the relative proportions of the essential MyLC isoforms. This was also true for the regulatory MyLC isoforms (slow MyLC2 and fast MyLC2) (Table 2).

**MAF-box mRNA Levels**

MAF-box has been shown to be associated with a spectrum of perturbed physiologic conditions that produce muscle atrophy (7), and as a consequence, it seemed logical to determine whether mechanical ventilation altered the mRNA levels of this gene in the diaphragm muscle. Unfortunately, our analyses were limited to the mRNA level given that neither a monoclonal nor polyclonal antibody is available for analyses at the protein level. Compared to the mRNA level given that neither a monoclonal nor polyclonal antibody is available for analyses at the protein level. Compared with the control group, CMV produced a large increase (174%) in the mRNA level of MAF-box (p < 0.03; Figure 6). Interestingly, the mRNA levels of MAF-box in the AMV group were similar to those of the control group.

**DISCUSSION**

There are three unique findings of this study. First, to our knowledge, this study is the first to demonstrate that partial diaphragm muscle activation associated with AMV is sufficient to mitigate the profound reduction in Po, that occurs as a result of CMV. This observation may have profound clinical importance. Second, we observed that neither CMV nor AMV produced significant changes in Vmax relative to the control group. Consistent with this observation, we found that both the MyHC and MyLC isoform compositions of the CMV and AMV muscles were also unaffected. Finally, we observed that CMV produced a significant increase in the mRNA levels of MAF-box, an E3 ligase in the ubiquitin–proteasome pathway that may play a key role in myofibrillar disassembly that accompanies CMV. The following discussion addresses each of these findings in more detail.

**AMV Minimizes the Loss of Diaphragmatic Function**

We and others have shown that CMV leads to a significant loss in diaphragmatic force generating capacity studied both in vivo (3, 15, 16) and in isolated diaphragm muscle strips (2–4, 17–19). Three days of CMV results in an approximately 50% decrease in P0 (3). This represents a rather dramatic loss in functional capacity, as defined by both its magnitude and the rapidity with which it occurs. Indeed, this response is faster than that typically observed in rat hind limb muscle (20) and causes us to speculate

**TABLE 2. PROPORTION OF MYOSIN, ACTIN, MYOSIN HEAVY CHAIN, AND MYOSIN LIGHT-CHAIN PROTEIN ISOFORMS**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AMV</th>
<th>CMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin, % of total protein pool</td>
<td>40.5 ± 2.2</td>
<td>45.9 ± 2.2</td>
<td>43.9 ± 2.9</td>
</tr>
<tr>
<td>Actin, % of total protein pool</td>
<td>21.4 ± 1.0</td>
<td>25.0 ± 1.3</td>
<td>20.9 ± 1.4</td>
</tr>
<tr>
<td>MyHCslow, %</td>
<td>34.9 ± 2.5</td>
<td>33.8 ± 3.8</td>
<td>34.6 ± 1.5</td>
</tr>
<tr>
<td>MyHCan, %</td>
<td>54.8 ± 2.4</td>
<td>56.1 ± 2.6</td>
<td>51.1 ± 1.8</td>
</tr>
<tr>
<td>MyHCfast, %</td>
<td>10.3 ± 1.7</td>
<td>10.1 ± 2.8</td>
<td>14.3 ± 2.2</td>
</tr>
<tr>
<td>sMyLC1, %</td>
<td>28.3 ± 2.9</td>
<td>31.7 ± 1.5</td>
<td>34.5 ± 0.9</td>
</tr>
<tr>
<td>fMyLC1, %</td>
<td>58.2 ± 3.1</td>
<td>55.9 ± 2.7</td>
<td>52.7 ± 1.1</td>
</tr>
<tr>
<td>fMyLC2, %</td>
<td>13.5 ± 0.8</td>
<td>12.4 ± 1.8</td>
<td>12.8 ± 0.5</td>
</tr>
<tr>
<td>sMyLC2, %</td>
<td>12.7 ± 0.7</td>
<td>16.2 ± 2.1</td>
<td>18.1 ± 1.8</td>
</tr>
<tr>
<td>fMyLC3, %</td>
<td>87.3 ± 0.7</td>
<td>83.7 ± 2.1</td>
<td>81.9 ± 1.8</td>
</tr>
</tbody>
</table>

*Definition of abbreviations: AMV = assisted mechanical ventilation; CMV = controlled mechanical ventilation; f = fast; MyHC = myosin heavy chain isoform; MyLC = myosin light chain isoform; s = slow.
Values are mean ± SE; n = 6 animals in each group.*
that the diaphragm is much more sensitive to a complete absence of electrical and mechanical activity than are other skeletal muscles. Others (1, 4) speculated that the rapid diaphragmatic force loss with CMV might be related to the reduced number of sarcomere in parallel due to myofibril shortening with the application of positive end-expiratory airway pressure. In this study, positive end-expiratory airway pressure was not applied to either AMV or CMV, and the length at which diaphragm muscle strip produced maximal isometric tension was not significantly different among groups (Table 1).

In this study, we made the novel observation that AMV can dramatically blunt the loss of function that occurs as a result of CMV. This is an important observation from both a clinical (see Critique and Clinical Implications later here) and basic science perspective. In experimental animal models of diaphragm muscle inactivity associated with phrenic denervation, tetrodotoxin nerve blockade, or C2 spinal cord hemisection, neurotrophic influence appears predominant over phrenic motoneuron transmission (21). With both AMV and CMV, phrenic motoneuron transmission and neurotrophic influence are intact, but with CMV, the diaphragm is completely inactive (both electrically and mechanically). Currently, it is unclear whether the protective effect of AMV is due to the maintenance of partial neural activation, mechanical activity, or a combination of both factors. One study suggests that in the human diaphragm, atrophy can be prevented by the delivery of brief periods of daily phrenic nerve stimulation (22) without mechanical loading, suggesting that neural activation is essential in preventing atrophy and force loss. Future studies are needed to determine clearly the degree of activation required to maintain diaphragmatic function and to identify the importance of neural activity versus mechanical loading.

**Neither CMV nor AMV Affects V\textsubscript{max}**

Diaphragm muscle function after CMV has been examined primarily using isometric measurements (twitch and tetanus). These types of measurements are highly constrained with respect to the functional spectrum of loading conditions under which skeletal muscle operates. Perhaps the single best measure of muscle function is the force–velocity relationship given that it describes the maximal force that can be produced at any given shortening velocity, including isometric conditions. Additionally, the shape of the force–velocity relationship reflects important structural features. For instance, the maximal force that a muscle can produce under isometric conditions is determined by the number of functional sarcomeres in parallel. At the other end of the force–velocity spectrum, the V\textsubscript{max} of skeletal muscle appears to be highly correlated with MyHC isoform composition (23), although thin filaments density, MyLC isoforms, and the geometry of the sarcomere are other potential factors contributing to V\textsubscript{max} (6). Finally, the mathematical product of force and velocity is mechanical power. Hence, the force–velocity curve also defines the spectrum of mechanical power that skeletal muscle can produce.

Given this background, AMV did not affect the force–velocity relationship as reflected by a lack of change in either P\textsubscript{o} or V\textsubscript{max}. In contrast, CMV induced a large reduction in the ability of the diaphragm to produce force, as reflected by both a decrease in P\textsubscript{o} and a significant alteration in the high force region of the force–velocity relationship (Figure 3, middle panel). This dramatically reduces the ability of the diaphragm to produce mechanical power in this region (Figure 4, middle panel). The V\textsubscript{max} of the CMV group was unaltered relative to that of the control group.

**Does Mechanical Ventilation Produce Myosin Isoform Transitions?**

The native myosin molecule is a complex hexameric structure that is composed of two MyHCs, two essential MyLCs, and two regulatory MyLCs. The globular head of each MyHC represents
the molecular motor that is responsible for developing force and shortening/lengthening, and the function of the globular head of the MyHC can be partially influenced by the type of essential MyLC isoform (23).

Previously, we observed that 3 days of CMV did not alter the MyHC isoform composition of the diaphragm (3). The findings of this study are consistent with this earlier observation, and importantly, this study extends our earlier findings by providing additional insight regarding (1) the affects of 3 days of AMV on MyHC protein isoform composition, (2) the affects of CMV and AMV on MyHC isoform mRNA levels, and (3) possible changes in MyLC isoform composition. At the protein level, we observed that neither CMV nor AMV influenced the MyHC and MyLC isoform compositions of the diaphragm. We also observed that the mRNA levels of the different MyHC isoforms were unaffected by CMV and AMV. Collectively, the mechanical (V_{max}) and protein (isoform) data demonstrate that short periods of CMV and AMV do not affect the high velocity–low force region of the force–velocity relationship. This perspective is also consistent with the findings of Yang and colleagues (4) who observed very minor changes in the muscle fiber composition of the diaphragm muscle after 44–93 hours of CMV.

Effect of AMV and CMV on MAF-box mRNA Levels

As noted previously here, CMV markedly reduces the ability of the diaphragm to produce force without influencing the high velocity–low force region of the force–velocity relationship. Currently, the underlying mechanisms responsible for this type of ventilator-induced diaphragm dysfunction remain unknown. However, as we have shown previously (3), 3 days of CMV produces a dramatic disassembly of the myofibrillar network. Some of the postulated mechanisms that might be responsible for such phenomenon include the potential involvement of (1) the ubiquitin–proteasome pathway, (2) calcium-activated proteases, and (3) free radicals.

The possible involvement of the ubiquitin–proteasome pathway was suggested by the findings of Shanelly and colleagues (24), who reported that 18 hours of CMV produced increased 20S proteasome activity. However, the elevated 20S proteasome can be an indicator of protein degradation via protein oxidation and not necessarily via the ubiquitin–proteasome pathway (25). Indeed, Zergeroglu and coworkers (26) demonstrated that myofibril protein oxidation occurred in rats very early in the course of CMV (approximately 6 hours). To explore the potential involvement of the ubiquitin–proteasome pathway, we examined the affects of 3 days of CMV and AMV on the mRNA levels of MAF-box, a so-called E3 ligase that plays a fundamental role in the ubiquitination of proteins targeted for degradation via the proteasome (27, 28). We focused on MAF-box because (1) it has been shown to be upregulated across a broad spectrum of altered physiologic conditions known to produce muscle atrophy, and (2) knockout of this gene markedly mitigates the atrophic response (7). The findings of this study demonstrate that CMV produces a significant increase in MAF-box mRNA levels, whereas AMV does not. Importantly, the upregulation of MAF-box appears to be correlated with the loss of P_E (Figure 7). Whether this represents a cause and effect relationship or simply is correlative remains to be determined.

Critique and Clinical Implications

This study was limited to examining the effects of 3 days of mechanical ventilation on healthy diaphragm muscles. As a consequence, it may not be valid to extrapolate the results of this study to the affects of mechanical ventilation on the human diaphragm under diseased conditions. It is clear, however, that when the rabbit diaphragm is allowed to contract with each breath during mechanical ventilatory support, the loss of diaphragmatic force-generating capacity observed with CMV was attenuated. On this basis, it seems prudent that from the outset critically ill patients receiving prolonged mechanical ventilation should receive partial support modes that allow for intermittent diaphragmatic contractions and that the use of paralytics or sedatives at doses that eliminate diaphragmatic contractions should be avoided.

Conflict of Interest Statement: C.S.H.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; E.Z. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; V.J.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this article.

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