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# Mechanical ventilation results in progressive contractile dysfunction in the diaphragm

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Abstract

These experiments tested the hypothesis that a relatively short duration of controlled mechanical ventilation (MV) will im-pair diaphragmatic maximal specific force generation (specific Po) and that this force deficit will be exacerbated with increased time on the ventilator. To test this postulate, adult Sprague-Dawley rats were randomly divided into one of six experimental groups: 1) control (n = 12); 2)12hofMV(n = 4); 3)18hofMV(n = 4); 4) 18 h of anesthesia and sponta-neous breathing (n = 4); 5)24hofMV(n = 7); and 6)24hof anesthesia and spontaneous breathing (n = 4). MV animals were anesthetized, tracheostomized, and ventilated with room air. Animals in the control group were acutely anesthe-tized but were not exposed to MV. Animals in two spontane-ous breathing groups were anesthetized and breathed spon-taneously for either 18 or 24 h. No differences (P = 0.05) existed in diaphragmatic specific Po between control and the two spontaneous breathing groups. In contrast, compared with control, all durations of MV resulted in a reduction (P = 0.05) in diaphragmatic specific tension at stimulation fre-quencies ranging from 15 to 160 Hz. Furthermore, the MV-induced decrease in diaphragmatic specific Po was time de-pendent, with specific Po being =18 and =46% lower (P = 0.05) in animals mechanically ventilated for 12 and 24 h, respectively. These data support the hypothesis that rela-tively short-term MV impairs diaphragmatic contractile function and that the magnitude of MV-induced force deficit increases with time on the ventilator.

# Mechanical ventilation results in progressive contractile dysfunction in the diaphragm

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These experiments tested the hypothesis that a relatively short duration of controlled mechanical ventilation (MV) will impair diaphragmatic maximal specific force generation (specif-ic $P_o$) and that this force deficit will be exacerbated with increased time on the ventilator. To test this postulate, adult Sprague-Dawley rats were randomly divided into one of six experimental groups: *1)* control ($n = 12$); *2)* 12 h of MV ($n = 4$); *3)* 18 h of MV ($n = 4$); *4)* 18 h of anesthesia and sponta-neous breathing ($n = 4$); *5)* 24 h of MV ($n = 7$); and *6)* 24 h of anesthesia and spontaneous breathing ($n = 4$). MV animals were anesthetized, tracheostomized, and ventilated with room air. Animals in the control group were acutely anesthe-tized but were not exposed to MV. Animals in two spontane-ous breathing groups were anesthetized and breathed spon-taneously for either 18 or 24 h. No differences ($P > 0.05$) existed in diaphragmatic specific $P_o$ between control and the two spontaneous breathing groups. In contrast, compared with control, all durations of MV resulted in a reduction ($P < 0.05$) in diaphragmatic specific tension at stimulation fre-quencies ranging from 15 to 160 Hz. Furthermore, the MV-induced decrease in diaphragmatic specific $P_o$ was time de-pendent, with specific $P_o$ being ~18 and ~46% lower ($P < 0.05$) in animals mechanically ventilated for 12 and 24 h, respectively. These data support the hypothesis that rela-tively short-term MV impairs diaphragmatic contractile function and that the magnitude of MV-induced force deficit increases with time on the ventilator.

respiratory muscles; muscle atrophy; weaning from mechan-ical ventilation; muscle injury

MECHANICAL VENTILATION (MV) is used clinically to sus-tain ventilation in patients who are incapable of main-taining adequate alveolar ventilation on their own. Respiratory failure, neuromuscular diseases, drug overdoses, and recovery from general anesthetics are common indications for the use of MV (11). Problems in weaning patients after prolonged MV have been re-ported to occur in a significant number of patients (3, 13, 24). It is postulated that a frequent cause of difficult weaning is respiratory muscle failure that occurs be-cause of inspiratory muscle (e.g., diaphragm) force and endurance defects (3, 14, 23). Therefore, understand-ing the effects of prolonged MV on respiratory muscle contractile function is important.

To date, only two published reports have addressed the effects of MV on diaphragm contractile perfor-mance in animals. Specifically, Anzueto et al. (1) dem-onstrated that 11 days of controlled MV in baboons resulted in a decrease in both maximal diaphragmatic force production ($P_o$) and endurance. Another investi-gation used a rat model and examined the effects of 48 h of MV on the contractile properties of the dia-phragm (15). A significant reduction (~60%) in maxi-mal diaphragmatic specific force (specific $P_o$; i.e., force per cross-sectional area) was reported after MV.

Collectively, the aforementioned studies indicate that prolonged MV impairs diaphragmatic contractile function. However, the time course of MV-induced di-aphragmatic contractile dysfunction is unknown. This is significant because an understanding of the time course of MV-induced diaphragmatic dysfunction might provide clues into the mechanism(s) responsible for this phenomenon. Specifically, identification of the time course of MV-induced diaphragmatic contractile dysfunction would set the stage for future experiments aimed at determining which biochemical alterations may be responsible for the observed contractile dys-function. Therefore, these experiments examined the temporal pattern of MV-induced contractile dysfunc-tion in the rat diaphragm. On the basis of preliminary experiments in our laboratory, we hypothesized that as few as 12 h of MV would result in diaphragmatic contractile dysfunction and that the magnitude of MV-induced contractile dysfunction increases with time on the ventilator.

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## METHODS

### Experimental Animals

These experiments were approved by the University of Florida Animal Use Committee and followed the guidelines for animal experiments established by the National Institutes of Health. Healthy, female young adult (4-mo-old) Sprague-Dawley rats were individually housed and fed rat chow and water ad libitum and were maintained on a 12:12-h light-dark cycle for 3 wk before initiation of these experiments.

### Preliminary Experiment

Before performing our primary experiments, we conducted a preliminary experiment that was designed to determine whether the diaphragm is mechanically active during controlled MV. Briefly, we placed wire electrodes in the costal diaphragm of four animals and measured electromyograph activity during 24 h of controlled MV (all breaths delivered by the ventilator). No electrical activity in the diaphragm was observed in any of these animals during the 24 h of MV. These results agree with the previous work of Le Bourdelles et al. (15) and support the idea that diaphragmatic contractions do not occur in anesthetized rats during prolonged, controlled MV.

### Experimental Design for Primary Experiments

To examine the time course of MV-induced contractile dysfunction in the diaphragm, animals were randomly assigned to one of six experimental groups (Fig. 1).

*Protocol for control animals.* The animals in the control group were free of intervention before measurement of diaphragmatic contractile properties. That is, these animals were not mechanically ventilated or exposed to long-term anesthesia before study. At the specified time, animals in the control group received an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). After a surgical plane of anesthesia was achieved, the diaphragm was rapidly removed for measurement of in vitro contractile properties.

*Protocol for anesthetized and spontaneously breathing animals.* To determine whether long-term anesthesia (i.e., pentobarbital sodium) impairs diaphragmatic contractile properties, our experimental design included two groups of anesthetized and spontaneously breathing animals. Diaphragmatic force production in one group of animals ($n = 4$) was studied after 18 h of spontaneous breathing, whereas the second group ($n = 4$) was studied after 24 h of spontaneous breathing. Animals in both of these groups were anesthetized and tracheostomized, and a surgical plane of anesthesia was maintained throughout the experimental period by repeated

doses of pentobarbital sodium (~10 mg/kg approximately every hour). These animals were not mechanically ventilated and breathed spontaneously during this time. Arterial catheters were placed in the carotid arteries, and arterial blood samples (~100 µl/sample) were removed at 1, 12, and 18 h from each experimental group. Arterial blood samples obtained from animals in the 18-h experimental group were analyzed for pH and blood-gas status by using a blood-gas analyzer (model 1610, Instrumentation Laboratories). Blood samples were not analyzed in the 24-h experimental group because of technical problems. Body temperature was maintained at ~37°C by use of a recirculating heating blanket. Animals in both of these groups were constantly monitoring by a technician and exposed to continuing care. Continuing care during anesthesia in both of these experimental groups included expressing the bladder, removing upper airway mucus, lubricating the eyes, rotating the animal, and passively moving the limbs. This care was maintained throughout the experimental period at 2-h intervals. Furthermore, both heart rate and blood pressure (tail cuff procedure) were measured at 2-h intervals throughout the experiment. Finally, to reduce airway secretions, animals in both spontaneous breathing groups received intramuscular injections of glycopyrrolate (0.04 mg/kg) every 2 h.

Throughout the period of spontaneous breathing, animals received enteral nutrition from the AIN-76 rodent diet with a nutrient composition of 15% proteins, 35% lipids, 50% carbohydrates, and vitamins and minerals (Research Diets, Brunswick, NY). Our planned feeding schedule was designed to provide an isocaloric diet with the nutrients administered every 2 h via a gastric tube; the total daily administration of 69 ml is equivalent to 69 kcal/day. Body fluid homeostasis was maintained via the administration of $2.0 \text{ ml·kg}^{-1} \cdot \text{h}^{-1}$ intravenous electrolyte solution. On completion of the prescribed time period (i.e., 18 or 24 h), a segment of the costal diaphragm was removed for in vitro measurement of contractile properties.

*Protocol for mechanically ventilated animals.* All surgical procedures were performed by using aseptic technique. Animals randomly selected for MV were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). After a surgical plane of anesthesia was reached, the animals were tracheostomized and mechanically ventilated using a volume-driven small-animal ventilator (model CIV-101, Columbus Instruments, Columbus, OH). All breaths were delivered by the ventilator; hence, this mode of ventilation (i.e., controlled MV) results in complete diaphragmatic inactivity. The tidal volume was established at ~1 ml/100 g body wt with a respiratory rate of 80 breaths/min; this respiratory rate was selected to mimic the breathing frequency of adult rats at rest. Additionally, positive end-
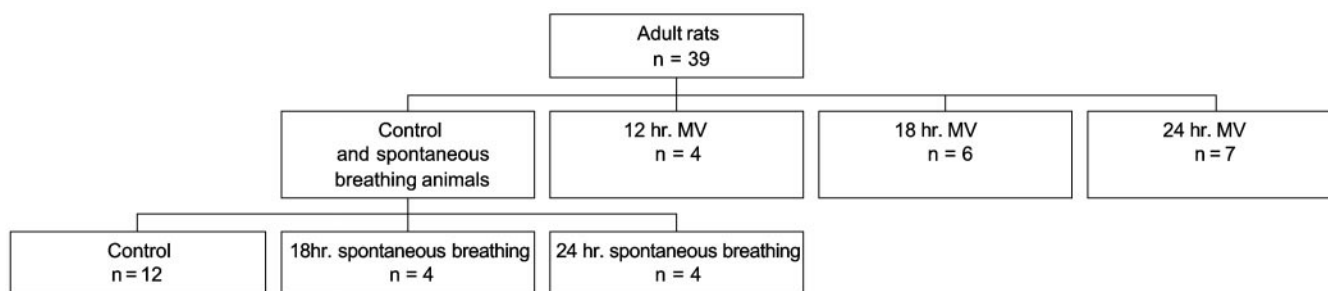


Fig. 1. Schematic illustration of the experimental design used in these experiments. MV, mechanical ventilation; *n*, no. of animals.

expiratory pressure of 1 cmH$_2$O was used throughout the protocol.

An arterial catheter was placed in the carotid artery for constant measurement of blood pressure and periodic blood sampling for analysis of arterial pH and blood gases. Arterial blood samples (~100 µl/sample) were removed at selected time periods and analyzed for arterial P$_{CO_2}$, P$_{O_2}$, and pH by using a blood-gas analyzer (model 1610, Instrumentation Laboratories). Before analysis, the analyzer was calibrated by using standardized gases and pH solutions.

A venous catheter was placed in the jugular vein to permit the infusion of isotonic saline and pentobarbital sodium when necessary. Anesthesia was maintained over the entire period of MV by repeating infusions of pentobarbital sodium (8–12 mg/kg body wt). Note that we continuously monitored the level of anesthesia in our animals by several methods (i.e., monitoring heart rate, blood pressure, and corneal and lid reflexes). Body temperature was maintained at ~37°C by use of a recirculating heating blanket. Additionally, heart rate and electrical activity of the heart were monitored via a lead II electrocardiogram by using needle electrodes placed subcutaneously.

Throughout MV animals received enteral nutrition from the aforementioned AIN-76 rodent diet. This planned feeding schedule was identical to the routine described for the spontaneously breathing animals. Furthermore, body fluid homeostasis was maintained via the administration of 2.0 ml·kg$^{-1}$·h$^{-1}$ intravenous electrolyte solution.

Continuing care during MV included expressing the bladder, removing airway mucus, lubricating the eyes, rotating the animal, and passively moving the limbs. This care was maintained throughout the experimental period at 2-h intervals. Finally, identical to the protocol used in the spontaneously breathing animals, repeated intramuscular injections of glycopyrrolate (0.04 mg·kg$^{-1}$·2 h$^{-1}$) were employed to reduce airway secretions. On completion of MV, a segment of the costal diaphragm was removed for in vitro measurement of contractile properties.

### Measurement of in Vitro Diaphragmatic Contractile Properties

*Description of the muscle organ bath.* Details of our in vitro protocol have been described in detail elsewhere (6, 10, 17, 18) and will be briefly described here. After a surgical plane of anesthesia was reached, the entire diaphragm was removed and placed in a dissecting chamber containing a Krebs-Hensleit solution equilibrated with a 95% O$_2$-5% CO$_2$ gas. A muscle strip, including the tendinous attachments at the central tendon and rib cage (dimensions = 20 ± 3 mm), was dissected from the midcostal region. The strip was suspended vertically between two lightweight Plexiglas clamps with one end connected to an isometric force transducer (model FT-03, Grass Instruments, Quincy, MA) within a jacketed tissue bath. The force output was recorded via a computerized data-acquisition system (Super Scope II, GW Instruments Somerville, MA; Apple Computer Cupertino, CA). The tissue bath was filled with Krebs-Hensleit saline and 12 µM $d$-tubocurarine to produce complete neuromuscular blockade. The saline within the bath was aerated with gas (95% O$_2$-5% CO$_2$), pH was maintained at 7.4, and the osmolality of the bath was ~290 mosmol/kgH$_2$O.

*Determination of the optimal length-tension relationship.* After a 15-min equilibration period (25°C), in vitro diaphragmatic contractile measurements were made. The muscle strip was stimulated along its entire length with platinum wire electrodes (modified S48 stimulator, Grass Instruments)

by using supramaximal (~150%) stimulation voltage to determine the optimum contractile length ($L_o$). $L_o$ was determined by systematically adjusting the length of the muscle by using a micrometer while evoking single twitches. Thereafter, all contractile properties were measured isometrically at $L_o$.

*Peak twitch and tetanic tension.* After $L_o$ was found, the bath temperature was increased to 37°C, and, after a 30-min equilibration period, peak twitch tension was determined by using supramaximal monophasic pulses of 2 ms; peak isometric twitch tension was then determined from a series of single pulses. Maximal isometric tetanic contractions (P$_o$) were then produced by using a supramaximal stimulus train of 500-ms duration (160 Hz). Force was monitored by the computerized system described in *Description of the muscle organ bath.* Peak isometric tetanic tension was measured from a series of three contractions with a 3-min recovery between measurements.

*Force-frequency response.* To measure the force frequency response each strip was stimulated supramaximally with 120-V pulses at 15–160 Hz. The duration of each train was 500 ms to achieve a force plateau. Contractions were separated by a 3-min recovery period.

*Normalization of diaphragm force production to muscle cross sectional area.* For comparative purposes, diaphragmatic (bundles of fibers) force production was normalized as specific P$_o$. The total muscle cross-sectional area at right angles to the long axis was calculated by the following algorithm (22)

Total muscle cross-sectional area (mm$^2$)

$$= [\text{muscle mass}/(\text{fiber length} \times 1.056)]$$

where 1.056 is the density of muscle (in g/cm$^3$). Fiber length was expressed in centimeters measured at $L_o$.

### Postmortem Examination

To determine whether our animals developed an infection during our experiments three separate blood samples were removed, smeared on microscope slides, and stained by using a bacteriological stain (Gram's stain, Sigma Chemical, St. Louis, MO); each sample was then microscopically examined for the presence of bacteria. Furthermore, lungs were visually examined for abnormalities. If macroscopic abnormalities of the lung were observed during necropsy, these animals were eliminated from the study.

### Statistical Analysis

Comparisons between groups for each dependent variable measured were made by a two-way ANOVA. Where significant differences existed, a Scheffé's test was used post hoc. Furthermore, the relationship between the duration of MV and diaphragmatic specific P$_o$ was examined by using a Pearson product-moment correlation. Significance was established at $P < 0.05$.

### RESULTS

### Systemic and Biological Response to MV

Two animals that completed the prescribed period of MV were eliminated from our analysis because postmortem analysis revealed lung barotrauma. No animals were eliminated because of infection. Of the animals included in our analysis, initial and final body weights did not differ ($P > 0.05$) between the experi-

**Table 1.** *Changes in arterial P$_{O_2}$, P$_{CO_2}$, and pH at selected time periods during mechanical ventilation*

| Parameter | 1 h of MV ($n = 17$) | 12 h of MV ($n = 17$) | 24 h of MV ($n = 7$) |
|---|---|---|---|
| Pa$_{O_2}$, Torr | 94±4 | 89±4 | 83±3 |
| Pa$_{CO_2}$, Torr | 37±2 | 38±2 | 36±1 |
| pH$_a$ | 7.44±0.04 | 7.42±0.04 | 7.44±0.05 |
| Systolic arterial blood pressure, mmHg | 110±6 | 103±5 | 98±4 |

Values are means ± SD; *n*, total no. of animals that were exposed to mechanical ventilation (MV) for each time period. Pa$_{O_2}$, arterial P$_{O_2}$; Pa$_{CO_2}$, arterial P$_{CO_2}$; pH$_a$, arterial pH.

mental groups (i.e., control vs. MV animals). Furthermore, no differences ($P > 0.05$) existed in the weight of the soleus muscles between control animals and any of the MV groups (data not shown). Importantly, 12–24 h of MV did not result in a significant change ($P > 0.05$) in animal body weights (data not shown). These results indicate that our program of enteral nutrition and rehydration was appropriate. Furthermore, it is noteworthy that animals in the MV groups urinated and experienced intestinal transit throughout the experimental period.

To determine whether our MV protocol was successful in maintaining homeostasis, we measured arterial blood pressures, pH, and the partial pressures of both $CO_2$ and $O_2$ in all animals at various time intervals during MV. Although small variations in arterial blood pressure, blood gases, and pH existed over time during MV, our data reveal that adequate arterial blood pressure and blood-gas/pH homeostasis were maintained during MV (Table 1). In contrast, our measurements of arterial blood gas tensions and pH in the spontaneously breathing animals (18 h) revealed that these animals experienced hypoxemia, hypercapnia, and mild acidosis during the experimental period (Table 2). It seems likely that these disturbances in blood-gas homeostasis during the 18 h of anesthesia were due to hypoventilation resulting from depressed ventilatory drive.

Because sepsis is associated with diaphragmatic contractile dysfunction, strict aseptic techniques were followed throughout the experiments. In this regard, our data indicate that the animals included in our analysis did not develop infections during MV. This statement is supported by the observation that microscopic examination of blood revealed no detectable bacteria and that postmortem examination of the lungs (visual) and peritoneal cavity (visual) yielded no detectable abnormalities. Furthermore, our MV animals were afebrile during the investigation, with body temperatures

ranging from 36.5 to 37.6°C. Finally, during the course of MV, no significant ($P > 0.05$) changes occurred in the body weights of our MV animals. Collectively, these results indicate that the MV animals used in our analysis were free of significant infection.

### MV and Diaphragmatic Contractile Properties

$L_o$ ranged from 1.7 to 2.1 cm across all diaphragm strips investigated. No differences existed in $L_o$ between the control group and any of the MV groups.

All durations of MV resulted in a significant ($P < 0.05$) reduction in diaphragmatic maximal twitch force production (Table 3). Furthermore, this MV-induced decrease in twitch tension was exacerbated with time (Table 2).

Figure 2 illustrates the diaphragmatic force-frequency response for control animals and the two groups of anesthetized and spontaneously breathing animals. No differences existed between control animals and the two groups of spontaneously breathing animals.

Compared with control, prolonged MV (all durations) was associated with a right shift in the diaphragmatic force-frequency curve (Fig. 3). Indeed, prolonged MV resulted in a reduction ($P < 0.05$) in diaphragmatic specific force production at both submaximal and maximal stimulation frequencies. Also, note that the magnitude of the right shift in the diaphragmatic force-frequency curve was exacerbated with time on the ventilator (Fig. 3). The relationship between the duration of MV and the decline in diaphragmatic specific P$_o$ is illustrated in Fig. 4. Note, that compared with control, as little as 12 h of MV resulted in a decrease in diaphragmatic specific P$_o$ in all animals. Furthermore, this MV-induced diaphragmatic contractile dysfunction is increased during an additional 12 h of MV. For example, 12 h of MV resulted in an 18% reduction ($P < 0.05$) in the mean diaphragmatic specific P$_o$, whereas 24 h of MV resulted in a 46% reduction ($P < 0.05$) in the mean diaphragmatic specific P$_o$.

### DISCUSSION

### Overview of Principal Findings

Although previous experiments have reported that prolonged MV results in diaphragmatic dysfunction, the present experiments provide important new information regarding the influence of pentobarbital sodium on the diaphragm and the time course of MV-induced diaphragmatic contractile dysfunction. Indeed, this is the first study to demonstrate that the diaphragmatic contractile dysfunction associated with

**Table 2.** *Changes in arterial P$_{O_2}$, P$_{CO_2}$, and pH at selected time periods during 18 h of spontaneous breathing*

| Parameter | 1 h of Spontaneous Breathing | 12 h of Spontaneous Breathing | 18 h of Spontaneous Breathing |
|---|---|---|---|
| Pa$_{O_2}$, Torr | 56±4 | 63±3 | 61±2 |
| Pa$_{CO_2}$, Torr | 50±4 | 52±2 | 48±3 |
| pH$_a$ | 7.36±0.02 | 7.36±0.02 | 7.37±0.02 |

Values are means ± SD; $n = 4$ animals for each time period.

Table 3. *Maximal isometric twitch force production in diaphragm strips obtained from mechanically ventilated and nonmechanically ventilated (control) animals*

| Experimental group | Maximal Isometric Twitch Force, N/cm$^2$ |
|---|---|
| Control | 6.6±0.4 |
| 18 h of spontaneous breathing | 6.4±0.5 |
| 24 h of spontaneous breathing | 6.9±0.7 |
| 12 h of MV | 4.3±0.3† |
| 18 h of MV | 3.4±0.1* |
| 24 h of MV | 3.8±0.4* |

Values are means ± SE. *Different from control and both spontaneous breathing groups, $P < 0.05$. †Different from all other experimental groups; $P < 0.05$.

prolonged MV in anesthetized animals is not due to a direct effect of pentobarbital sodium on the diaphragm. Furthermore, our results indicate that as few as 12 h of controlled MV result in decreased submaximal diaphragmatic specific force production and $P_o$. Finally, the magnitude of diaphragmatic contractile dysfunction increased progressively with time on the ventilator. Therefore, these data support the hypothesis that the onset of MV-induced diaphragm dysfunction is rapid and that this contractile impairment is exacerbated with increased time on the ventilator.

### Prolonged MV and Diaphragmatic Contractile Dysfunction

The present experiments support previous reports (1, 15) that prolonged MV results in a significant reduction in both submaximal diaphragmatic specific force production and specific $P_o$. Importantly, the MV-induced decrease in diaphragmatic specific $P_o$ was time dependent, with specific $P_o$ being ~18 and ~46% lower in animals ventilated for 12 and 24 h, respectively (Fig. 3).

Fig. 3. Effects of prolonged MV on the diaphragmatic force-frequency response (in vitro) in rats. Values are means ± SE. Compared with control, MV (all durations) resulted in a significant (*$P < 0.05$) reduction in diaphragmatic specific force production at all stimulation frequencies.

Although these experiments were not designed to determine the mechanism(s) responsible for the MV-induced decrease in diaphragmatic specific force production, a discussion of this issue is warranted. There are at least four possible explanations for the diaphragmatic contractile dysfunction associated with prolonged MV: *1)* negative impact of anesthesia on muscle function, *2)* infection, *3)* arterial pH imbalance, and/or *4)* intrinsic alterations in diaphragm muscle fibers. We postulate that the MV-induced diaphragmatic contractile dysfunction was not due to anesthesia, infection, or a pH/blood-gas disturbance. Arguments to support this position follow.

High levels of barbiturates have been reported to influence mammalian muscle contractile properties (25). Nonetheless, in the present experiments, the level of pentobarbital sodium required to maintain a surgical plane of anesthesia did not impair diaphrag-
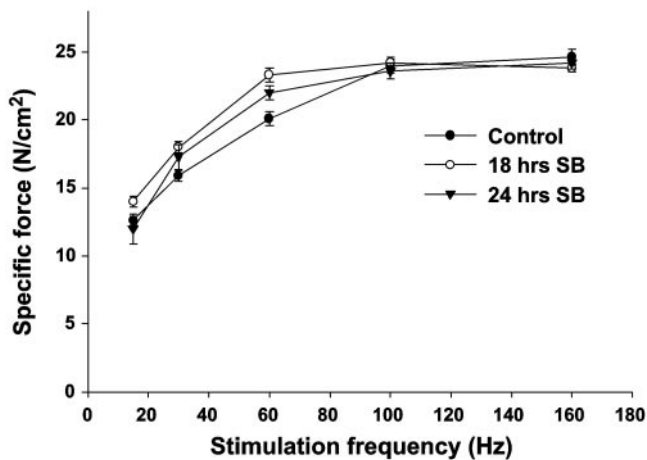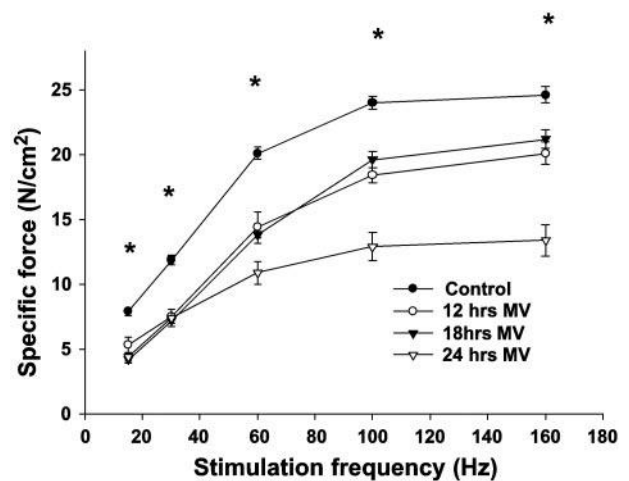
Fig. 2. Comparison of diaphragmatic force-frequency relationship (in vitro) between control animals and the 2 groups (i.e., 18 and 24 h) of anesthetized and spontaneously breathing (SB) animals. Values are means ± SE. No significant differences ($P > 0.05$) existed between control and either of the spontaneous breathing groups at any stimulation frequency.
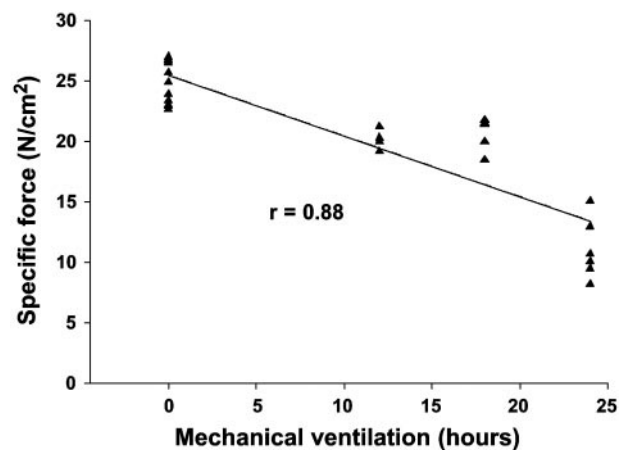
Fig. 4. Relationship between 12–24 h of MV and maximal diaphragmatic maximal specific force generation. Each point represents data from 1 animal. Note that individual data points sometimes overlap, and thus some symbols are hidden from view.

matic function in the two groups of spontaneously breathing animals. Hence, these data indicate that the level of pentobarbital sodium anesthesia used in our experiments does not contribute to the diaphragmatic contractile dysfunction associated with prolonged MV.

Although sepsis is associated with impairment of diaphragmatic force production (2), our data indicate that our animals did not develop infections during MV. Therefore, these results indicate that sepsis was not responsible for the diaphragmatic force deficit associated with MV.

It is well known that arterial acidosis decreases muscle force production and increases fatigue in the diaphragm (4, 8). Nonetheless, our measurements of arterial pH indicate that acid-base status was well maintained during 24 h of MV (Table 1). Hence, we do not believe that the diaphragmatic contractile dysfunction associated with prolonged MV is linked to systemic acidosis. Our finding that diaphragmatic contractile properties were not impaired in our spontaneously breathing animals that experienced 18–24 h systemic acidosis supports this notion.

By elimination, we conclude that the MV-induced contractile dysfunction is due to intrinsic changes within diaphragmatic muscle fibers. Theoretically, the MV-induced diaphragmatic force deficit could stem from one or several different mechanisms. Specifically, a reduction in myofibrillar protein concentration, abnormalities of contractile or cytoskeletal proteins, and/or impaired excitation-contraction (E-C) coupling could individually or collectively contribute to the observed force deficit (5, 10). If individual myosin cross bridges produce the same force when activated, the force produced by a muscle is proportional to the number of parallel cross bridges in the strong binding state (5). It follows that a reduction in the number of cross bridges (i.e., myofibrillar protein) per cross-sectional area of muscle would result in a reduced specific force production. Therefore, it is possible that a reduction in myofibrillar protein concentration is a potential contributory factor to the MV-induced diaphragmatic specific force deficit.

A second potential mechanism that may contribute to the MV-induced diaphragmatic force deficit is an abnormality in either contractile or cytoskeletal proteins. That is, intrinsic alterations in the structure and function of key contractile or cytoskeletal proteins could impair diaphragmatic force generation. Although preliminary experiments indicate that diaphragmatic contractions do not occur during prolonged controlled MV, we cannot eliminate the possibility that an occasional, nonsynchronized inspiratory effort occurred during our experiments. This type of diaphragmatic contraction could result in muscle injury and therefore reduce diaphragmatic force production. Also, it is possible that prolonged MV is associated with elevated diaphragmatic proteolysis and increased oxidative damage to muscle proteins. Each of these factors can promote intrinsic alterations in muscle proteins and could impair force production (9, 12, 19–21).

A final potential mechanism to explain the MV-induced diaphragmatic force deficit is impairment in E-C coupling. For example, a reduction in calcium release from the sarcoplasmic reticulum is associated with reduced muscle force generation (21). Furthermore, a reduction in myofilament sensitivity to calcium is also associated with lowered force production (21). Hence, any factor that reduces the magnitude of calcium release from the sarcoplasmic reticulum and/or calcium sensitivity would negatively impact muscle force production. The data of the present study do not provide evidence for or against the possibility that E-C coupling in the diaphragm was impaired by MV. Nonetheless, our experiments reveal that the onset of MV-induced diaphragmatic contractile dysfunction occurs rapidly and is progressive during the first 24 h of MV. These observations indicate that the pathways responsible for this contractile dysfunction are activated quickly after the induction of MV. In this regard, it seems possible that the activation of oxidant production and/or proteolytic pathways in the diaphragm during prolonged MV are candidate mechanisms that could negatively impact E-C coupling and muscle force production (7, 19, 21).

In summary, we postulate that MV-induced diaphragmatic contractile dysfunction is due to intrinsic changes within the diaphragmatic muscle fibers. In theory, the MV-induced diaphragmatic force deficit could result from a reduction in myofibrillar protein concentration, abnormalities of contractile or cytoskeletal proteins, and/or impairment in calcium handling (i.e., E-C coupling) in the diaphragm. Which of these specific mechanisms is responsible for the observed force deficit is unknown and is an important area for future research.

### Critique of Experimental Model

Because of the invasive nature of obtaining a biopsy from the diaphragm in human patients, animal models must be used to study the effects of MV on respiratory muscle biochemistry and in vitro contractile properties. Considerations in the choice of an animal model include both practical considerations (i.e., size of the animal and cost) and the applicability of the animal model to humans. In this regard, the rat is a relatively inexpensive model to study the effects of MV. Most importantly, human and rat diaphragms are similar in fiber-type composition, gross anatomic features, and function (16, 18). Therefore, we chose the rat model to study the effects of prolonged MV on diaphragmatic function.

Experiments that use animal models are obligated to use only the number of animals required to answer the question addressed in a particular experiment; this was the approach used in the present experiments. Our results reveal that only a small sample size in each of our experimental groups was required to observe a statistically significant difference in diaphragmatic

force production between control animals and MV animals. This is a reflection of the large between-group mean differences (i.e., control vs. MV) in muscle force production and the relatively low variability in the data. Importantly, the application of parametric statistics with a specified alpha level (i.e., $P < 0.05$) to a given data set ensures that the chances of making a type I error is the same in all experiments independent of the number of observations. Hence, the results of the present experiments can be viewed with the same level of confidence as any other experiments using an alpha of $P < 0.05$.

It is noteworthy that most adult patients receiving MV in intensive care units are maintained on some form of pressure-assist MV. Nonetheless, to investigate the effects of prolonged MV on diaphragmatic contractile function, we chose to use controlled MV for two primary reasons. First, compared with assist modes of MV, an advantage of controlled MV is that this mode is postulated to result in rapid diaphragmatic atrophy (26). This is experimentally advantageous because it permits the investigation of the adverse effects of MV on the diaphragm after relatively short periods of MV. Second, controlled MV has clinical relevance because this mode of ventilation is used in adult patients in several circumstances, including drug overdose, spinal cord injury, and surgery. Furthermore, controlled MV is commonly used in numerous pediatric situations (11).

We chose pentobarbital sodium as the general anesthetic in these experiments because of evidence that this anesthetic, when used in low doses, does not negatively impact locomotor and diaphragm contractile function. For example, work by Le Bourdelles et al. (15) reveals that the level of pentobarbital sodium required to maintain a surgical plane of anesthesia in rats over a 48-h period does not promote locomotor muscle atrophy, nor does it impair locomotor muscle maximal tetanic force production. Importantly, the present experiments with anesthetized, spontaneously breathing animals clearly indicate that the level of pentobarbital sodium required to maintain a surgical plane of anesthesia does not impair diaphragm contractile function.

### Summary and Conclusions

These novel experiments examined the time course of MV-induced contractile dysfunction in the rat diaphragm. Our data indicate that as few as 12 h of MV result in decreased diaphragmatic specific force production and that the magnitude of this force deficit increases as a function of time on the ventilator. The mechanism(s) responsible for this MV-induced contractile dysfunction is unclear. Nonetheless, we postulate that the observed MV-induced diaphragmatic contractile dysfunction is linked to intrinsic changes within diaphragm muscle fibers. Future experiments designed to ascertain those factors that trigger these injurious processes in the diaphragm will be required to develop a clinical intervention to prevent diaphragmatic injury during prolonged MV.

## REFERENCES

1. **Anzueto A, Peters J, Tobin M, de los Santos R, Seidenfeld J, Moore G, Cox W, and Coalson J.** Effects of prolonged controlled mechanical ventilation on diaphragmatic function in healthy baboons. *Crit Care Med* 25: 1187–1190, 1997.
2. **Boczkowski J, Dureuil B, Branger C, Pavlovic D, Murciano D, Pariente R, and Aubier M.** Effects of sepsis on diaphragmatic function in rats. *Am Rev Respir Dis* 138: 260–265, 1988.
3. **Chevrolet J.** Difficult weaning from mechanical ventilation. *Lung, Suppl* 168: 829–832, 1990.
4. **Coast J, Shanely R, Lawler J, and Herb R.** Lactic acidosis and diaphragmatic function in vitro. *Am J Respir Crit Care Med* 152: 1648–1652, 1995.
5. **Criswell D, Powers S, Herb R, and Dodd S.** Mechanism of specific force deficit in the senesent rat diaphragm. *Respir Physiol* 107: 149–155, 1997.
6. **Dodd S, Powers S, Vrabas I, Criswell D, Stetson S, and Hussain R.** Effects of clenbuterol on contractile and biochemical properties of skeletal muscle. *Med Sci Sports Exerc* 28: 669–676, 1996.
7. **Eley D, Eley J, Korecky B, and Fliss H.** Impairment of cardiac contractility and sarcoplasmic reticulum calcium ATPase activity by hypochlorous acid: reversal by dithioreitol. *Can J Physiol Pharmacol* 69: 1677–1685, 1991.
8. **Esau S.** Hypoxic, hypercapnic acidosis decreases tension and increases fatigue in hamster diaphragm muscle in vitro. *Am J Respir Crit Care Med* 139: 1410–1417, 1989.
9. **Haycock JW, Falkous G, and Mantle D.** Differential susceptibility of skeletal muscle proteins to free radical-induced oxidative damage in vitro. In: *Oxidative Stress in Skeletal Muscle*, edited by Reznick A. Basel: Birkhauser, 1998, p. 157–176.
10. **Herb R, Powers S, Criswell D, Caiozzo V, Vrabas I, and Dodd S.** Alterations in phenotypic and contractile properties of the rat diaphragm: influence of hypothyroidism. *J Appl Physiol* 80: 2163–2170, 1996.
11. **Hess D and Kacmarek R.** *Essentials of Mechanical Ventilation.* New York: McGraw-Hill, 1996.
12. **Horowitts R, Kempner E, Bisher M, and Podolsky R.** A physiological role for titin and nebulin in skeletal muscle. *Nature* 323: 160–164, 1986.
13. **Jabour E, Rabil D, Truwit J, and Rochester D.** Evaluation of a new weaning index based on ventilatory endurance and efficiency of gas exchange. *Am Rev Respir Dis* 144: 531–537, 1991.
14. **Kelly S, Rosa A, Field S, Coughlin M, Shizgal H, and Macklem P.** Inspiratory muscle strength and body composition in patients receiving total parenteral nutrition therapy. *Am Rev Respir Dis* 130: 33–37, 1984.
15. **Le Bourdelles G, Viires N, Boczkowski J, Seta N, Pavlovic D, and Aubier M.** Effects of mechanical ventilation on diaphragmatic contractile properties in rats. *Am J Respir Crit Care Med* 149: 1539–1544, 1994.
16. **Mizuno M.** Human respiratory muscles: fibre morphology and capillary supply. *Eur Respir J* 4: 587–601, 1991.
17. **Powers S, Criswell S, Herb R, Demirel H, and Dodd S.** Age-related changes in diaphragmatic maximal shortening velocity. *J Appl Physiol* 80: 445–451, 1996.
18. **Powers S, Demirel H, Coombes J, Fletcher L, Calliaud C, Vrabas I, and Prezant D.** Myosin phenotype and bioenergetic characteristics of rat respiratory muscle. *Med Sci Sports Exerc* 29: 1573–1579, 1997.
19. **Powers S, Lawler L, and Vincent HH.** The diaphragm and oxidative stress. In: *Oxidative Stress in Skeletal Muscle*, edited by Reznick A. Basel: Birkhauser, 1998, p. 223–238.

20. **Reid M.** Reactive oxygen species and nitric oxide in skeletal muscle. *News Physiol Sci* 11: 114–119, 1996.
21. **Reid M.** Muscle fatigue: mechanisms and regulation. In: *Handbook of Oxidants and Antioxidants in Exercise*, edited by Sen C, Packer L, and Hanninen O. Amsterdam: Elsevier, 2000, p. 599–630.
22. **Segal S, White T, and Faulkner J.** Architecture, composition, and contractile properties of rat soleus and muscle grafts. *Am J Physiol Cell Physiol* 250: C474–C479, 1986.
23. **Sporn P and Morganroth M.** Discontinuation of mechanical ventilation. *Clin Chest Med* 9: 113–126, 1988.
24. **Tahvanainen J, Salmenpera M, and Nikki P.** Extubation criteria after weaning from intermittent mandatory ventilation and continuous positive airway pressure. *Crit Care Med* 11: 702–707, 1983.
25. **Taylor R, Abresch R, Lieberman J, Fowler W, and Portwood M.** Effects of pentobarbital on contractility of mouse skeletal muscle. *Exp Neurol* 83: 254–263, 1984.
26. **Tobin M, Juban A, and Hines E.** Pathology of failure to wean from mechanical ventilation. *Schweiz Med Wochenschr* 124: 2139–2149, 1994.