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Mechanical ventilation reduces rat diaphragm blood flow and impairs O₂ delivery and uptake

Robert T. Davis 3rd, MS¹, Christian S. Bruells, M.D.², John N. Stabley, M.S.¹, Danielle J. McCullough, M.S.¹, Scott K. Powers, Ph.D. and Ed.D.¹, and Bradley J. Behnke, Ph.D.¹

¹Department of Applied Physiology & Kinesiology, Center for Exercise Science, University of Florida, Gainesville, FL. 32611

²Department of Anesthesiology, Aachen University Hospital of the Rhenish-Westphalian Technical University, Aachen, Germany

Abstract

Objectives—Although mechanical ventilation (MV) is a life-saving intervention in patients suffering from respiratory failure, prolonged MV is often associated with numerous complications including problematic weaning. In contracting skeletal muscle, inadequate O₂ supply can limit oxidative phosphorylation resulting in muscular fatigue. However, whether prolonged MV results in decreased diaphragmatic blood and induces an O₂ supply-demand imbalance in the diaphragm remains unknown.

Design—We tested the hypothesis that prolonged controlled MV results in a time-dependent reduction in rat diaphragmatic blood flow and microvascular PO₂ and that prolonged MV would diminish the diaphragm's ability to increase blood flow in response to muscular contractions.

Measurements and Main Results—Compared to 30 min of MV, 6 hrs of MV resulted in a 75% reduction in diaphragm blood flow (via radiolabeled microspheres), which did not occur in the intercostal muscle or high-oxidative hindlimb muscle (e.g., soleus). There was also a time-dependent decline in diaphragm microvascular PO₂ (via phosphorescence quenching). Further, when contrasted to 30 min of MV, 6 hrs of MV significantly compromised the diaphragm's ability to increase blood flow during electrically-induced contractions which resulted in a ~80% reduction in diaphragm O₂ uptake. In contrast, 6 hrs of spontaneous breathing in anesthetized animals did not alter diaphragm blood flow or the ability to augment flow during electrically-induced contractions.

Conclusions—These new and important findings reveal that prolonged MV results in a time-dependent decrease in the ability of the diaphragm to augment blood flow to match O₂ demand in response to contractile activity and could be a key contributing factor to difficult weaning. Although additional experiments are required to confirm, it is tempting to speculate that this ventilator-induced decline in diaphragmatic oxygenation could promote a hypoxia-induced

Address correspondence to: Bradley J. Behnke, Ph.D, PO Box 118205, Department of Applied Physiology & Kinesiology, University of Florida, Gainesville, FL. 32611, bjbehnke@hhp.ufl.edu, Phone: (352) 392-0584 ext. 1324.

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generation of reactive oxygen species in diaphragm muscle fibers and contribute to ventilator-induced diaphragmatic atrophy and contractile dysfunction.

Keywords

Mechanical ventilation; diaphragm; oxygen delivery; microvascular PO₂; oxygen uptake; contractions; weaning

Introduction

Mechanical ventilation (MV) is a life-saving intervention frequently used in critical care medicine to maintain alveolar ventilation in patients incapable of doing so independently (e.g., respiratory failure, spinal cord injury, and drug overdoses). However, prolonged MV results in the rapid development of diaphragmatic atrophy and contractile dysfunction which is collectively known as ventilator-induced diaphragmatic dysfunction (VIDD) (1). Importantly, it is predicted that VIDD is a contributor to the inability to wean patients from the ventilator (2). Indeed, the failure to wean patients from MV is a major clinical problem as ~30% of patients experience weaning difficulties (3), resulting in additional time on the ventilator and increased patient mortality (2, 3).

At present, the precise mechanisms responsible for the rapid development of VIDD remain an enigma (4). However, it is clear that diaphragmatic production of reactive oxygen species (ROS) is a prerequisite for the diaphragmatic atrophy and contractile dysfunction that occurs during prolonged MV (5–7). Although the cause of the MV-induced ROS generation is unclear, it is possible that a reduced O₂ supply to the diaphragm during prolonged MV could result in tissue hypoxia and, subsequently, increase mitochondrial ROS production (8).

To sustain diaphragmatic contractile function there must be a coordinated balance in the rates of O₂ delivery ($\dot{Q}O_2$) to those of O₂ utilization ($\dot{V}O_2$) (9, 10), as an imbalance in oxygen supply-demand will promote muscle fatigue (reviewed in (11)). At the onset of MV, a reduction in diaphragm blood flow occurs (12) due to the reduced recruitment (and thus metabolic load) of the respiratory muscles. In critically ill patients a reduced diaphragm perfusion elicited by MV allows for an effective redistribution of cardiac output to sustain perfusion of vital organs (13, 14). However, it is unknown if prolonged MV results in a decrease in diaphragm blood flow or has a negative impact on the diaphragm's ability to increase O₂ delivery during muscular contractions (e.g., during weaning). Therefore, we investigated the temporal pattern of changes in diaphragm O₂ delivery (i.e., blood flow), microvascular PO₂ (which allows the quantification of the $\dot{Q}O_2$ -to- $\dot{V}O_2$ relationship (15, 16)) and $\dot{V}O_2$ during prolonged MV. Using a well-established animal model of MV, we tested the hypotheses that prolonged MV promotes: 1) a progressive decline in diaphragm blood flow and O₂ supply; 2) a diminished microvascular PO₂; and 3) a compromised ability to augment diaphragm blood flow during electrically-induced contractions *in vivo* (to induce a metabolic load on the diaphragm).

Our results reveal that MV induces a time-dependent functional loss in the ability to increase blood flow to the diaphragm during an elevated metabolic demand, which was associated with a significantly reduced $\dot{V}O_2$ during contractions following 6 hrs of MV. Clearly, an imbalance in oxygen delivery-demand to the diaphragm during contractions would force reliance on anaerobic energy sources with finite reserves to sustain contractions and hasten the onset of diaphragmatic fatigue. These findings in the rodent diaphragm with MV and the weaning difficulties observed in many human patients are consistent with the concept that a reduced O₂ supply may contribute difficult weaning.

Methods

All procedures performed herein were approved by the University of Florida Institutional Animal Care and Use Committee and conform to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and the American Veterinary Medical Association Guidelines on Euthanasia.

Animals

Forty-one young female Sprague-Dawley (4 mo. old, ~250 g) were obtained from Charles River Laboratories (Boston, MA, USA) and used for this investigation. All rats were housed in a temperature-controlled ($23 \pm 2^\circ\text{C}$) room with a 12:12-h light-dark cycle. Water and rat chow were provided *ad libitum*.

Experimental Design

To investigate the effects of the duration of MV on diaphragm blood flow, microvascular oxygenation (PO_2m), and O_2 uptake ($\dot{V}\text{O}_2$) at rest and during electrically-induced contractions we performed four separate experiments. All animals received the same surgeries to prevent variability between experiments. See “Online data supplement” and the next segment for detailed methodology.

Experiment 1—This experiment was performed to measure diaphragm blood flow during spontaneous breathing (i.e., blood flow measured at 30 min of spontaneous breathing under anesthesia) and during acute and prolonged controlled mechanical ventilation (MV) (i.e., blood flow measured at 30 min and 6 hrs of MV, respectively) using the radioactive microsphere technique (see below). As blood flow could be measured during two time points in each animal, blood flow was determined: 1) after 30 min of spontaneous breathing and after 30 min of MV (Group A; $n=7$), and 2) after 30 min and 6 hrs of MV (Group B, $n=8$). Given the high daily duty cycle (i.e., ratio of active to inactive times) and oxidative capacity of the diaphragm we wanted to measure blood flow in skeletal muscle(s) that demonstrate similar properties. Therefore, blood flow was also measured in the soleus muscle (i.e., highest daily duty cycle of the hindlimb locomotory muscles (17)) and the red portion of the gastrocnemius muscle (Gast_{Red} ; similar oxidative capacity and fiber type profile as the diaphragm (18)). Further, arterial O_2 concentration (CaO_2) and mean arterial pressure (MAP) were measured to calculate O_2 delivery (i.e., $\text{CaO}_2 \times \text{blood flow}$) and vascular conductance (i.e., $\text{blood flow}/\text{MAP}$), respectively.

Experiment 2—This experiment was performed to determine if microvascular PO_2 was reduced from the spontaneous breathing condition to 30 min of MV, and if there was a further reduction after 6 hrs of MV. We used phosphorescence quenching techniques *in vivo* (see below) to quantify changes in diaphragm PO_2m over time in 11 animals. The PO_2m values were then time aligned with the blood flow values and diaphragm $\dot{V}\text{O}_2$ was calculated (19, 20) for the three time points.

Experiment 3—To test the hypothesis that there will be an attenuated blood flow response with electrically-induced contractions in the diaphragm after 6 hrs versus 30 min of MV resulting in a reduced contracting $\dot{V}\text{O}_2$, we measured blood flow and PO_2m and calculated $\dot{V}\text{O}_2$ in the contracting diaphragm (see below) after 30 min and 6 hrs of MV in 5 animals.

Experiment 4—To determine whether 6 hrs of anesthesia, *per se*, versus MV affected diaphragm perfusion, blood flow was measured after 6 hrs of spontaneous breathing at rest and during electrically-induced contractions ($n=5$). These values were then compared to those temporally aligned from animals subjected to 6 hrs of MV.

Mechanical Ventilation

All surgical procedures were performed using aseptic techniques. Animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), tracheostomized and connected to a volume-cycled ventilator (Harvard Apparatus; Cambridge, MA). A catheter was implanted in the carotid artery for measurement of MAP and periodic blood sampling (every 3 hrs) for analyses of blood gases (GemPremier 3000, Instrumentation Laboratory, Bedford, MA). Arterial O₂ saturation was monitored continuously using a Mouse OX (Asbury Park, PA) placed around the left foot. Expired PCO₂ was measured continuously using a microcapnograph (Model Columbia Instruments, Columbus, Ohio). Arterial PO₂, PCO₂, and pH were maintained within the normal range (80 to 110 torr, 30 to 40 torr, and 7.35 to 7.45, respectively) by minor adjustments to minute volume. A catheter was placed in the jugular vein for infusion of sodium pentobarbital (10 mg/kg/h) and fluids, when necessary. Body temperature was maintained at 37 ± 1°C (via rectal thermometer) by use of a recirculating heating blanket. Body fluid homeostasis was maintained by administering electrolyte solution (i.v., 2 ml/kg/hr). Operative care during the MV period included expressing the bladder, removal of airway mucus, lubricating the eyes and rotating the animal. All operative procedures were performed using aseptic techniques. The ventilator was set to an average breathing frequency of 70 ± 10 breaths/min and a tidal volume of 2.2 ± 0.5 ml/breath with a positive end-expiratory pressure set at 0 cm H₂O.

Measurement of Blood Flow

Blood flow to respiratory and selected muscles of the hindlimb, as well as the kidneys, was determined using radiolabelled microspheres as described previously (21, 22). Blood withdrawal from the caudal artery was initiated at 0.25 ml/min and 2.5 × 10⁵ microspheres were then infused into the carotid artery catheter. Upon completion of each experiment, the diaphragm, intercostal muscles (from the 5th interspace), soleus, red portion of the gastrocnemius muscle and kidneys were removed. Tissue radioactivity was measured and individual tissue flows, including the separate regions of the diaphragm (i.e., medial costal, ventral, dorsal and crural diaphragm), were determined by the reference sample method (23) and expressed in milliliters per minute per 100 grams of tissue. To normalize for any change in arterial blood pressure vascular conductance was calculated and expressed as milliliters per minute per 100 grams of tissue per mmHg. For more detailed methods see “Online data supplement”.

Phosphorescence Quenching

Phosphorescence quenching was used to measure diaphragm muscle PO_{2m} during spontaneous breathing and after 30 min and 6 hrs of MV according to the methods of Poole and colleagues (9, 15, 24). Detailed methodological information on this technique, measurements and calculations are listed in the “Online data supplement”. The phosphorimeter light guide was positioned 2–5 mm from the surface of the medial costal diaphragm and the phosphor, palladium meso-tetra-(4-carboxyphenyl)-porphyrin dendrimer (R2; Oxygen Enterprises Ltd., Philadelphia, PA), was infused at a dose of 15 mg/kg and PO_{2m} measurements were recorded every 2 s for 60 s to provide an average steady-state PO_{2m} during spontaneous breathing and after 30 min and 6 hrs of MV. R2 is tightly bound to albumin in the plasma and is negatively charged and has been demonstrated to remain compartmentalized to the vascular space in skeletal muscle (25). In a separate group of rats (n=5) diaphragm PO_{2m} was assessed after 6 hrs of MV with the R2 probe infused immediately before PO_{2m} was measured to compare these values to those in animals that have had the R2 circulating for 6 hrs. No differences were found between groups confirming compartmentalization of the R2 probe to the vascular space as previously demonstrated by Poole et al. (25). Methods and data for this additional group of rats can be found in the “Online data supplement”.

Calculation of Diaphragm $\dot{V}O_2$

Muscle oxygen uptake ($\dot{V}O_2$) of the medial costal diaphragm (i.e., region of PO_2m measures) was calculated from blood flow and PO_2m measurements in the diaphragm during the resting and contracting (see below) conditions according to the methods of Behnke et al. (19). Both data sets were obtained under identical conditions. $\dot{V}O_2$ was calculated using the Fick equation using PO_2m as an analogue for venous PO_2 (26, 27) and, from the O_2 dissociation curve, microvascular blood O_2 content (CmO_2) (28). Muscle $\dot{V}O_2$ was expressed as ml/min/per 100 g tissue. See “Online data supplement” for further information.

Diaphragm Contractions

The diaphragm was exposed as described above and stainless steel electrodes were sutured to the right ventral costal (cathode) and the right dorsal costal (anode) diaphragm according to the methods of Geer et al. (24). Electrically stimulated twitch contractions were induced at 1 Hz (3–6 V, 2-ms pulse duration) with a Grass S88 stimulator (Quincy, MA) for 3 min during mechanical ventilation. The stimulation protocol was initiated after 30 min and 6 hrs of MV and PO_2m was measured at 2-s intervals across the rest-to-contractions transition. Blood flow was measured at 180 s of stimulation to assess the contracting steady-state muscle hyperemia. $\dot{V}O_2$ was then calculated as described above for the steady-state contracting condition. For more information see “Online data supplement”.

Statistical analyses

Resting, steady-state contracting PO_2m , blood pressure, arterial blood gases, and pH across time were analyzed with repeated measures ANOVA. Blood flow, vascular conductance, O_2 delivery, and $\dot{V}O_2$ were analyzed with one-way ANOVA. Linear regression analysis was performed to compare MAP and arterial PO_2 to PO_2m , $\dot{V}O_2$ and the PO_2m dynamics. Individual significant differences were examined *post hoc* using Tukey’s test. Data are presented as means $\pm$ SE. Significance was accepted at $P < 0.05$. For more details about the statistical analyses see “Online data supplement”.

Results

Diaphragm blood flow and vascular conductance are diminished as a function of time with controlled mechanical ventilation

During spontaneous breathing, diaphragm blood flow averaged 36 ± 5 ml/min/100 g and did not change over the 6 hrs measurement period (Figure 1A). Compared to spontaneous breathing, blood flow was reduced by ~25% after 30 min of controlled MV to ~26 ml/min/100 g (Figure 1A). After 6 hrs of MV there was an additional ~75% reduction in diaphragm blood flow versus that measured at 30 min MV, resulting in an average flow of 7 ml/min/100 g (Figure 1A). Reductions in vascular conductance (i.e., blood flow $\div$ MAP) paralleled changes in blood flow with the duration MV (Figure 1B). Consistent with what others have found (29, 30) there was a marked heterogeneity in regional distribution of blood flow within the diaphragm, with all regions demonstrating a reduced blood flow (Figure 2) and vascular conductance (supplemental Figure 2) after 6 hrs of MV versus both spontaneous breathing and 30 min of MV. Blood flow and vascular conductance to the intercostal muscles, which would be subject to similar alterations in intrathoracic pressures as the diaphragm, did not change across the measurement period (Figure 3A & B). Unlike the diaphragm, blood flow and vascular conductance remained unchanged in the soleus and $Gast_{Red}$ muscles at every time point measured herein (i.e., up to 6 hrs; Figure 3A & B).

Mechanical ventilation reduces resting diaphragm microvascular PO₂ (PO_{2m}) and O₂ uptake (V̇O₂) in a time-dependent manner

Figure 4A shows representative resting diaphragm PO_{2m} responses from an individual animal during the measurement periods. During spontaneous breathing diaphragm PO_{2m} was ~53 torr and was significantly reduced to ~37 torr after 30 min of MV (Figure 4B). PO_{2m} decreased by an additional ~50% after 6 hrs of MV versus that after 30 min of MV to 18 ± 5 torr (Figure 4B).

Relative to spontaneous breathing, 30 minutes of MV significantly decreased diaphragm V̇O₂ (spontaneous breathing 1.44 ± 0.10; 30 min MV, 1.19 ± 0.08 ml/min/100 g; P<0.05). After 6 hrs of MV there was a trend (P=0.056) for a further reduction in diaphragm V̇O₂ (1.02 ± 0.06 ml/min/100 g) versus after 30 min of MV.

Mechanical ventilation reduces the ability to augment diaphragm blood flow, match O₂ delivery-to-utilization, and increase V̇O₂ during muscular contractions

To determine whether the intrinsic capacity to increase blood flow and V̇O₂ during a metabolic challenge in the diaphragm is compromised after 6 hrs of MV, we elicited twitch contractions and quantified the resultant blood flow, PO_{2m} and V̇O₂ responses in the diaphragm. During electrically-induced contractions there was a 2.8-fold increase in diaphragm blood flow from the resting value after 30 min of MV (Figure 5). However, after 6 hrs of MV the increase in blood flow with contractions was only 1.3-fold from the resting value (Figure 5). Six hrs of spontaneous breathing did not alter the hyperemic response to diaphragmatic contractions as compared to that measured after the onset of MV (Figure 5). The resultant change in diaphragm blood flow from rest-to-contractions (i.e., steady-state contracting value minus pre-contracting baseline value) was ~90% greater after 30 min of MV (40 ± 6 ml/min/100 g) versus 6 hrs of MV (4 ± 1 ml/min/100 g).

Average and representative diaphragm PO_{2m} profiles across the rest-to-contractions transition after 30 min and 6 hrs of MV are demonstrated in Figure 6A and 6B, respectively. Upon initiation of contractions, diaphragm PO_{2m} after 30 min of MV decreased by ~15.9 ± 3.0 torr from the pre-contracting value to a low PO_{2m} value of ~20.5 torr (Figure 6A). After 6 hrs of MV the decrease in PO_{2m} with contractions was significantly less (i.e., 8.1 ± 1.0 torr or roughly 55% of that observed after 30 min of MV) resulting in a low PO_{2m} value of 7.7 ± 1.1 torr. The time delay before PO_{2m} fell was shorter (i.e., 6 hrs MV, 7.8 ± 1.4 vs. 30 min MV, 10.8 ± 1.1 s, P<0.05) and the rate of decline faster (i.e., time-constant; 6 hrs MV, 6.8 ± 1.2 vs. 30 min MV, 11.3 ± 2.1 s, P<0.05) in the 6 hrs MV group versus that after 30 min of MV. The faster PO_{2m} dynamics suggest a slower relative increase in O₂ delivery than that of O₂ uptake (31) during contractions after 6 hrs versus 30 min of MV.

We also wanted to determine whether MV affected the ability to increase V̇O₂ during a metabolic challenge (e.g., diaphragm muscular contractions). After 30 min of MV, electrically-induced contractions elicited a marked increase in diaphragm V̇O₂ from ~1.4 at rest to ~7.0 ml/min/100 g during the contracting steady-state (Figure 7A). However, the change in V̇O₂ with contractions was significantly blunted after 6 hrs versus 30 min of MV from ~1.0 at rest to ~1.6 ml/min/100 g during contractions (Figure 7A), which was only ~22% of that achieved after 30 min of MV during contractions.

In addition, there was a reduced diaphragm O₂ delivery (i.e., arterial O₂ content blood flow; Figure 7B) and increased O₂ extraction (i.e., V̇O₂ ÷ O₂ delivery; Figure 7C) both at rest and during contractions after 6 hrs versus 30 min of MV.

Animals weight, hemodynamic data, arterial blood gases and pH

Body and diaphragm weight, mean arterial pressure (MAP), blood gases, pH, hemoglobin and hematocrit are summarized in Table 1 for spontaneous breathing and MV groups. There was a reduction in MAP after 6 hrs of MV, however, it remained above levels that affect PO_2m dynamics (32). Further, we did not observe any relation between MAP and PO_2m dynamics (i.e., mean response time (time delay + τ ; $r^2=0.007$, $P=0.72$). Arterial PO_2 decreased slightly over time but no relations were observed between arterial PaO_2 and $\dot{V}O_2$ ($r^2=0.09$, $P=0.66$) or PO_2m ($r^2=0.07$, $P=0.17$). Based on the rightward-shifted O_2 dissociation curve ($P_{50}=38$ torr) small changes in PaO_2 above 80 torr would have very little impact on O_2 content as these PO_2 values would fall on the flat portion of the O_2 dissociation curve.

Discussion

These experiments provide several new and clinically relevant findings regarding the impact of prolonged controlled MV on blood flow and oxygen delivery to the diaphragm. Specifically, this is the first investigation to demonstrate: 1) a time-dependent reduction in diaphragm blood flow and O_2 delivery ($\dot{Q}O_2$) during MV that does not occur in locomotor or other respiratory skeletal muscles in the ventilated animal or in the diaphragm of time-matched spontaneously breathing animals; 2) diaphragm microvascular PO_2 (PO_2m), which represents the sole driving force for capillary-to-myocyte O_2 diffusion, is reduced as a function of time of MV; 3) the ability to increase blood flow in the diaphragm with electrically-induced muscular contractions is severely reduced after 6 hrs of MV; and 4) diaphragm O_2 uptake ($\dot{V}O_2$) during muscular contractions is reduced by ~80% after 6 hrs versus the onset of MV. Overall, our findings clearly demonstrate an O_2 supply-demand imbalance in the diaphragm after mechanical ventilation that occurs within 6 hrs after the onset of MV. Further, as neither blood flow nor the ability to augment diaphragm flow with contractions were altered after 6 hrs of spontaneous breathing, the results demonstrate that the decrements in diaphragm O_2 delivery and utilization resulted from MV and were not due to the impact of anesthesia. The time-dependent reduction in microvascular O_2 supply (and ultimately O_2 pressure) in the diaphragm with MV present several potential consequences, including 1) possible local areas of hypoxia and/or anoxia that may promote ROS generation within diaphragm mitochondria and activate apoptotic pathways; and 2) an O_2 delivery limitation and exacerbated fall in intramyocyte PO_2 during elevated respiratory muscle O_2 demand (e.g., during the weaning process), hastening the onset of respiratory muscle fatigue, although these remain to be determined.

Mechanistic basis for the diminished diaphragm blood flow following mechanical ventilation

Given the large vasodilator reserve evident in the diaphragm even at maximal exercise (33), the finding of a relative inability to increase blood flow during contractions following 6 hrs of MV (Figure 5A) is surprising and suggests dysfunction within the resistance vasculature of the diaphragm. To our knowledge there have been no investigations examining the effect of MV over time on diaphragm vasomotor function. However, the greatly reduced blood flow during contractions (Figure 5A) suggests a reduced vasodilation (possibly due to increased oxidative stress/antioxidant imbalances and NO bioavailability (34)) and/or structural modifications (e.g., short-term remodeling) associated with an increased tonic vasoconstriction (35, 36) when the diaphragm is inactive. Both of these potential vascular mechanisms may act in concert to restrict the hyperemic response in the diaphragm with longer durations of MV. It is unclear why the diaphragm displays a time-dependent reduction in blood flow which was not apparent in other respiratory (i.e., intercostal) and skeletal (i.e., soleus and gastrocnemius) muscles that were also inactive (Figure 3). There are

several unique properties of the diaphragm in relation to other skeletal muscle (for review see (37)) which, when coupled with its chronic activation, may expedite the time-course of vasomotor dysfunction with disuse in this muscle. Given the vasomotor dysfunction observed after prolonged inactivity (e.g., after bed rest; (38)), we cannot preclude that similar reductions in blood flow to other skeletal muscle occur over longer time periods than utilized in our protocol. Nonetheless, the reduced blood flow, PO_2m , and O_2 delivery to the diaphragm at rest and during contractions following MV (Figures 5, 6 and 7B, respectively) can have potential ramifications on intracellular ATP production and the production of ROS. Both of these important issues will be addressed in the next segments.

Implications from reduced PO_2m with mechanical ventilation

In addition to the faster decline of PO_2m , which is also observed with aging (39) and some diseases (e.g., chronic heart failure (40, 41) and type II diabetes (42)) and attributed to a blunted increase in muscle blood flow (43), there was a greatly reduced contracting steady-state PO_2m after 6 hrs versus the onset of MV (Figure 6). Under conditions of altered O_2 supply, compensatory changes in the regulatory parameters of mitochondrial respiration (i.e. cytosolic $[ATP]/[ADP][P_i]$ and mitochondrial $[NAD^+]/[NADH]$) occur to maintain a given rate of ATP production (and thus $\dot{V}O_2$) (44, 45). In the current study, 6 hrs of MV lowered contracting PO_2m (Figure 6), thereby reducing intracellular PO_2 , and the maintenance of a given rate of oxidative ATP production would mandate lowered intracellular energy levels (i.e., reduced $[ATP]/[ADP][P_i]$, $[NAD^+]/[NADH]$, and $[PCr]$) (46).

The finding of a reduced PO_2m in the diaphragm during acute MV relative to spontaneous breathing is intriguing and suggests that spontaneous breathing elicits a greater increase in $\dot{Q}O_2$ compared to that of $\dot{V}O_2$ such that PO_2m is elevated. Interestingly, in contracting muscle there can be a transient increase in PO_2m (16) or decrease in fractional O_2 extraction (47) compared to resting conditions, which suggests a temporally enhanced $\dot{Q}O_2$ -to- $\dot{V}O_2$ ratio. Currently, it is unclear why diaphragm PO_2m is reduced during acute MV but this could be related to the cessation of metabolic signals that maintain arterial tone, reduced contributions of the respiratory and muscle pump, the unique microcirculation of the diaphragm (e.g., greater proportion of counter-current capillary flow (48)) and/or differential vasomotor regulation in diaphragm arterioles (49).

Potential biological links between reduced PO_2 and ventilator-induced diaphragmatic atrophy

It is well-established that prolonged MV results in the swift development of both diaphragmatic atrophy and contractile dysfunction in humans and other animals (50–52). This rapid MV-induced diaphragmatic atrophy greatly exceeds the rate of disuse muscle atrophy observed in locomotor skeletal muscles during periods of unloading (e.g., prolonged bed rest) (53). Identical to inactivity-induced muscle atrophy in locomotor skeletal muscle, MV-induced diaphragmatic atrophy occurs due to reduced protein synthesis and increased proteolysis. The MV-induced increase in diaphragmatic proteolysis is due to activation of key proteolytic systems including the calpain, caspase-3, proteasome, and autophagy pathways (4). Although the precise molecular mechanisms that regulate this rapid activation of these proteolytic systems remains unclear, strong evidence indicates that MV-induced production of ROS is essential for the activation of both calpain and caspase-3 (5, 7). The primary sources of ROS production in the diaphragm appear to be NADPH oxidase, xanthine oxidase, and mitochondria with mitochondria playing the dominant role (5, 54, 55).

The mechanism(s) responsible for the MV-induced increases in mitochondrial ROS production in the diaphragm remain unknown. Nonetheless, the current results suggest that MV-induced diaphragmatic hypoxia could play a contributing role. Although there is no

direct evidence that prolonged MV induces cellular hypoxia, the reduced diaphragm PO_{2m} observed during our experiments suggest a large reduction in intracellular PO_2 with 6 hrs of MV.

Specifically, blood-tissue O_2 transfer within the diaphragm can be described by Fick's law: $\dot{V}O_2 = DO_{2m} (PO_{2m} - PiO_2)$, where DO_{2m} is the effective diffusing capacity for O_2 , and PO_{2m} and PiO_2 are the mean microvascular and intracellular PO_2 s, respectively. In our study PO_{2m} is ~50% lower at 6 hrs versus 30 min of MV. Consequently, to maintain a given resting $\dot{V}O_2$, either DO_{2m} must increase or PiO_2 decrease. As blood flow is greatly reduced after 6 hrs of MV (Figure 1), DO_{2m} would likely be reduced and an exaggerated fall in PO_{2i} is predicted to occur to facilitate any given $\dot{V}O_2$ under these conditions. Further, the PO_{2m} measurements performed in this investigation reflect the composite PO_2 of all the plasma within the sampled region and it is likely that some areas of the diaphragm vasculature would have considerably lower PO_2 s than the mean value. Therefore, the current results suggest a lower than normal intracellular PO_2 in the diaphragm during prolonged MV, which could result in cellular hypoxia, although this remains to be determined. If prolonged mechanical ventilation results in physiologically significant decrease in diaphragmatic PO_2 , a buildup of reducing equivalents (i.e., NADH and FADH₂) could occur in the mitochondria resulting in increased electron availability and the production of superoxide anions (8, 56). Hence, given the large reduction in PO_{2m} following 6 hrs of MV in the current study, it is feasible that hypoxic loci within many diaphragm myocytes could promote mitochondrial ROS generation. This is a testable hypothesis and is worthy of further study.

Although the current results are consistent with the concept that MV-induced reductions in diaphragmatic blood flow could promote cellular hypoxia and the increased production of ROS, our data do not demonstrate cause and effect. Nonetheless, these new and important findings provide the foundation for future experiments to determine if MV-induced reductions in diaphragmatic blood flow and oxygen delivery is a contributory factor to MV-induced mitochondrial ROS production in the diaphragm.

$\dot{Q}O_2$ and $\dot{V}O_2$ during contractions: Ramifications on the weaning process

During muscular work under healthy conditions there is a tight coupling between the increase in $\dot{V}O_2$ and $\dot{Q}O_2$ (i.e., metabolic demands are precisely met by O_2 delivery; (57)). However, after 6 hrs of ventilation there is an uncoupling between these two variables, resulting in an increased fractional O_2 extraction at rest and during contractions (Figure 7C). In human patients who failed spontaneous breathing trials, there was an increased fractional O_2 extraction until failure, which was attributed to a reduced O_2 supply (58). Further, it has been demonstrated that impairments in the mechanical-chemical coupling in the diaphragm can result, in part, from a decreased O_2 availability (59). In the current study, we observed a ~80% reduction in $\dot{V}O_2$ during contractions after 6 hrs of MV compared to that after 30 min of MV. This large reduction in oxidative metabolism would hasten the onset of diaphragm fatigue and may predispose the patient to weaning difficulties, although low-frequency diaphragmatic muscle fatigue has not been detected during weaning failure (60). However, from the current study it is not possible to delineate the contributions of a reduced O_2 supply from mitochondrial and contractile dysfunction to this impaired $\dot{V}O_2$ response after MV.

Conclusions

In summary, we have demonstrated that controlled mechanical ventilation elicits a significant reduction in diaphragm blood flow and microvascular PO_2 in as short as 6 hrs, and time-matched spontaneously breathing animals did not demonstrate reductions in diaphragm blood flow. Further, upon initiation of muscular contractions, there is an inability to augment diaphragm blood flow and O_2 delivery which results in an inadequate matching

of O₂ delivery-to-O₂ uptake. Taken together, the diminished O₂ delivery results in a ~80% reduction in the aerobic metabolism of the diaphragm during contractions. These data provide strong support that a diminished O₂ supply (in addition to mitochondrial dysfunction) contributes to mechanical ventilation-induced diaphragm dysfunction which may predispose patients to weaning failure. Important questions to address in future work are the role(s) of the diminished O₂ supply on mitochondrial function and what vasomotor pathways contribute to the reduced blood flow response after mechanical ventilation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Resting diaphragm muscle blood flow (A) and vascular conductance (B) measured after 30 min of spontaneous breathing (n=7) or MV (n=8) and after 6 hrs of spontaneous breathing (n=5) or MV (n=8). *P<0.05 versus spontaneous breathing; †P<0.05 versus 30 min MV.

Figure 2. Resting blood flow to regionally delineated portions of the diaphragm muscle during spontaneous breathing (n=7) and after 30 min (n=8) and 6 hrs (n=8) of MV. *P<0.05 versus same muscle during spontaneous breathing; †P<0.05 versus same muscle after 30 min MV. ¥P<0.05 versus ventral diaphragm. #P<0.05 versus medial costal diaphragm.

Figure 3.

Comparison of blood flow (A) and vascular conductance (B) measured at rest between the diaphragm, red portion of the gastrocnemius muscle complex, soleus and intercostal muscles during spontaneous breathing (n=7) and after 30 min (n=8) and 6 hrs (n=8) of MV. The diaphragm was the only muscle that demonstrated a reduction in blood flow over time with MV. *P<0.05 versus spontaneous breathing; †P<0.05 versus 30 min MV.

Figure 4. Representative resting diaphragm microvascular PO₂ profiles measured over time (A) and the average diaphragm microvascular PO₂ (B) measured during spontaneous breathing and after 30 min and 6 hrs of MV (n=11). *P<0.05 versus spontaneous breathing; †P<0.05 versus 30 min MV.

Figure 5.

Blood flow at rest and during the steady-state of electrically stimulated contractions in the diaphragm muscle after 30 min and 6 hrs of MV (rest, n=8; contractions, n=5) and 6 hrs of spontaneous breathing (rest and contractions, n=5). For further information see 'Experimental Design' of methods section in text. *P<0.05 versus spontaneous breathing; †P<0.05 between 30 min and 6 hrs of MV; ¥P<0.05 versus rest in the same condition.

Figure 6.

Mean microvascular PO₂ profiles including 95% CI (dashed lines) (A) and representative diaphragm microvascular PO₂ profiles (B) in response to electrically stimulated muscle contractions after 30 min and 6 hrs of MV (n=5). Contractions were initiated at time zero.

Figure 7.

Diaphragm oxygen uptake (A), O₂ delivery (B) and fractional O₂ extraction (C) during spontaneous breathing and after 30 min and 6 hrs of MV in the quiescent (i.e., rest n=7 for SB and n=8 for MV) and contracting (via electrical stimulation; n=5) diaphragm. *P<0.05 versus spontaneous breathing; †P<0.05 between 30 min and 6 hrs of MV; ‡P<0.05 versus rest in the same condition; #P=0.056 between 30 min and 6 hrs of MV.

Table 1

Body and diaphragm mass, blood gasses and hematocrit.

	Spontaneous Breathing	30 min MV	6 hrs MV
Body mass (g)	274 ± 3	268 ± 3	271 ± 2
Diaphragm wt (mg)	873 ± 11	843 ± 30	855 ± 13
MAP (mmHg)	100 ± 4	105 ± 4	81 ± 4 *
pH	7.4 ± 0.1	7.4 ± 0.1	7.4 ± 0.1
Arterial PO₂ (torr)	96 ± 0.4	90 ± 6	80 ± 5 *
Arterial PCO₂ (torr)	47 ± 1	38 ± 4	30 ± 3 *
Hematocrit (%)	44 ± 0.3	40 ± 1	37 ± 1 *
[Hb] (g/dL)	14.0 ± 0.3	13.5 ± 0.4	13.3 ± 0.4 *
Arterial Saturation (%)	89 ± 3	86 ± 2	85 ± 3

MAP, mean arterial pressure. [Hb], arterial hemoglobin concentration.

*

P<0.05 versus both spontaneous breathing and 30 min MV. Values are means ± SE.