Mechanical Ventilation Depresses Protein Synthesis in the Rat Diaphragm

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Abstract

Prolonged mechanical ventilation results in diaphragmatic atrophy and contractile dysfunction in animals. We hypothesized that me-chanical ventilation–induced diaphragmatic atrophy is associated with decreased synthesis of both mixed muscle protein and myosin heavy chain protein in the diaphragm. To test this postulate, adult rats were mechanically ventilated for 6, 12, or 18 hours and dia-phragmatic protein synthesis was measured in vivo. Six hours of mechanical ventilation resulted in a 30% decrease ($p < 0.05$) in the rate of mixed muscle protein synthesis and a 65% decrease ($p < 0.05$) in the rate of myosin heavy chain protein synthesis; this depression in diaphragmatic protein synthesis persisted through-out 18 hours of mechanical ventilation. Real-time polymerase chain reaction analyses revealed that mechanical ventilation, in compari-son with time-matched controls, did not alter diaphragmatic levels of Type I and IIx myosin heavy chain messenger ribonucleic acid levels in the diaphragm. These data support the hypothesis that mechanical ventilation results in a decrease in both mixed muscle protein and myosin heavy chain protein synthesis in the diaphragm. Further, the decline in myosin heavy chain protein synthesis does not appear to be associated with a decrease in myosin heavy chain messenger ribonucleic acid.

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Prolonged mechanical ventilation results in diaphragmatic atrophy and contractile dysfunction in animals. We hypothesized that mechanical ventilation–induced diaphragmatic atrophy is associated with decreased synthesis of both mixed muscle protein and myosin heavy chain protein in the diaphragm. To test this postulate, adult rats were mechanically ventilated for 6, 12, or 18 hours and diaphragmatic protein synthesis was measured *in vivo*. Six hours of mechanical ventilation resulted in a 30% decrease ($p < 0.05$) in the rate of mixed muscle protein synthesis and a 65% decrease ($p < 0.05$) in the rate of myosin heavy chain protein synthesis; this depression in diaphragmatic protein synthesis persisted throughout 18 hours of mechanical ventilation. Real-time polymerase chain reaction analyses revealed that mechanical ventilation, in comparison with time-matched controls, did not alter diaphragmatic levels of Type I and IIx myosin heavy chain messenger ribonucleic acid levels in the diaphragm. These data support the hypothesis that mechanical ventilation results in a decrease in both mixed muscle protein and myosin heavy chain protein synthesis in the diaphragm. Further, the decline in myosin heavy chain protein synthesis does not appear to be associated with a decrease in myosin heavy chain messenger ribonucleic acid.

Keywords: atrophy; skeletal muscle; weaning

Controlled mechanical ventilation (MV) provides life support for patients who are unable to maintain adequate alveolar ventilation. However, unloading the diaphragm via MV for extended periods (i.e., 3 days or more) leads to weaning difficulties in as many as 20% of patients (1). Although the causes responsible for weaning difficulties continue to be investigated, respiratory muscle weakness is a key potential mechanism (2, 3). Indeed, patients exposed to prolonged MV have severely weakened diaphragms (4). Further, we and others have observed diaphragmatic atrophy (5–9) and contractile dysfunction (6–11) in experimental animals exposed to prolonged MV.

Similar to other skeletal muscles, the mammalian diaphragm is malleable and rapidly adapts to the demands placed on it. Skeletal muscle adapts to inactivity by incurring a net loss of muscle protein (i.e., atrophy). Muscle atrophy results in a decrease in fiber cross-sectional area, which is functionally important because force generation is directly related to fiber cross-sectional area (12). In theory, MV-induced diaphragmatic atrophy can occur as a result of a decrease in protein synthesis and/or an increase in the rate of protein degradation. In this regard, we have demonstrated that MV-induced diaphragmatic atrophy is associated with an increased rate of protein degradation and a decrease in fiber cross-sectional area after 18 hours of MV (5). In contrast, it is currently unknown whether prolonged MV results in a decrease in the rate of diaphragmatic protein synthesis; this forms the basis for the current experiment.

Locomotor skeletal muscle protein synthesis is sensitive to contractile activity and rapidly decreases within the first hours of reduced use. Specifically, during the first 5 hours of reduced contractile activity, mixed muscle protein synthesis (i.e., an average synthesis rate for all muscle proteins) and myosin heavy chain (MHC) protein synthesis rates decrease in rat soleus muscle by about 16 and 22%, respectively (13). On the basis of these data in locomotor skeletal muscle, we postulated that controlled MV would diminish protein synthesis rates in the diaphragm. In these experiments, we tested the hypothesis that mechanical ventilation-induced diaphragmatic atrophy is associated with decreased synthesis of diaphragmatic mixed muscle protein and myosin heavy chain protein. Further, by measuring MHC mRNA levels in the diaphragm of rats exposed to MV, we investigated the impact of MV on posttranscriptional events leading to protein synthesis. Portions of this work have been reported in abstract form (14).

METHODS

Animals

These experiments were approved by the University of Florida (Gainesville, FL) Animal Use Committee. Sprague-Dawley rats (4 months old) were randomly assigned to one of three experimental groups: control, MV, or spontaneously breathing (SB). For complete details *see* the online supplement.

Mechanical Ventilation Protocol

The MV group was subdivided into three groups ($n = 10$/group) and the rate of protein synthesis in the diaphragm was determined over 6 hours (MV 6), 12 hours (MV 12), and 18 hours (MV 18) of MV.

All surgical procedures were performed under aseptic conditions. Animals received glycopyrrolate (0.04 mg/kg, intramuscularly) before and every 2 hours after anesthesia. Animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally), tracheostomized, and mechanically ventilated (controlled-MV) using a volume-cycled ventilator (Inspira; Harvard Apparatus, Cambridge, MA). The tidal volume was about 1 ml/100 g of body weight, the respiratory rate was 80 breaths/minute, and positive end-expiratory pressure was 1 cm H_2O.

Heart rate was monitored via a Lead II electrocardiograph. The carotid artery was catheterized for constant measurement of systolic blood pressure and two blood samples (1 ml) were drawn at the end of the experiment for analysis of [^{13}C]leucine. The jugular vein was catheterized to add fluids, sodium pentobarbital (about 10 mg/kg per hour), and [^{13}C]leucine (*see below*). Body (rectal) temperature was maintained at $37 \pm 1^{\circ}$C. Animals received continuous care and monitoring throughout.

Spontaneous Breathing Protocol

The SB animals were subdivided into three groups (n = 10/group) to serve as time-matched controls for the MV groups. The SB animals were not mechanically ventilated (i.e., they were spontaneously breathing during the experiment) and received the same anesthetic dose, surgical intervention, [^{13}C]leucine infusion paradigm, and continuing care as the MV animals. Note that postmortem examinations were conducted on all MV and SB animals.

Control Animal Protocol

Control animals (n = 10) (acute anesthesia; sodium pentobarbital administered intraperitoneally at 50 mg/kg) were not mechanically ventilated or infused with [^{13}C]leucine before removal of the diaphragm. These animals were used to determine the natural abundance of [^{13}C]leucine in the rat diaphragm.

In Vivo Protein Synthesis

The fractional rate of protein synthesis in each MV and SB diaphragm was measured with a primed, constant intravenous infusion of [^{13}C]leucine (Cambridge Isotope Laboratories, Andover, MA) during the last 6 hours of each experimental period. The fractional rate of protein synthesis was computed as the magnitude of [^{13}C]leucine enrichment in protein divided by the enrichment of [^{13}C]leucine in the precursor pool. Plasma and tissue fluid (i.e., tissue fluid without amino acids) [^{13}C]leucine enrichments were analyzed according to Hasten and coworkers (15) and were used to reflect ^{13}C enrichment in the precursor pools for protein synthesis. Mixed muscle protein and myosin heavy chain (MHC) protein were isolated and their [^{13}C]leucine enrichments quantified according to Hasten and coworkers (15) and Yarasheski and coworkers (16). The *in vivo* rates of mixed muscle protein and MHC protein synthesis were calculated on the basis of each of the precursor pool measurements (15). Finally, it is important to appreciate that the effect that an elevated rate of muscle proteolysis has on this method of measuring muscle protein synthesis is extremely small.

Note that the use of [^{13}C]leucine precluded feeding the MV and SB animals during the experiments. ^{13}C is a naturally occurring stable isotope present in all foods. Likewise, leucine is a branched-chain amino acid present in dietary protein sources. Thus, feeding the animals during the experiments would introduce an unknown amount of [^{13}C]- and [^{12}C]leucine into their circulation and tissue and bias the results.

Real-time Quantitative Polymerase Chain Reaction

Total RNA was isolated from the costal diaphragm and reverse transcribed. Type I and IIx MHC mRNA levels were determined via real-time quantitative polymerase chain reaction (PCR). The relative standard curve method was used with a reference gene, hypoxanthine-guanine phosphoribosyltransferase, that did not differ between groups ($p = 0.76$).

Statistics

Data were analyzed by two-way analysis of variance. Where significant differences existed, a Newman–Keuls test was used post hoc. Significance was established at $p < 0.05$. Data are reported as means ± SE.

RESULTS

Morphologic, Physiological, and Postmortem Examination

No significant differences existed in preexperiment or postexperiment body mass between groups (*see* Table E1 in the online supplement). Therefore, no group experienced a significant loss of body mass during the experiment, indicating adequate hydration during the experimental period. In addition, all animals urinated and experienced intestinal transit during the experimental period.

Cardiovascular homeostasis during the experimental period was monitored via heart rate and systolic blood pressure. Heart rate (about 360 beats/minute) and systolic blood pressure (more than 90 mm Hg) were normal and well maintained during the experiments, that is, heart rate and blood pressure did not significantly decrease during the experiments. Further, heart rate and systolic blood pressure responses did not differ between SB animals and MV animals at each time point.

Postmortem examination of SB and MV animals included necropsy and blood culture. No animals demonstrated any signs of infection, weight loss, or postmortem abnormalities, and all blood cultures were negative for bacteria. The colonic temperature of all MV and SB animals remained constant, 37 ± 0.5°C, during the experiments. Collectively, these results indicate that our aseptic surgical technique successfully prevented infection. Finally, no MV animals displayed visible evidence of barotrauma.

Influence of Mechanical Ventilation on Protein Synthesis

A record of all changes in precursor pools and diaphragmatic protein enrichment of [^{13}C]leucine across the experimental groups is located in the online supplement (*see* Table E2 and Figures E2–E4). Briefly, plasma enrichment of [^{13}C]leucine reached a steady state plateau by Hour 6 of infusion in each experimental group. Note that the fractional rate of protein synthesis in the diaphragm was calculated on the basis of measurements from each of the three measured precursor pools. Regardless of which precursor pool was used for these calculations, the rate of change in diaphragm protein synthesis was similar. Therefore, for clarity, only the protein synthetic rate calculated on the basis of the tissue fluid [^{13}C]leucine precursor pool is presented.

MV significantly ($p < 0.05$) slowed the fractional synthetic rate of both mixed muscle protein and MHC protein synthesis (Figures 1 and 2). Mixed muscle protein synthesis, an average synthesis rate for all proteins present in muscle, slowed significantly (-30%) during the first 6 hours of MV as compared with the time-matched SB 6 group (Figure 1). The 30% decrease in the rate of mixed muscle protein synthesis persisted at 12 hours of MV (i.e., MV 12 compared with SB 12, -26%) and remained depressed at 18 hours of MV (MV 18 compared with SB 18, -29%).

Myosin heavy chain protein synthesis was measured to estimate the impact of MV on the rate of contractile protein synthesis. Within the first 6 hours of MV, MHC protein synthesis slowed significantly (-66%) compared with the time-matched SB 6 group (Figure 2). Similar to the mixed muscle protein synthesis rates, the MV-induced decrease in MHC protein synthesis rates remained

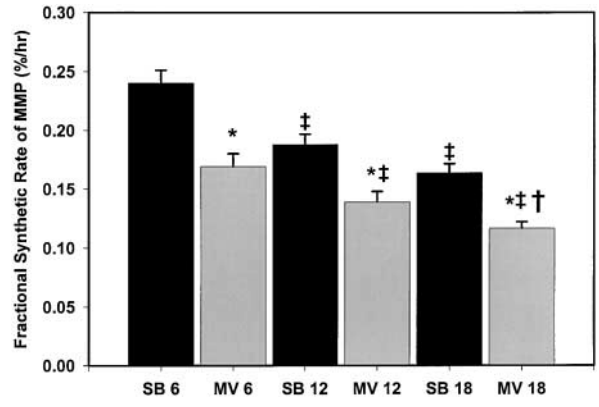

Figure 1. Fractional synthetic rates of mixed muscle protein (MMP) by calculation with tissue fluid [^{13}C]leucine as a surrogate measure of the [^{13}C]leucyl-tRNA precursor pool. Values are expressed as percent per hour (%/hour). MV = mechanically ventilated animals; SB = spontaneously breathing animals. *Significantly different ($p < 0.05$) from time-matched SB group; ‡significantly different ($p < 0.05$) from SB 6; †significantly ($p < 0.05$) different from MV 6.

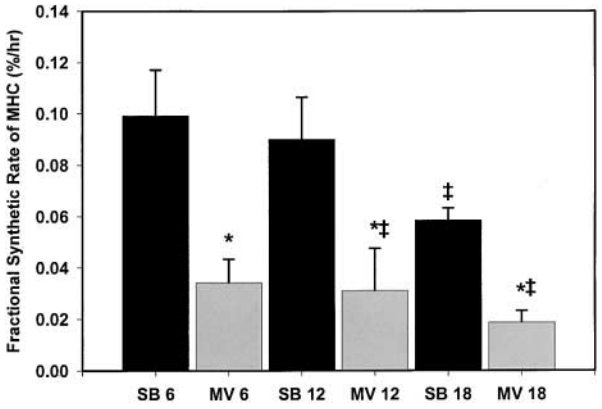

Figure 2. Fractional synthetic rates of myosin heavy chain (MHC) protein by calculation with tissue fluid [^{13}C]leucine as a surrogate measure of the [^{13}C]leucyl-tRNA precursor pool. Values are expressed as percent per hour (%/hour). MV = mechanically ventilated animals; SB = spontaneously breathing animals. *Significantly different ($p < 0.05$) from time-matched SB group; ‡significantly different ($p < 0.05$) from SB 6.

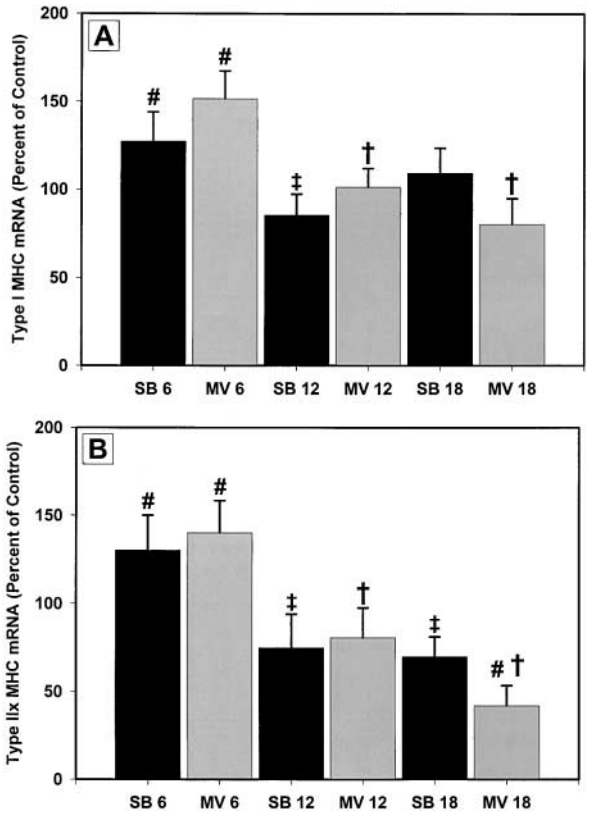

Figure 3. Real-time quantitative PCR was used to measure mRNA levels for Type I and Type IIx myosin heavy chain in the diaphragm of control, spontaneously breathing (SB), and mechanically ventilated (MV) animals. Values are expressed as a percentage of control. #Significantly different ($p < 0.05$) from control group; ‡significantly different ($p < 0.05$) from SB 6; †significantly different ($p < 0.05$) from MV 6.

constantly depressed when contrasted with each time-matched SB group.

Influence of Mechanical Ventilation on Total RNA and Myosin Heavy Chain mRNA in the Diaphragm

Total RNA is about 80–85% rRNA and can be used as an index of the quantity of ribosomal subunits and as an indirect index of the synthetic capacity of the tissue. In contrast, mRNA constitutes about 2–3% of the total RNA pool. Total RNA was isolated from each diaphragm and the mRNAs encoding Type I and Type IIx MHC were then measured to determine whether the observed decrease in protein synthesis was due, in part, to a decrease in total RNA and/or MHC mRNA. Type I and Type IIx MHC mRNAs were measured because the mRNAs that encode these proteins represent about 26 and 41%, respectively, of MHC protein in the costal diaphragm of the rat (17). Exposure to the anesthetic (SB groups) or MV did not affect the amount of total RNA in the diaphragm (*see* Table E3 in the online supplement).

Real-time quantitative PCR is a highly specific, sensitive, and reliable method to quantify specific mRNA. Animals in both the SB and MV groups were found to have significantly ($p < 0.05$) higher levels of both Type I and Type IIx MHC mRNAs compared with the control, acutely anesthetized, animals (Figures 3A and 3B). At the 12-hour time point the Type I and Type IIx mRNA levels in diaphragms from SB and MV animals were not different from those of the control animals. Compared with the control group, 18 hours of spontaneous breathing or MV did not alter the Type I mRNA levels. However, animals exposed to 18 hours of MV were found to have 60% lower Type IIx mRNA levels than controls, whereas the time-matched controls, SB 18, were not different from controls. Importantly, at no time point did the diaphragm levels of Type I or Type IIx mRNA differ between the mechanically ventilated animals and their time-matched controls.

DISCUSSION

Overview of Principal Findings

These experiments investigated the effect of controlled MV on protein synthesis and MHC mRNA in the rat diaphragm. Our results support the hypothesis that mechanical ventilation-induced diaphragmatic atrophy is associated with decreased synthesis of diaphragmatic mixed muscle protein and MHC protein. Indeed, within the first 6 hours of MV, mixed muscle protein synthesis decreased by about 30% and MHC protein synthesis decreased by about 65%. These decrements in protein synthesis persisted during the ensuing 12 hours of MV. Our data also reveal that this early decrease in protein synthesis during MV does not appear to be due to reduced MHC mRNA levels in the diaphragm. A detailed discussion of these points follows.

Impact of Mechanical Ventilation on Protein Synthesis in the Diaphragm

Mixed muscle protein synthesis. Mixed muscle protein synthesis is the average synthetic rate of all proteins (e.g., contractile proteins, sarcoplasmic reticulum proteins, etc.) in a muscle sample, and was measured as an index of total protein synthesis in the diaphragm. Our data indicate that MV was associated with a 30% decrease in mixed muscle protein synthesis in the diaphragm within the first 6 hours of MV and remained at this suppressed level during the following 12 hours of MV. This rapid reduction in skeletal muscle protein synthesis has also been observed during periods of inactivity in rat gastrocnemius muscle. Furthermore, similar to our findings, a decrease in mixed muscle protein synthesis in immobilized rat gastrocnemius has been reported during 2 days of immobilization (18). Therefore, the observed decrease in mixed muscle protein synthesis during 18 hours of MV in the rat diaphragm is consistent with data

from rat hindlimb immobilization (18) and indicates that the diaphragm, like other skeletal muscles, is sensitive to loading state. Once the diaphragm is unloaded via controlled MV, mixed muscle protein synthesis decreased rapidly and a new steady state of protein synthesis was established.

The synthesis rate for mixed muscle protein in our spontaneous breathing animals (i.e., SB 6 hours) was about 0.2%/hour, which is lower than previously published synthetic rates for mixed muscle protein in the rat diaphragm (i.e., about 0.4 to 0.6%/hour) (19–21). It seems likely that these differences in protein synthesis rates are due to the fact that young and rapidly growing animals were used in previous studies whereas the current study employed young adult animals that were not rapidly growing. Nonetheless, our measured protein synthetic rates are similar to those measured in other adult rat skeletal muscles (e.g., 0.16%/hour in quadriceps [22], 0.23%/hour in gastrocnemius [23], and 0.33%/hour in soleus [13]). Hence, the measured rates of mixed muscle protein synthesis in the rat diaphragm in the current study are consistent with rates reported in the literature for adult animals.

Myosin heavy chain protein synthesis. MHC is an essential component of the contractile apparatus and constitutes about 25% of skeletal muscle mass (24, 25). Importantly, force generation is proportional to the amount of myofibrillar protein within the fiber and thus a decrease in the rate of MHC synthesis would lead to a decrease in the force-generating ability of the diaphragm (10, 12, 26). Our results indicate that diaphragmatic MHC protein synthesis decreased by about 65% during the first 6 hours of MV. Furthermore, this decrease in MHC protein synthesis was maintained through 18 hours of MV. This rapid decrease in the rate of diaphragmatic MHC protein synthesis is more severe than the reported change after 5 hours of hindlimb unloading, where a nonsignificant decrease in MHC protein synthesis has been reported in the soleus muscle (13). A possible explanation for the divergent findings is that during controlled MV the diaphragm is not only silent but is being passively shortened (27), whereas the unloaded soleus is free to contract (against little resistance). Hence, this level of activation in the soleus may serve to attenuate the decrease in protein synthesis during hindlimb unloading compared with controlled MV. The change in MHC protein synthesis has not been measured during hindlimb immobilization (i.e., casting), but the synthetic rate of another essential contractile protein, Q-actin, has been examined (28). During the first 6 hours of hindlimb immobilization, Q-actin protein synthesis in the rat gastrocnemius decreases about 66% (28). The MHC protein synthesis data in the current study and the Q-actin protein synthesis data after 6 hours of hindlimb immobilization (28) indicate that skeletal muscle rapidly adapts to unloading by significantly decreasing the rate of contractile protein synthesis.

To our knowledge, these are the first experiments to measure the rate of MHC protein synthesis in the rat diaphragm. In our spontaneously breathing animals the rate of MHC protein synthesis was about 0.1%/hour (i.e., SB 6). Previous investigations report MHC protein synthesis rates of about 0.1%/hour in the rat quadriceps muscle (22) and about 0.25%/hour in the soleus muscle (13). It is unclear why MHC synthesis rates in the diaphragm are not in line with those of the more active soleus. Finally, it is noteworthy that the rates of MHC protein synthesis in the current study are less than the mixed muscle protein synthesis rate. This observation suggests that MHC protein pool turns over slower (longer half-life) than other proteins in the mixed muscle protein pool. Similar results have been reported in locomotor skeletal muscle in the rat (13, 22).

Protein synthesis rates in SB animals. The rate of muscle protein synthesis decreased over time in the SB group. In comparison with the SB 6 group, SB 18 animals experienced a 32% decrease in the rate of mixed muscle protein synthesis and a 41% decrease in the rate of MHC synthesis. The observed changes in the synthetic rate are consistent with the literature. Goldspink and coworkers (29) report a 48% decrease in diaphragm mixed muscle protein synthesis 23 hours postfeeding and Bates and coworkers (30) report a 44% decrease in the rate of limb-locomotor MHC synthesis 24 hours postfeeding. However, in comparing the MV18 results with those of the time-matched SB group a 29% decrease in mixed muscle protein synthesis and a 68% decrease in MHC synthesis are still realized. Therefore, the impact of MV on protein synthesis in the diaphragm was not obscured by the nutrient status of the animals.

Primed, constant infusion of [^{13}C]leucine. The method used to measure the rate of mixed protein and MHC protein synthesis in our experiments quantifies the amount and rate of [^{13}C]leucine incorporation into muscle proteins. This was done by a primed, constant infusion method during the time the animal was spontaneously breathing or mechanically ventilated (up to 18 hours). The probability that an elevated rate of muscle proteolysis confounded this method of measuring muscle protein synthesis is infinitesimally small. Once the [^{13}C]leucine leaves the circulation and enters the muscle amino acid free pool, it will either be incorporated into a muscle protein, oxidized, or transported back out of the muscle cell. Once incorporated into a long-lived muscle protein such as MHC (skeletal muscle MHC has been reported to have a half-life as long as about 30 days) the probability that the same MHC molecule will undergo proteolysis and release the same [^{13}C]leucine back into the muscle free pool during an 18-hour experiment is extremely small, especially given the trace amount of [^{13}C]leucine that is incorporated into muscle protein during the short course of these experiments. If this pathway for [^{13}C]leucine "recycling" were occurring during the relatively short duration of this tracer infusion study, muscle free pool [^{13}C]leucine enrichment would be greater in muscle undergoing high rates of proteolysis (MV) versus the comparison muscle undergoing basal rates of proteolysis (SB). Clearly, this was not the case (*see* Figure E3 in the online supplement). Although it is known that MV significantly elevates diaphragmatic protein degradation (5) the muscle free pool [^{13}C]leucine enrichments were not different between MV and SB at each time point (6, 12, and 18 hours), indicating that our protein synthesis measurements were not influenced by muscle proteolysis.

Anesthesia and protein synthesis. The anesthetic agent, sodium pentobarbital, could have affected the rate of muscle protein synthesis in the diaphragm. However, both MV and SB animals were anesthetized with sodium pentobarbital for the same time period, and therefore comparisons between groups are valid. Moreover, a previous study has reported that rats acutely anesthetized with sodium pentobarbital do not experience a significant decrease in protein synthesis in skeletal muscle (31). In addition, general anesthesia does not decrease protein synthesis in skeletal muscle in healthy humans undergoing abdominal surgery (32). Collectively, these data indicate that protein synthesis is not altered by anesthesia per se. The influence of continued exposure to any given anesthetic agent (e.g., 18 hours) would be difficult to separate from reduced use during that state. However, the experiments reviewed above (31, 32) report normal rates of protein synthesis in limb-locomotor skeletal muscle during periods of time that reduced use would not be expected to have an affect on protein synthesis. These reports (31, 32) indicate that anesthesia does not affect protein synthesis; therefore, the decreased rate of protein synthesis in the diaphragm during MV is attributable to MV, not the anesthetic.

Impact of Mechanical Ventilation on Diaphragmatic MHC mRNA

The fractional synthetic rate of specific proteins can be altered by pretranslational events leading to a decrease in the amount of a given mRNA (e.g., the rate of transcription or turnover of MHC mRNA). As discussed previously, MV significantly slows both mixed muscle protein synthesis and MHC protein synthesis. To determine whether MV-induced reductions in MHC protein synthesis in the diaphragm were influenced by pretranslational events, we measured Type I and Type IIx MHC mRNA levels. Interestingly, compared with control, diaphragm levels of Type I and IIx MHC mRNAs were elevated after 6 hours of MV, but this increase was transient. After 12 hours of MV the mRNA levels did not differ from control whereas after 18 hours of MV the Type IIx MHC mRNA levels were depressed compared with control. By comparison, the MHC protein synthesis rates were significantly decreased after 6 hours of MV and remained depressed for the remaining period of the experiment (18 hours). These observations are consistent with previous studies of locomotor skeletal muscle disuse (13, 28). Therefore, similar to other skeletal muscles, the reduced rate of protein synthesis during the first 12 hours of inactivity in the diaphragm does not appear to be due to a change in MHC mRNA content. One report indicated that extended periods of MV (i.e., more than 48 hours) does alter MHC mRNA (8). In these experiments, Yang and coworkers (8) reported that more than 48 hours of MV increases MHC IIa (70%) and MHC IIx (22%) mRNA levels, with little change in MHC IIb mRNA (4%), and no change in Type I mRNA (8). In addition, Yang and coworkers (8) report that more than 48 hours of MV causes a significant decrease in the number of diaphragmatic fibers expressing Type I MHC protein and a significant increase in fibers coexpressing both Type I and II MHC protein (8). These findings (8), in conjunction with the present study, suggest that there is little change in diaphragmatic levels of MHC mRNA during the first 18 hours of MV, whereas during the next 30 hours MHC mRNA expression is altered, leading to a slow-to-fast shift in MHC protein expression in the diaphragm.

Protein synthesis is the culmination of many events, including transcription and translation, all of which are highly regulated. The rapid decrease in protein synthesis during MV could be due to the inhibition of one or both of these steps. In healthy active locomotor skeletal muscle, MHC protein expression appears to be regulated by transcriptional events (33). For example, 13-MHC promoter region activity in the soleus is significantly decreased after 7 days of inactivity (34, 35). In addition, changes in mRNA expression precede changes in protein expression measured from Day 4 to Day 90 of inactivity (36). Collectively, these data suggest that over a period of weeks to months, MHC protein expression is regulated by transcriptional events, but that during the first hours of reduced use protein expression is regulated by post-transcriptional mechanisms.

MV did not change the amount of total RNA, nor were MHC mRNA levels in the diaphragm different from those of time-matched controls (Figure 3). The MV-induced decrease in protein synthesis therefore suggests a decrease in translational efficiency (i.e., amount of MHC protein synthesized per quantity of MHC mRNA). A decrease in translational efficiency occurs when one or more steps of translation are hindered. In this regard, each of three major processes of translation (initiation [37–39], elongation [40], and termination [41]) is impacted in skeletal muscle during periods of reduced use and one or more of these steps could contribute to the rapid decrease in protein synthesis induced by MV.

Comparison of MV and Denervation on Diaphragmatic MHC mRNA Levels

The current experiments did not measure diaphragmatic EMG activity in the diaphragm during MV. Nonetheless, previous work in our laboratory indicates that the diaphragm is devoid of EMG activity during controlled MV (10); we interpret this observation as evidence that MV eliminates mechanical activation of the diaphragm. Hence, it is interesting to compare the present experiments with previous studies imposing diaphragmatic inactivity via interventions such as denervation. In this regard, one study demonstrated that diaphragmatic inactivity imposed by denervation results in a time-dependent change in diaphragmatic MHC mRNA and MHC protein isoforms (39). Specifically, these experiments reported that 3 days of denervation decreased diaphragmatic levels of both Type I and IIx MHC mRNAs whereas no change occurred in Type IIa and IIb MHC mRNAs (39). In comparison, the present study revealed that 18 hours of MV decreased MHC Type IIx but not Type I mRNA levels. It is unclear whether these contrasting results are due to the variation in the time course of the two experiments or to differences in the experimental model.

Summary

These experiments investigated the effect of MV on protein synthesis in the rat diaphragm. Our results support the hypothesis that MV-induced diaphragmatic atrophy is associated with decreased synthesis of diaphragmatic mixed muscle protein and myosin heavy chain protein. Further, the data suggest that the decrease in diaphragmatic protein synthesis is due to an impairment of posttranscriptional events. Given that we have previously reported that MV increases the rate of proteolysis in the diaphragm (5), and that the current experiments demonstrate that the rate of protein synthesis decreases significantly during MV, we conclude that MV-induced diaphragmatic atrophy is due to both increased proteolysis and a decreased rate of protein synthesis.

***Conflict of Interest Statement*:** R.A.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; D.V.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; K.C.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; A.M.Z. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.J.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; K.E.Y. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.K.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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