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Mechanical Ventilation-Induced Diaphragm Atrophy Strongly Impacts Clinical Outcomes

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ECG and NDF conceived the study. EG, EF, GDR, BPK, MSH, LJB and NDF designed the study. ECG, SV, MCS, NR, AL, AM, DB, CU, and GT acquired and analyzed the study measurements. ECG, MD, EF, GDR, DCS, WDR, GT, LJB and NDF planned the statistical analysis. ECG and GT conducted the statistical analysis. All authors contributed to the interpretation of the findings. ECG, LJB and NDF prepared the first draft of the manuscript and all authors critically revised the manuscript for intellectually important content. All authors gave final approval for the publication of the work, and all accepted responsibility for the integrity of the work.

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Running Head
Diaphragm Atrophy and Clinical Outcomes

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At A Glance Commentary
Scientific Knowledge on the Subject
Diaphragm dysfunction is associated with prolonged ventilator-dependence and poor outcomes in critically ill patients. Many factors contribute to diaphragm dysfunction including diaphragm atrophy due to mechanical ventilation. However, the specific impact of diaphragm atrophy due to ventilation on clinical outcomes is unknown.

What This Study Adds to the Field
Diaphragm atrophy developing during mechanical ventilation was specifically associated with substantial delays in liberation from mechanical ventilation and a significant increase in the risk of serious complications including reintubation, tracheostomy and prolonged ventilation. When patients were breathing spontaneously at a level of inspiratory effort (assessed by diaphragm thickening fraction on ultrasound) similar to that seen in healthy subjects at rest, changes in diaphragm thickness were significantly attenuated and the duration of ventilation, duration of ICU admission, and risk of complications were minimized.

This article has an Online Data Supplement, which is accessible from this issue's Table of Contents online at www.atsjournals.org

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Abstract

Rationale
Diaphragm dysfunction worsens outcomes in mechanically ventilated patients but the clinical impact of potentially preventable changes in diaphragm structure and function due to mechanical ventilation is unknown.

Objectives
To determine whether diaphragm atrophy developing during mechanical ventilation leads to prolonged ventilation.

Methods
Diaphragm thickness was measured daily by ultrasound in adults requiring invasive mechanical ventilation; inspiratory effort was assessed by thickening fraction. The primary outcome was time to liberation from ventilation. Secondary outcomes included complications (reintubation, tracheostomy, prolonged ventilation, or death). Associations were adjusted for age, severity of illness, sepsis, sedation, neuromuscular blockade, and comorbidity.

Measurements and Main Results
Of 211 patients enrolled, 191 had two or more diaphragm thickness measurements. Thickness decreased more than 10% in 78 patients (41%) by median day 4 (IQR 3-5). Development of decreased thickness was associated with a lower daily probability of liberation from ventilation (adjusted HR 0.69, 95%CI 0.54-0.87, per 10% decrease), prolonged ICU admission (duration ratio 1.71, 95%CI 1.29-2.27), and a higher risk of complications (OR 3.00, 95%CI 1.34-6.72). Development of increased thickness (n=47, 24%) also predicted prolonged ventilation (duration ratio 1.38, 95%CI 1.00-1.90). Decreasing thickness was related to abnormally low inspiratory effort; increasing thickness was related to excessive effort. Patients with thickening fraction between 15-30% (similar to breathing at rest) during the first 3 days had the shortest duration of ventilation.

Conclusions
Diaphragm atrophy developing during mechanical ventilation strongly impacts clinical outcomes. Targeting an inspiratory effort level similar to that of healthy subjects at rest might accelerate liberation from ventilation.

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250/250

MeSH Key Words for Indexing
Respiration, Artificial; Weaning; Diaphragm; Acute Respiratory Failure.

Introduction

Mechanical ventilation is a life-saving intervention employed worldwide in an estimated 15 million patients annually (1). While many patients who recover from their initial critical illness are readily liberated from the ventilator, approximately 30% require prolonged weaning (2). Prolonged mechanical ventilation is associated with an increased risk of death (2, 3), poor long-term functional outcomes (3-5), and markedly higher healthcare costs (6).

Diaphragm weakness is a leading cause of difficult weaning from mechanical ventilation (7-9). A wide variety of factors can give rise to acute or chronic diaphragm weakness in critically ill patients, including preadmission injury, sepsis, medications, and multi-organ dysfunction syndrome (10). Mechanical ventilation *per se* can cause acute diaphragm injury and weakness (11-14). Ventilatory assistance suppressing inspiratory effort results in rapid diaphragm atrophy (13, 15-17). Diaphragm atrophy has now been documented repeatedly in clinical studies and many patients are affected: our previous prospective study found that diaphragm thickness decreased rapidly following intubation in nearly 50% of ventilated patients (16). Conversely, insufficient ventilatory support may fail to adequately unload the respiratory muscles, potentially resulting in load-induced diaphragmatic inflammation and injury (18-20).

It remains uncertain whether the changes in the diaphragm specifically due to mechanical ventilation significantly impact clinical outcomes. Multiple studies have shown that diaphragm weakness predicts prolonged ventilator-dependence and poor clinical outcomes (7-9, 21, 22). However, such diaphragm weakness reflects the global functional impact of all causes of diaphragm dysfunction in critically ill patients ("ICU-acquired dysfunction") (21) and not the specific effect of mechanical ventilation. In fact, two-thirds of ventilated patients exhibit diaphragm weakness at the time of ICU admission prior to any effect of mechanical ventilation (10) and some patients actually demonstrate improvements in diaphragm function during the

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early course of ventilation (21). Therefore, the specific effect of diaphragm atrophy and injury due to ventilation on clinical outcomes remains unknown. Indeed, one recent small study found no relationship between changes in diaphragm thickness and extubation outcome (23). The potential improvement in outcome that might be obtained by preventing diaphragm atrophy due to ventilation is therefore also uncertain.

To establish the impact of diaphragm injury resulting from ventilation on clinical outcomes, we undertook to examine prospectively whether ventilation-induced changes in diaphragm thickness (T_{di}) ascertained by ultrasound (24) were associated with prolonged ventilator-dependence and related complications including reintubation, tracheostomy, or death. Since previous observations suggest that increases in T_{di} during ventilation might indicate load-induced diaphragm injury (16), we evaluated the effect of both decreases and increases in T_{di} on the risk of prolonged ventilator-dependence. Finally, given that the rate of decrease or increase in T_{di} varied with diaphragm thickening fraction (a surrogate measure of inspiratory effort) in our prior study (16), we examined whether mean thickening fraction over the first 3 days of ventilation predicted the duration of ventilation, hypothesizing *a priori* that the duration of ventilation would be lowest at an intermediate level (i.e. a J-shaped curve). Some of the results of this study were previously reported in the form of an abstract (25).

Methods

Study Population and Setting

Patients were enrolled in two closely related cohorts: a large prospective study involving daily measurements of diaphragm thickness (Cohort A; N=191), and a smaller prospective study involving daily measurements of diaphragm thickness together with continuous monitoring of diaphragm electrical activity by crural electromyography (Cohort B; N=25) (see Table E1 for

details). In Cohort A, patients were included if they were ventilated for fewer than 36 hours (the first 53 patients were enrolled up to 72 hours after intubation; the protocol was then modified to limit the time period for enrolment to 36 hours). Patients were excluded if they were expected to be liberated within 24 hours of screening or if they had received invasive ventilation for greater than 48 hours in the previous six months. Eligible patients were identified by regular screening (Monday-Thursday). Approximately half of the patients (n=122) were included in our previous report on changes in diaphragm thickness during ventilation (16).

In Cohort B, patients were included if they were intubated for less than 36 hours because of acute brain injury, acute respiratory distress syndrome, septic shock, or pneumonia. Patients were excluded if they were deemed unlikely to remain on the ventilator for at least 7 days or if there were clinical conditions that interfered with reliable crural EMG measurements.

Informed consent was obtained from substitute decision makers prior to enrolment. For both cohorts, if no substitute decision maker was available, eligible patients were enrolled by deferred consent and consent for the use of study data was obtained from study participants once they regained capacity. The Research Ethics Boards at University Health Network (#12-5582, #13-5953) and St. Michael's Hospital (#14-229) approved the study protocols. We followed the STROBE guidelines for reporting cohort studies (26).

Measurement of Diaphragm Thickness, Inspiratory Effort, and Diaphragm Function

Right hemidiaphragm thickness (T_{di}) was measured using a high frequency (13 MHz) linear array transducer in the zone of apposition between the anterior and mid-axillary lines at the level of the 9^{th} or 10^{th} intercostal space; measurements were made daily on weekdays until extubation or day 14 of invasive mechanical ventilation using a technique we previously validated (27). Inspiratory effort was indirectly quantified daily by measuring diaphragm thickening fraction on

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ultrasound. In Cohort B, inspiratory effort was also indirectly quantified by hourly recordings of diaphragm electrical activity (EA_{di}).

Diaphragm function was assessed on the day of extubation or after 1 week of mechanical ventilation once patients were awake and breathing spontaneously. Diaphragm function was quantified by measuring maximal diaphragm thickening fraction during coached maximal inspiratory efforts under CPAP mode (9, 28). Severe diaphragm dysfunction was defined as maximal thickening < 20% (29). Measurement techniques are detailed in the Online Data Supplement.

Patient Characteristics and Clinical Outcomes

Demographic data, comorbidities, admission diagnosis, and severity of illness (Simplified Acute Physiology Score [SAPS] II) (30) were collected at baseline. Ventilator settings, arterial blood gas tensions, criteria for sepsis (31), Riker Sedation-Agitation Scale (32), exposure to neuromuscular blockade, and Sequential Organ Failure Assessment (SOFA) (33) scores were recorded daily.

Patients were assessed for the following events until hospital discharge: extubation, reintubation, tracheostomy, ICU discharge, hospital discharge, and death. Liberation from ventilation was defined as separation from ventilation (extubation or tracheostomy mask breathing for 24 hours) without resumption of invasive ventilatory support during the index ICU admission. Our primary end-point was the time from intubation until liberation from ventilation (or death). Ventilator-free days were computed to 60 days; patients who required more than 60 days of ventilatory support or who died on or before day 60 were assigned 0. Complications of acute respiratory failure were defined as the occurrence of any of the following events: reintubation, tracheostomy, prolonged ventilation (>14 days), or death (3, 34). Investigators

responsible for analysis of diaphragm ultrasound images were blinded to patient outcomes. Clinicians responsible for medical decisions including weaning were not aware of ultrasound measurement data. Routine weaning practices (described in the Supplement) were similar across participating ICUs but were not uniformly standardized for the study.

Statistical Analysis

The analysis plan, developed and executed in collaboration with a senior biostatistician (GT), is detailed in the Online Supplement. Patients with only 1 measurement of T_{di} were excluded from the analysis.

The primary analysis ("time-varying exposure" approach) examined the association between T_{di} (as a percentage of the initial T_{di}) on any given day (time-varying covariate) and the hazard of liberation from ventilation on that day using a Cox proportional hazards model of the time to liberation from ventilation. This approach was selected as the primary analysis to avoid potential time-dependent confounding and immortal time bias related to variation in the timing of changes in T_{di}. The model treated death as a censored event and adjusted for age, baseline SAPS II, presence of sepsis, SOFA score, baseline PaO_2/FiO_2, SAS score, use of neuromuscular blockade in the first 48 hours of ventilation, initial measurement of T_{di}, and the presence of at least one chronic comorbidity. We conducted sensitivity analyses to assess whether the model was robust to varying conditions (see Supplement for rationale and details of these analyses).

In secondary analyses, we employed an alternate analytical framework ("classification-based" approach) to assess the relationship between the initial change in T_{di} and clinical outcomes. Under this framework, patients were classified as having diaphragm atrophy if T_{di} decreased by at least 10% from baseline. Patients were classified as having increased T_{di} if T_{di} increased by at least 10% increase from baseline. Subjects without a 10% increase or decrease in Tdi over the

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first week of ventilation were classified as 'unchanged'. To mitigate against time-dependent confounding, patients were classified on the first day that the percentage change in T_{di} exceeded 10% of baseline T_{di} (Figure E1, Online Supplement). This threshold was selected in accordance with our previous study (16), based on the measurement resolution of the ultrasound technique (27), and in accordance with previous studies of myopathy in critical illness (35).

The association between changes in T_{di} and the risk of severe diaphragm dysfunction was evaluated using bivariate logistic regression. The effects of patient inspiratory effort, ventilator settings, and fluid balance on changes in diaphragm thickness over time were examined using linear mixed effects regression modelling. To conduct the primary analysis, our estimated sample size requirement was 210 subjects. All statistical analyses were conducted using R version 3.3.2 (www.R-project.org).

Results

Study Cohort

Between May 2013 and January 2016, 222 patients were enrolled; of these, 191 patients had at least 2 T_{di} measurements and were available for the primary analysis (Figure E2, Online Supplement). Demographic and clinical characteristics of enrolled patients are shown in Table 1. Forty-five patients (21%) died without being liberated from the ventilator and 29 (14%) were liberated from ventilation but died in hospital. Over half of patients (56%) experienced at least one complication of acute respiratory failure (Table 2).

Development of Decreased Diaphragm Thickness and Clinical Outcome

In the primary (time-varying exposure) analysis, the daily probability of liberation from mechanical ventilation was significantly lower on days when T_{di} was decreased from baseline

(Figure 1, adjusted hazard ratio (HR) 0.69, 95% CI 0.54-0.87, per 10% decrease in T_{di}). The daily probability of liberation was also lower with increased SOFA, decreased SAS, and lower initial T_{di} (Table E3). In sensitivity analyses of the primary model, similar effects were obtained when the analysis was restricted to patients in Cohort A (adjusted HR 0.63, 95% CI 0.48-0.81, per 10% decrease in T_{di}), after excluding patients enrolled on day 3 of ventilation (adjusted HR 0.73, 95% CI 0.54-0.98), after removing initial T_{di} from the model (adjusted HR 0.78, 95% CI 0.62-0.98), or after excluding patients requiring ventilation for fewer than 7 days (adjusted HR 0.75, 95% CI 0.55-1.01).

In the alternate (classification-based) analysis framework, patients were classified according to their initial change in T_{di} during the first week of ventilation. Diaphragm atrophy (defined as >10% decrease in T_{di}) developed in 78 patients (41%) within a few days of intubation (median day 4, IQR 3-5) (Table 1). After accounting for the competing risk of death, the risk of remaining on ventilation for at least 3 weeks was significantly higher in patients with diaphragm atrophy (Figure 2, 39% vs. 22%, p=0.008). The risk of death was not significantly different (p=0.46). The development of diaphragm atrophy was also associated with fewer ventilator-free days at day 60, a prolonged duration of ventilation, prolonged ICU admission, and a higher risk of complications including reintubation and tracheostomy (Table 2).

Development of Increased Diaphragm Thickness and Clinical Outcome

In the primary (time-varying exposure) analysis framework, there was a non-significant trend towards a lower daily probability of liberation on days when T_{di} was increased from baseline (Figure 1, adjusted HR 0.81, 95% CI 0.62-1.06, per 10% increase in T_{di}). Under the alternate (classification-based) analysis framework, 47 patients (25%) developed increased T_{di} during the first week of ventilation. After accounting for the competing risk of death, the risk of requiring

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ventilation for at least 3 weeks was significantly higher in patients with increased T_{di} (Figure 2, 43% vs. 22%, p=0.006). The risk of death was not significantly different (p=0.73). The development of increased T_{di} was also associated with prolonged ICU admission and a higher risk of complications, though these associations were not significant after multivariable adjustment (Table 2).

Changes in Diaphragm Thickness and Diaphragm Function

To substantiate the mechanistic basis for the relationship between changes in T_{di} and outcome, diaphragm function (measured by maximal diaphragm thickening fraction) was evaluated in 84 patients after 7 (IQR 5-9) days of ventilation (Figure E3). In comparison to patients with unchanged T_{di} (n=51 measurements), patients with >10% decrease in T_{di} (n=23 measurements) exhibited lower maximal thickening (26±17% vs. 38±24%, p=0.03, Figure E4) and more frequent severely reduced maximal thickening (48% vs 22%, p=0.03, Figure E4). Patients with increased T_{di} (n=8 measurements) had similarly reduced maximal thickening and increased frequency of severely reduced maximal thickening, but these differences did not reach statistical significance (30±22% vs. 38±24%, p=0.70; and 38% vs 22%, p=0.61, respectively, Figure E4).

Effect of Inspiratory Effort and Ventilator Settings on Diaphragm Thickness

T_{di} tended to decrease over time at low diaphragm thickening fraction levels and increase over time at high thickening fraction levels (Figure E5, p<0.001 for interaction between thickening fraction and time). Similarly, T_{di} tended to decrease at low EA_{di} levels and increase at high EA_{di} levels (Figure E5, p=0.02 for interaction between mean daily EA_{di} and time). Consistent with these findings, T_{di} declined more rapidly with higher levels of ventilator assistance (Figure E5).

In similar models, T_{di} was unaffected by SOFA score, SAS score, the presence of sepsis, fluid balance, or the set PEEP level (see also Table 1).

Inspiratory Effort and Duration of Ventilation

Given the foregoing results, we hypothesized that intermediate thickening fraction levels would be associated with the shortest duration of ventilation. In a *post hoc* exploratory analysis, duration of ventilation was lowest in patients with intermediate mean thickening fraction values between 15-30% over the first 3 days of ventilation (Figure 3). ICU length-of-stay and the risk of complications were also lowest in patients with this range of inspiratory effort (Figure E6).

Discussion

The central finding of this study is that the progressive development of diaphragm atrophy during mechanical ventilation is associated with prolonged mechanical ventilation and ICU admission and an increased risk of complications of acute respiratory failure. We also found that rapid early increases in diaphragm thickness predicted prolonged ventilation, raising the possibility of clinically significant diaphragm injury due to insufficient respiratory muscle unloading during ventilation (see discussion below). The rate and direction of change in diaphragm thickness varied with the level of inspiratory effort, either assessed by ultrasound or quantified by diaphragm electrical activity; diaphragm thickness was relatively stable at intermediate levels of inspiratory effort. We also report for the first time that the average level of diaphragm thickening fraction (an indirect measure of inspiratory effort) during the early course of ventilation is associated with the duration of ventilation: subjects with thickening fraction levels similar to those observed in healthy subjects at rest during the first three days of ventilation had the shortest duration of mechanical ventilation and ICU admission, while patients

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with either relatively lower or higher thickening fraction levels had a longer duration of ventilation and ICU admission (and a higher risk of complications). Taken together, these findings strongly suggest that changes in diaphragm structure and function due to mechanical ventilation are an important and potentially avoidable determinant of poor outcomes.

Several considerations support the hypothesis that diaphragm atrophy may be causally related to the risk of prolonged ventilation (36). First, there is a strong biological rationale for causation given the abundant experimental evidence of diaphragm atrophy due to ventilation. Experiments in a range of animal models have demonstrated that ventilation causes acute disuse atrophy, sarcomeric disarray, and contractile dysfunction in the diaphragm (11, 15, 17, 37). Diaphragm atrophy has been repeatedly demonstrated in mechanically ventilated patients by histology (13, 14, 38), computed tomography scanning (39), and ultrasound (16, 40-42). Second, diaphragm atrophy and injury due to mechanical ventilation would be expected to impact clinical outcomes by impairing diaphragm function (the putative causal pathway to poor outcome). Diaphragm dysfunction has been strongly linked to difficult weaning and poor clinical outcomes in a number of studies (7, 9, 22). Building on this work, we found that the development of diaphragm atrophy during ventilation was associated with impaired diaphragm function. Third, there was a consistent dose-response relationship between diaphragm atrophy and clinical outcomes across multiple end-points even after multivariable adjustment. Fourth, we found that inspiratory effort (the main modulator of changes in diaphragm thickness) predicted the duration of ventilation in a non-linear relation as predicted from the relationship between inspiratory effort and changes in diaphragm thickness. This finding strengthens the argument in support of a causal pathway linking the effect of ventilation on inspiratory effort to clinical outcomes and provides a basis to conceive of approaches to muscle-protective ventilation. Nevertheless, our findings cannot definitively confirm causality, which can only be demonstrated in the context of a randomized

study of interventions known to prevent deleterious changes in the diaphragm during mechanical ventilation.

Increases in T_{di} during ventilation also predicted prolonged ventilation and increased complications. The histological basis of increases in T_{di} during ventilation has not been characterized. Increased T_{di} may represent the accumulation of tissue edema related to inflammation, although changes in T_{di} were unrelated to fluid balance in our study and in a previous report (42). Increases in T_{di} were associated with relatively high levels of inspiratory effort, raising the possibility that such changes reflect load-induced muscle injury. Load-induced skeletal muscle injury is known to induce acute increases in muscle thickness and cross-sectional area on ultrasound (43). The diaphragm is vulnerable to load-induced injury (18), particularly when sensitized to mechanical stress by endotoxemia and muscular inflammation (20). Suppressing inspiratory effort by mechanical ventilation has been shown to mitigate load-induced diaphragm injury and inflammation (19, 20); failure to unload the respiratory muscles during ventilation may increase the risk of load-induced injury. Histological studies of diaphragm specimens obtained from mechanically ventilated patients have demonstrated features consistent with load-induced injury, including sarcomere disruption (14) and inflammation (38). This hypothesis merits further investigation.

Our findings suggest that prolonged ventilator-dependence due to deleterious diaphragmatic changes resulting from ventilation might be potentially mitigated by targeting levels of inspiratory effort during ventilation similar to that of healthy subjects breathing at rest. Previous work has shown that diaphragm inactivity causes diaphragm atrophy while excess inspiratory efforts can exacerbate ventilator-induced lung injury (44) and injure the diaphragm (18). Using two independent methods to indirectly estimate inspiratory effort (diaphragm thickening fraction measurements and diaphragm electrical activity) (27, 45-48), we found that T_{di} tended to

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decrease at lower inspiratory effort levels and increase at higher inspiratory effort levels, suggesting that some intermediate level may be optimal. Because competing issues of respiratory muscle oxygen consumption and lung injury can modify the safest inspiratory effort level during ventilation (44, 49), the optimal level of patient inspiratory effort during ventilation has been uncertain. In our study, the duration of ventilation was minimized in patients with thickening fraction levels similar to those observed in healthy subjects breathing at rest (50). These data raise the possibility that titrating ventilatory support to maintain this range of inspiratory effort might accelerate liberation from ventilation. This hypothesis of a 'muscle-protective' ventilation strategy requires confirmation in future trials.

This study has limitations. First, despite attempts to account for confounding, observed associations may derive from residual confounding related to unmeasured patient or illness characteristics, so that we cannot definitively conclude a causal effect. To account for immortal time bias in the association between changes in diaphragm thickness and duration of ventilation, we constructed a proportional hazards model where diaphragm thickness was modelled as a time-varying covariate. We also found consistent effects in a sensitivity analysis of patients who remained on the ventilator for at least 7 days.

Second, we did not record the use of non-invasive ventilation after extubation and consequently the frequency of non-invasive ventilation for post-extubation respiratory distress in our study is uncertain. This is not a routine practice in the ICUs participating in this study and the available evidence suggests that non-invasive ventilation probably merely delays re-intubation in patients with post-extubation respiratory distress (51, 52).

Third, we have no histological data correlating with the observed changes in diaphragm thickness. Diaphragm atrophy, however, has been documented repeatedly in clinical studies and animal models (13-15, 38) and decreases in diaphragm thickness were associated with decreased

myofiber cross-sectional area in an animal model (37); we believe it reasonable to assume that decreases in diaphragm thickness observed in our study represent atrophy. As mentioned earlier, the histological basis of increases in diaphragm thickness during ventilation remains uncertain.

Fourth, measurements were not collected on weekends, resulting in some missing values for T_{di} measurements. To mitigate this problem, we did not enroll patients on Fridays. Neither the distribution of changes in T_{di} nor the clinical outcome were related to the day of the week on which the patient was intubated. Consequently, any such missing values would be expected to bias the effect towards the null.

Fifth, quantifying diaphragm function by maximal diaphragm thickening fraction relies upon patient volitional effort, potentially limiting measurement reliability in a clinical context where it is sometimes challenging to obtain maximal volitional efforts. Previous studies have shown that maximal thickening is correlated with maximal inspiratory pressure in critically ill patients and predicts weaning trial success (28, 53). We did not record maximal inspiratory pressures or respiratory mechanics; maximal airway pressures also require adequate volitional effort and respiratory mechanics can be challenging to reliably assess in spontaneously breathing patients. Thickening fraction has been correlated with airway pressures obtained by the gold standard technique, twitch magnetic phrenic nerve stimulation (9, 22).

Sixth, we did not measure post-hospital outcomes. The importance of patient-centered outcomes including functional status and health-related quality of life is now widely appreciated (54). Prolonged ICU admission has been shown to increase the risk of poor functional outcomes (5, 55); it is therefore possible that changes in diaphragm structure and function during ventilation (strongly associated with prolonged ICU admission in our study) could impact functional recovery in survivors. Future studies should address whether preventing or treating

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diaphragm atrophy and dysfunction in ventilated patients can improve their long-term functional status.

In summary, the progressive development of diaphragm atrophy during the early course of mechanical ventilation predicts prolonged ventilation and an increased risk of complications of acute respiratory failure. Similar but weaker results were found for increased T_{di}. Efforts to prevent and treat diaphragm atrophy or increased T_{di} may significantly improve outcomes in patients with acute respiratory failure.

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Table 1. Demographic and clinical characteristics of the study population

Characteristics	Study Population (n=191)	Patients at risk of change in diaphragm thickness (≥ 2 diaphragm thickness measurements) (n=191)			p-value
		≥10% decrease in thickness (n=78, 41%)	<10% change in thickness (n=66, 35%)	≥10% increase in thickness (n=47, 24%)	
Age (years) mean (SD)	59 (15)	59 (16)	61 (13)	57 (16)	0.32
Female sex (n, %)	74 (39%)	33 (42%)	24 (36%)	16 (34%)	0.61
Body mass index (kg/m^2)	26.0 (22.3-30.1)	24.1 (21.3-28.5)	27.6 (22.8-31.2)	26.4 (23.4-30.1)	0.07
SAPS II	48 (34-58)	46 (35-57)	50 (36-58)	45 (33-64)	0.63
SOFA (mean over first 72 hours)	10 (8-14)	10 (8-14)	10 (7-13)	11 (8-15)	0.35
Primary cause of acute respiratory failure (n, %)					0.37
Respiratory	60 (31%)	27 (35%)	22 (33%)	11 (23%)	
Cardiovascular	26 (14%)	11 (14%)	9 (14%)	6 (13%)	
Sepsis (non-pulmonary)	26 (14%)	10 (13%)	12 (18%)	4 (9%)	
Neurological	18 (9%)	5 (6%)	5 (8%)	7 (15%)	
Post-operative	17 (9%)	5 (6%)	5 (8%)	7 (15%)	
Post-transplantation	29 (15%)	14 (18%)	10 (15%)	5 (11%)	
Other (hepatic, renal, intoxication)	16 (8%)	6 (8%)	3 (5%)	7 (15%)	
Sepsis-3 criteria present in first 48 hours (n, %)	170 (89%)	70 (90%)	59 (89%)	40 (85%)	0.71
Comorbidity at baseline (≥1) (n, %)	121 (63%)	46 (59%)	47 (71%)	28 (60%)	0.26
Baseline PaO_2/FiO_2 (mm Hg)	159 (105-233)	154 (115-215)	160 (96-242)	166 (105-212)	0.85
Initial diaphragm thickness (mm)	2.3 (2.0-2.6)	2.6 (2.2-3.0)	2.3 (2.0-2.4)	2.0 (1.7-2.3)	<0.01
Cumulative fluid balance on day 3 of ventilation (litres)	4.6 (2.1-8.1)	4.7 (2.1-8.7)	3.8 (1.7-7.3)	5.7 (3.2-8.4)	0.17
Number of measurements prior to classification	2 (2-3)	2 (2-3)	3 (2-4)	2 (2-3)	0.16
Days of ventilation before classification into thickness change group	4 (3-5)	4 (3-5)	4 (3-5)	3 (3-4)	0.13
Baseline Ventilator Settings					
Mode of Ventilation					0.28
Controlled	156 (81%)	67 (86%)	53 (80%)	35 (74%)	
Partially Assisted	36 (19%)	11 (14%)	13 (20%)	12 (26%)	
Vt (ml/kg PBW)	6.6 (5.8-7.7)	6.4 (5.4-7.6)	7.1 (6.2-8.1)	6.4 (5.7-7)	0.02
Peak airway pressure (cm H_2O)	20 (14-26)	21 (16-25)	20 (13-27)	20 (15-26)	0.99
Set inspiratory pressure above PEEP (cm H_2O)[a]	12 (6-15)	12 (8-16)	12 (8-15)	11 (5-15)	0.50
PEEP (cm H_2O)	8 (5-10)	8 (5-10)	8 (5-10)	8 (5-10)	0.97
Frequency (min^{-1})	21 (18-25)	22 (19-26)	21 (18-25)	21 (18-24)	0.79
FiO_2	0.45 (0.35-0.5)	0.44 (0.39-0.5)	0.45 (0.35-0.55)	0.45 (0.35-0.52)	0.93
Arterial Blood Gas Tensions					
pH	7.37 (7.33-7.41)	7.37 (7.33-7.42)	7.38 (7.33-7.41)	7.37 (7.33-7.42)	0.99
$PaCO_2$	40 (34-47)	41 (36-49)	41 (34-46)	38 (34-44)	0.13
PaO_2	97 (80-115)	103 (81-113)	98 (80-122)	88 (80-108)	0.55

All distributions are reported as median (interquartile range) unless otherwise noted

[a]Set inspiratory pressure above PEEP = peak airway pressure - PEEP

Table 2. Clinical outcomes in relation to changes in diaphragm thickness during mechanical ventilation

Outcome	Initial Change in Diaphragm Thickness During First Week of Ventilation Patients with ≥2 measurements (n=191)			Statistical Comparisons Adjusted count ratio or adjusted odds ratio (95% CI)[c]	
	≥10% decrease in thickness (n=78, 41%)	<10% change in thickness (n=66, 35%)	≥10% increase in thickness (n=47, 24%)	≥10% decrease in thickness vs. <10% change in thickness	≥10% increase in thickness vs. <10% change in thickness
Ventilator-free days to day 60	46 (0-53)	51 (0-55)	37 (0-51)	0.77 (0.59-1.00)	0.91 (0.67-1.22)
Duration of ventilation (in ICU survivors) (days)	9 (5-17)[d]	5 (4-9)	10 (6-22)[d]	1.69 (1.28-2.24)	1.38 (1.00-1.90)
Duration of ICU admission (in ICU survivors) (days)	12.5 (7-21)[d]	8 (5-12)	14 (7-24)[d]	1.71 (1.29-2.27)	1.31 (0.94-1.83)
Duration of hospitalization (in hospital survivors) (days)	29 (16-58)[d]	22 (11-51)	30 (17-65)	1.44 (1.01-2.05)	1.23 (0.71-1.60)
Complications of acute respiratory failure[b] (%)	49 (64%)[d]	31 (48%)	31 (67%)[d]	3.00 (1.34-6.72)	1.84 (0.77-4.43)
Reintubation (%)	16 (21%)[d]	5 (8%)	12 (26%)[d]	3.55 (1.14-11.05)	3.24 (0.97-10.88)
Tracheostomy (%)	20 (26%)[d]	7 (11%)	11 (23%)	3.58 (1.29-9.97)	2.11 (0.66-6.70)
Mechanical ventilation > 14 days (%)	27 (35%)[d]	14 (21%)	20 (43%)[d]	2.97 (1.26-6.97)	2.16 (0.87-5.40)
Readmission to ICU during same hospital admission (%)	5 (7%)	9 (15%)	9 (20%)	0.78 (0.21-2.84)	2.32 (0.70-7.67)
Death in ICU (%)	19 (24%)	12 (18%)	11 (23%)	1.55 (0.61-3.95)	1.28 (0.45-3.65)
Death in hospital (%)	28 (37%)	21 (33%)	17 (37%)	1.66 (0.73-3.76)	0.94 (0.38-2.34)

All distributions are reported as median (interquartile range) unless otherwise noted

[a]Primary end-point; computed for all patients (including patients with only 1 measurement of diaphragm thickness), n=211

[b]Complications of acute respiratory failure include reintubation, tracheostomy, duration of ventilation > 14 days, or death in hospital

[c]Adjusted for age, SAPS II score, baseline Riker Sedation Agitation Scale score, exposure to neuromuscular blockade during first week of ventilation, presence of sepsis at baseline, baseline SOFA, baseline PaO_2/FiO_2 ratio, initial diaphragm thickness, and presence of any chronic comorbidity

[d]$p<0.05$ for difference in outcome in comparison to patients with <10% change in diaphragm thickness from baseline in bivariate (unadjusted) analysis

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Figure Legends

Figure 1. The probability of liberation from mechanical ventilation on any given day is related to the magnitude of change in diaphragm thickness from baseline. The probability of liberation was lower on days when diaphragm thickness was decreased from baseline (adjusted hazard ratio 0.69, 95% CI 0.54-0.87, per 10% decrease in thickness below baseline). The probability of liberation was not significantly lower on days when diaphragm thickness was increased from baseline though a similar trend was observed (adjusted hazard ratio 0.81, 95% CI 0.62-1.06, per 10% increase in thickness above baseline). Grey shaded areas represent 95% confidence intervals for estimated relative hazards.

Figure 2. Changes in diaphragm thickness during the first week of ventilation predict an increased risk of prolonged mechanical ventilation. Patients are disconnected from the ventilator because of either liberation (solid lines) or death (dashed lines). Compared to patients without changes in diaphragm thickness, those in whom diaphragm thickness decreased or increased during the first week of ventilation had a significantly lower cumulative incidence of liberation from the ventilator at day 21 (*, p=0.008 and p=0.006, respectively). The cumulative incidence of death was not significantly different between groups at day 21 (p=0.46 and p=0.90, respectively).

Figure 3. The duration of ventilation varies with the level of inspiratory effort (assessed indirectly by diaphragm thickening fraction) during the early course of ventilation. The study population was divided according to quantiles of the average thickening fraction over the first 3 days of ventilation. The number of patients in each quantile is displayed on the plot. Duration of ventilation was prolonged in patients with either relatively low or relatively high average levels of inspiratory effort over the first 3 days of ventilation (p=0.02 for non-linear relation). The shortest duration of ventilation was observed in patients with intermediate levels of thickening fraction in the range of 15-30%, similar to values observed during resting quiet breathing in healthy adults (50). After multivariable adjustment, the duration of ventilation remained significantly higher in patients with mean thickening fraction < 15% (n=94, adjusted duration ratio 1.42, 95% CI 1.08-1.88). Adjusted duration of ventilation was not significantly different in patients with mean thickening fraction > 30% (n=12) compared to patients with intermediate thickening fraction of 15-30% (n=38), although the adjusted effect size was similar (adjusted duration ratio 1.42, 95% CI 0.87-2.29).

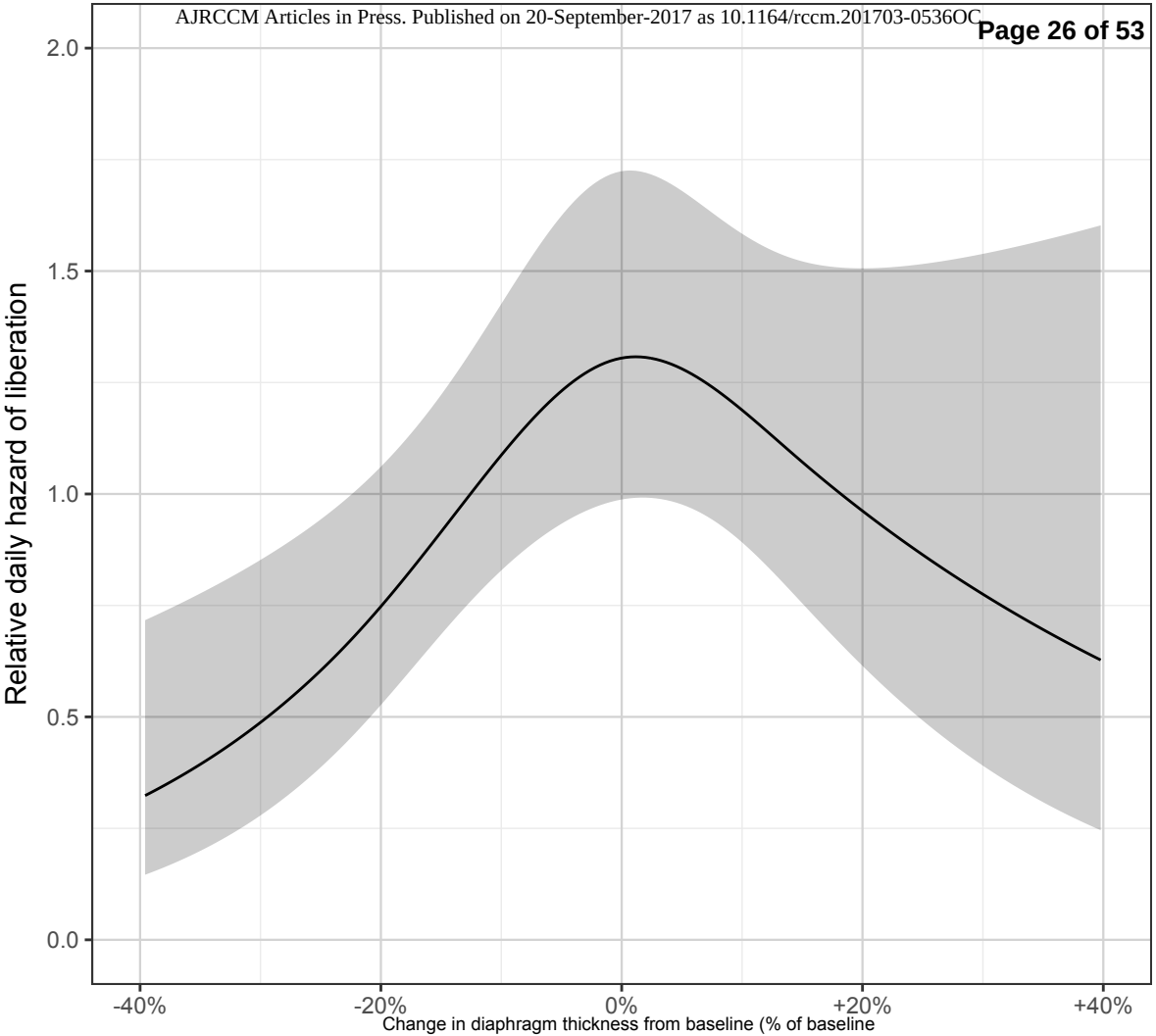

Figure 1

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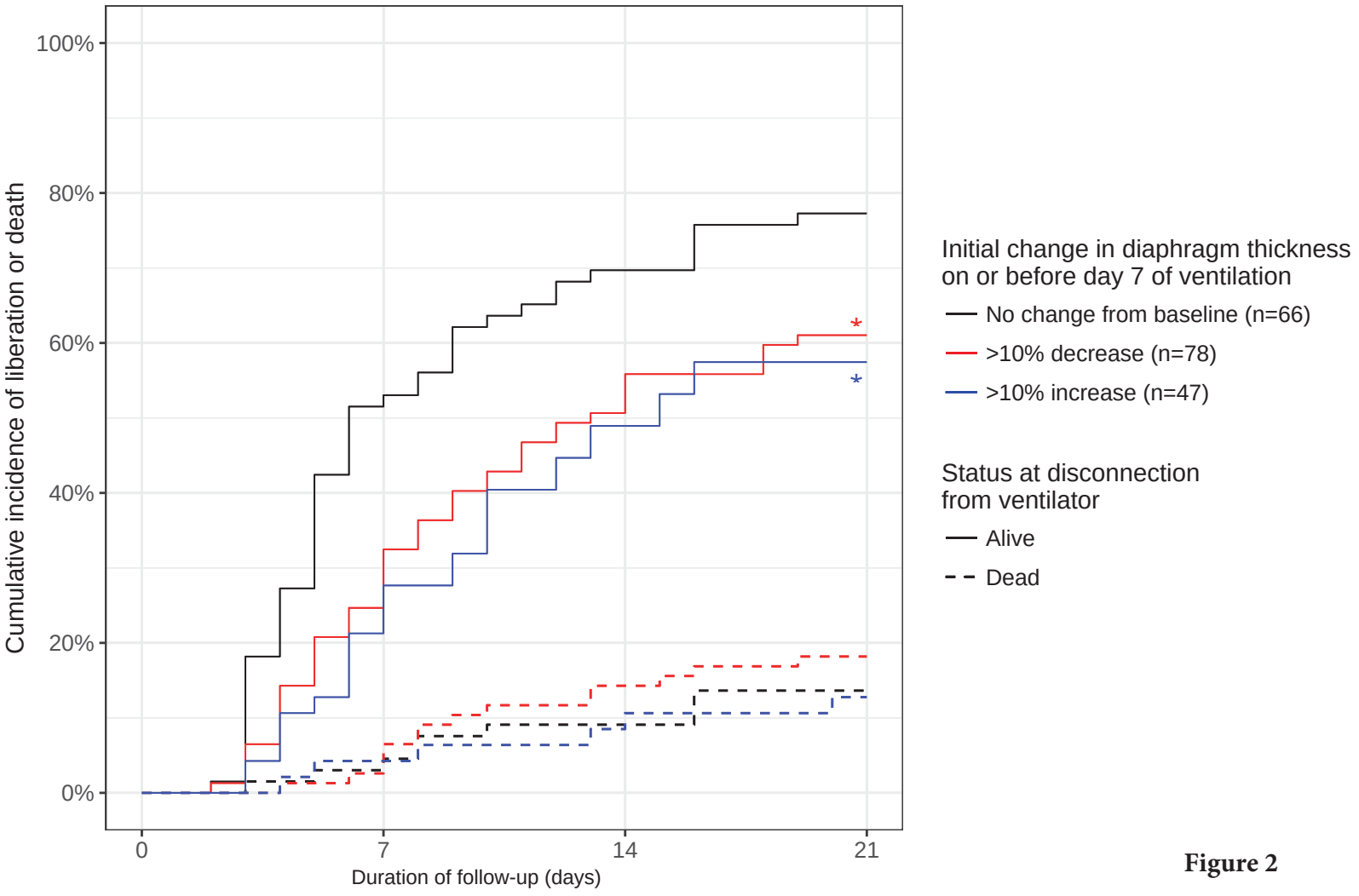

Figure 2

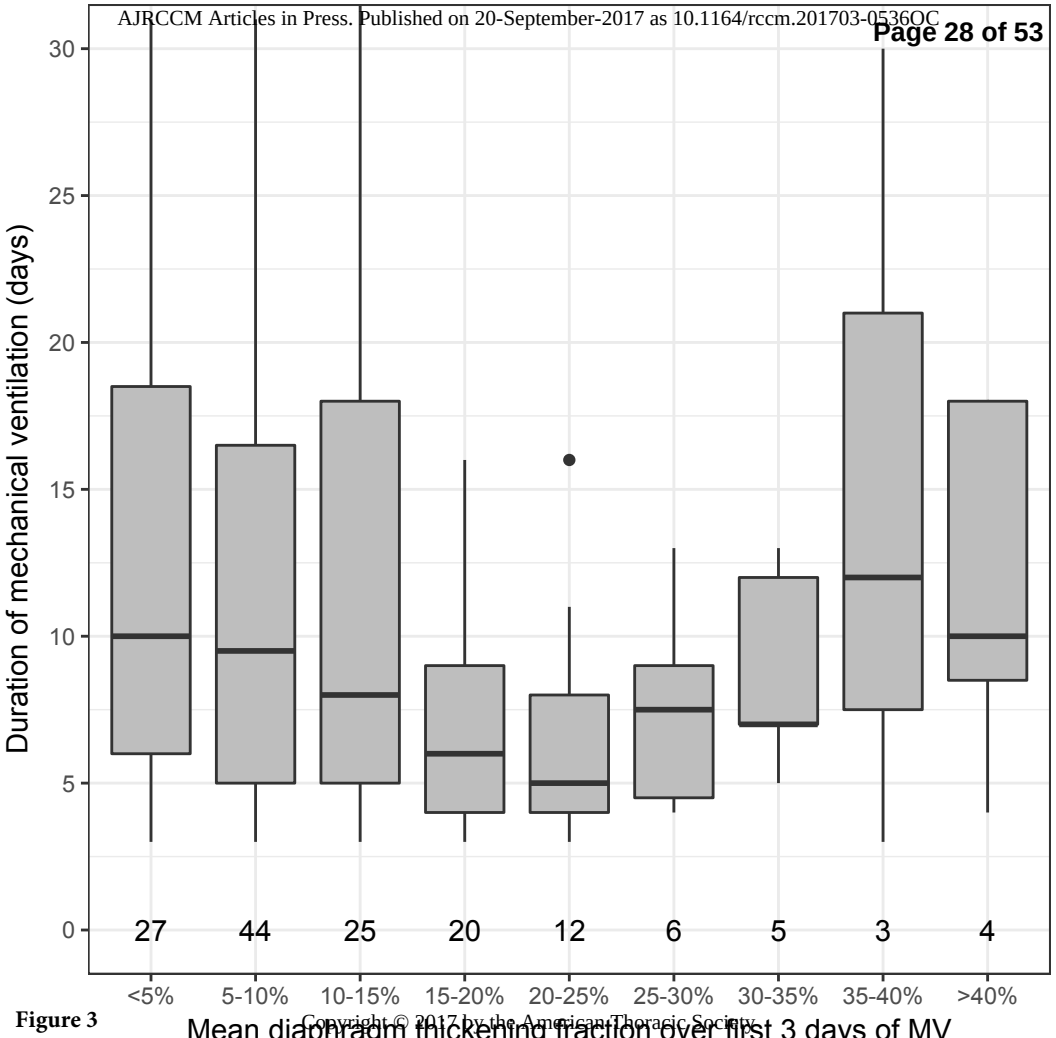

Figure 3

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Online Data Supplement

Mechanical Ventilation-Induced Diaphragm Atrophy Strongly Impacts Clinical Outcomes

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1 Inclusion and Exclusion Criteria for the Research Protocols

For this study of clinical outcomes in patients with acute respiratory failure, patients were drawn from two separate but closely-related and complementary cohorts studies (both of which received full approval from the research ethics boards of the participating institutions). The similarities and differences of the design characteristics of these two research studies are summarized in Table E1 below. Differences in the studies are highlighted and primarily reflect differences in the target study population. Eligibility criteria for Cohort A were designed to maximize feasibility of measurements and sample size. Eligibility criteria for Cohort B were designed to acquire detailed physiological data on diaphragm activity and changes in diaphragm function over time. Given the time- and resource-intensive measurements in Cohort B, the study was designed to enroll patients at high risk of remaining on the ventilator for at least 7 days to maximize data collection and opportunity for changes in diaphragm thickness and function (if present) to occur. Some findings from an early subset of the cohort enrolled in Protocol A have been published (1) and some preliminary findings from Cohort B have been presented at international meetings (2).

These studies were deliberately planned so that patient outcomes could be analyzed in combination and outcome analysis commenced once the combined studies reached the pre-specified sample size target.

Table E1. Design characteristics of the two cohort studies enrolling patients for this clinical outcomes study

Study Parameter	Cohort A	Cohort B
Objective	• To describe the changes in diaphragm thickness over time during mechanical ventilation • To ascertain the influence of mechanical ventilation settings and inspiratory effort under mechanical ventilation on diaphragm thickness • To examine the relationship between diaphragm thickness and clinical outcomes	• To ascertain the relationship between diaphragm inactivity and changes in diaphragm thickness over the early course of mechanical ventilation • To precisely quantify the variation in diaphragm activity within and between patients during the early course of mechanical ventilation • To examine the impact of changes in diaphragm thickness and function on clinical outcomes
Inclusion Criteria	Patients receiving invasive ventilation for less than 36 hours* for acute respiratory failure of any cause.	Patients receiving invasive ventilation for less than 36 hours** for: • Acute severe brain injury (GCS < 8 prior to intubation) • Moderate or severe acute respiratory distress syndrome (P/F < 200 mm Hg) • Septic shock (proven or suspected infection, 2 SIRS criteria satisfied, patient requiring vasopressor support) • Pneumonia (clinically suspected or confirmed)
Exclusion Criteria	• Anticipated duration of ventilation from time of screening is less than 24 hours • Mechanical ventilation for more than 48 hours in the preceding 6 months	• Predicted probability of remaining alive and on the ventilator on ICU day 7 is less than 50% (PI judgment based on clinical data) • Liberation from MV anticipated within 24 hours of screening • High cervical spine injury • Previously diagnosed neuromuscular disease • Acute exacerbation of obstructive lung disease • Known esophageal varices or esophageal injury or recent upper GI tract surgical procedures • Mechanical ventilation for more than 48 hours in the preceding 6 months
Number of centers participating in study	Multi-center (3 ICUs at 2 institutions)	Single-center (2 ICUs at 1 institution)
Time period of patient enrolment	May 2013 to January 2016	January 2014 to March 2016
Ultrasound measurements	Diaphragm thickness and thickening fraction were measured once daily for the first 14 days of mechanical ventilation (or until death or extubation, if earlier)	Diaphragm thickness and thickening fraction were measured once daily for the first 14 days of mechanical ventilation (or until death or extubation, if earlier)
Clinical measurements	• Demographic and clinical characteristics • Critical illness characteristics (cause, baseline severity, evolution of illness)	• Demographic and clinical characteristics • Critical illness characteristics (cause, baseline severity, evolution of illness)
Diaphragm electrical activity measurements	n/a	Recorded on an hourly basis from time of enrolment until completion of 7 days of mechanical ventilation (or liberation or death, if prior to 7 days of MV) – see below for details of measurement technique
Diaphragm function	Maximal diaphragm thickening fraction was measured after 1	Maximal diaphragm thickening fraction was measured after

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Study Parameter	Cohort A	Cohort B
measurements¶	week of MV (or on day of extubation if earlier than 1 week)	completing the monitoring protocol (after 7 days of MV or just prior to liberation from MV)
Clinical outcome measurements	• Duration of mechanical ventilation • Survival status at hospital discharge • Ventilator-free days to day 60 • Reintubation • Tracheostomy	• Duration of mechanical ventilation • Survival status at hospital discharge • Ventilator-free days to day 60 • Reintubation • Tracheostomy

*Initially, patients were enrolled up 72 hours from intubation; this was modified to minimize time from intubation to first measurement after the first 53 subjects were enrolled upon observing that most subjects could be enrolled within 36 hours.
**Initial duration of ventilation prior to enrolment was 12 hours; this was modified to 36 hours after observing that patients could not feasibly enrolled within that time frame.
¶This measurement was added to the study after the first 100 subjects were enrolled (Cohort 1) and the first 10 subjects were enrolled (Cohort 2) upon recognition that this measurement could be employed as a feasible means of assessing diaphragm function.

2 Measurement Techniques

2.1 Technique for measuring diaphragm thickness and thickening fraction by ultrasound

The thickness of the right hemidiaphragm was measured using a high frequency (13 MHz) linear array transducer placed in the 9th or 10th intercostal space between the anterior and mid-axillary lines in the zone of apposition (3). Ultrasound probe placement location was marked to enhance day-to-day measurement consistency. Four separate consecutive measurements of end-expiratory diaphragm thickness were obtained at each measurement session (4). Diaphragm contractile activity was quantified by measuring the percentage change in diaphragm thickness in the zone of apposition from end-expiration to end-inspiration. Measurements were repeated and averaged over several consecutive breaths. The observers (no prior ultrasound experience) underwent two weeks of one-to-one training (2-3 ultrasound exams/day) with an experienced investigator; competence was demonstrated by achieving agreement within 0.1 mm on 15 consecutive ultrasound examinations.

Diaphragm thickness measurements were obtained on a daily basis (from Monday to Friday) for the first 14 days of mechanical ventilation.

2.2 Diaphragm electrical activity monitoring protocol

Upon enrollment, a nasogastric catheter fitted multiple-array electrodes to acquire crural diaphragm electromyograms (EMG) was placed. EMG signals were acquired, filtered and processed to obtain the diaphragm EMG root mean square (diaphragm electrical activity, ΔEdi) by the Servo-I mechanical ventilator (Maquet, Solna, Sweden) according to previously published methods (5). Airway pressure (Paw), flow and EAdi were recorded in real time at a sampling frequency of 62.5 Hz by a personal computer connected to the ventilator using dedicated software (Neurovent Inc., Toronto, Canada). Study catheter positioning was confirmed by the electrode signals. Positioning was reconfirmed on a daily basis for the duration of the study.

Hourly signal recordings were analyzed using automated analysis software (Orion, Neurovent Inc., Toronto, Canada). This software uses a previously validated algorithm (6) to identify the onset, peak and end of inspiratory effort using the EAdi signal and to identify the onset and end of mechanical 'breaths' delivered by the ventilator. To quantify the hourly diaphragm activity level and ventilator support level, ΔEAdi and ΔPaw were averaged across all mechanical or spontaneous breaths for each 5-minute recording.

2.3 Diaphragm function measurement protocol

At a later stage in the study, we undertook to estimate diaphragm function in study participants by measurement of maximal diaphragm thickening fraction ($TF_{di,max}$). $TF_{di,max}$ was measured after 1 week of mechanical ventilation (once the patient was awake and breathing spontaneously) during coached maximal inspiratory efforts (7-9) while in CPAP mode. We employed a variety of maneuvers to achieve maximal inspiratory effort (transient reduction of ventilator setting, coached inspiratory effort, transient occlusion of the endotracheal tube if necessary). The observer measuring $TF_{di,max}$ was blinded to the change in diaphragm thickness over time. If the patient was extubated before 1 week of ventilation was completed, $TF_{di,max}$ was measured on the day of extubation (either before or immediately after extubation). In participants who were unable to follow instructions, the endotracheal tube was transiently occluded to stimulate

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maximal inspiratory efforts during $TF_{di,max}$ measurement (10). The highest value obtained for $TF_{di,max}$ during repeated measurements was taken as the measurement of muscle function.

3 Routine Weaning Practices in Participating Intensive Care Units

This study enrolled patients admitted to medical-surgical intensive care units in three hospitals in Toronto. Weaning technique was not standardized in this study but weaning practices were generally similar at all three sites. At all sites, weaning is managed by respiratory therapists using locally established protocols. Respiratory therapists assess mechanically ventilated patients on a daily basis for readiness-to-wean criteria. These criteria, which vary slightly between intensive care units, include the presence of patient-triggered ventilator breaths, $FiO_2 \leq 40\text{-}50\%$, PEEP≤8-10 cm H_2O, and absence of hemodynamic and respiratory instability). Patients who satisfy these criteria are routinely subjected to a daily trial of spontaneous breathing. Over the period that the study was conducted, spontaneous breathing trials were conducted with low levels of PEEP and inspiratory pressure augmentation, typically CPAP 5 cm H_2O or PSV 5 cm H_2O with PEEP 5 cm H_2O, depending on clinician discretion and preference. Spontaneous breathing trial success was defined according to conventional criteria including presence or absence of respiratory distress, hemodynamic instability, hypoxemia, and a rapid shallow breathing pattern (11). The decision to extubate was made by the interprofessional clinical team, incorporating the results of the spontaneous breathing trial along with other relevant clinical data.

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4 Variables Under Analysis: Rationale For Inclusion And Handling Of Missing Data

4.1 Selection of covariates for statistical model – a priori rationale

Here we provide a rationale for the pre-specified inclusion of the key covariates in the statistical models of clinical outcomes and the rates of missingness for each covariate.

Table E2. Rates of missing values for variables under analysis

Variable	Rationale for inclusion	Rate of missingness
Time to final disconnection from mechanical ventilation (due to liberation or death)	Primary outcome of interest	2/216 (0.9%)
Survival status at time of final disconnection from mechanical ventilation (alive or dead)	Primary outcome of interest	1/216 (0.5%)
Survival status at time of hospital discharge	Secondary outcome	6/216 (2.7%)
Survival status at day 60	Secondary outcome	5/216 (2.3%)
Ventilator-free days to day 60	Secondary outcome	6/216 (2.7%)
Reintubation	Secondary outcome	0/216 (0%)
Tracheostomy	Secondary outcome	0/216 (0%)
Prolonged ventilator-dependence (time to liberation > 14 days)	Secondary outcome	2/216 (0.9%)
Complications of respiratory failure (composite of at least one of reintubation, tracheostomy, time to liberation > 14 days, death in hospital)	Secondary outcome	4/216 (1.9%)
Time to ICU discharge	Secondary outcome	3/164 (1.8%) [due to transfers to other institutions prior to ICU discharge]
Time to hospital discharge	Secondary outcome	8/142 (5.6%) [due to transfers to other institutions prior to hospital discharge]
Change in diaphragm thickness as a percentage of baseline (see details re classification below)	Primary covariate under analysis – measurement intended to reflect the development of structural changes in the diaphragm during mechanical ventilation	3/216 (1.4%) [due to failure to obtain baseline diaphragm thickness measurement within 72 hours of intubation]
Baseline diaphragm thickness (measured within 72 hours of intubation)	Covariate under analysis – found to be predictive of changes in diaphragm thickness during descriptive analysis, so added to model to ensure that effect of change in diaphragm thickness on outcome is independent of baseline thickness	3/216 (1.4%) [due to failure to obtain baseline diaphragm thickness measurement within 72 hours of intubation]
Age (years)	Clinical characteristic with important impact on clinical outcomes	0/216 (0%)
SAPS II score	Baseline severity of illness – clinical characteristic with important impact on clinical outcome	0/216 (0%)

SOFA score (average value over first 3 days of study)	Daily severity of multi-organ failure – clinical characteristic with important impact on both changes in diaphragm thickness (as per previous report (1) and clinical outcome).	11/216 (5.1%) - see discussion below for handling of missing data in SOFA score computation
Sepsis at baseline (defined as suspected/presumed infection resulting in systemic organ dysfunction on either day 1 or day 2, ascertained by SOFA score ≥ 2 and on treatment with antibiotics) (12)	Known determinant of muscle function and clinical outcomes.	0/216 (0%) – for 11 subjects where SOFA score was missing at baseline, antibiotic exposure was known to be negative, so that sepsis was classified as absent.
Exposure to neuromuscular blockade at any time in the first week of ventilation	Known determinant of muscle function and clinical outcomes	0/216 (0%)
Riker Sedation Agitation Scale score (average value over first 3 days of study)	Sedation may suppress inspiratory effort and is a known determinant of clinical outcomes	0/216 (0%)
Presence of baseline comorbidity (of any of the following conditions: congestive heart failure, chronic obstructive pulmonary disease, interstitial lung disease, end-stage renal disease, liver cirrhosis, metastatic cancer, or immunosuppression)	Clinical characteristic with important impact on clinical outcomes (13)	0/216 (0%)
PaO_2/FiO_2 at baseline	Clinical characteristic with important impact on clinical outcomes	0/216 (0%)

4.2 Handling of missingness in SOFA score components

Because the availability of SOFA score data was contingent upon the clinical decision to collect the relevant measurements (i.e. arterial blood gas), rates of missingness in some components were high, as follows:

Platelet count – 29/1564 (1.9%)
Bilirubin – 203/1564 (13.0%)
Hypotension – 13/1564 (0.9%)
Glasgow Coma Scale – 14/1564 (0.9%)
Creatinine – 29/1564 (1.9%)
P/F ratio – 200/1564 (12.8%)

To maximize availability of data for SOFA computation, the following procedure was carried out:

1. Missing data for individual components were replaced by the last observation carried forward technique, which was deemed suitable given the correlated nature of sequential laboratory measurements and given that it seems likely that such data are missing at random (14).

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2. Missing data in SOFA components that persisted after this procedure were then imputed using the subject median value (50 measurements of bilirubin, 7 measurements of P/F ratio)
3. Missing data in SOFA components that persisted after this procedure (i.e. 19 measurements of bilirubin in 3 subjects in whom bilirubin was never measured, missing values of bilirubin were imputed from the study population mean).
4. SOFA was then computed from the various components (0 missing values).

4.3 Handling of missing data in parameters employed to ascertain sepsis

Details on handling SOFA score missingness discussed above. Sepsis was defined as per the Sepsis-3 definition. Patients were considered to have a suspected/presumed infection when any antibiotics were prescribed. Exposure to antibiotics was documented on each study day. Missing data for antibiotic exposure (26/1564, 1.6%, were replaced using the last observation carried forward technique). After this procedure, there were no persistent missing values.

4.4 Handling of extreme outliers in change in diaphragm thickness from baseline

Diaphragm thickness was computed as the percentage change in thickness from baseline. Inspection of the distribution of the percentage change in baseline revealed a limited number of extreme outliers in percentage increase in T_{di} from baseline ($\geq$60% increase in T_{di}, >99th percentile of distribution, 14 out of 954 measurements). We chose to remove these measurements from the dataset on the grounds that they were highly likely to represent measurement error.

5 Method for Classifying Diaphragm Thickness Change for Secondary Analyses

When the analysis plan was initially developed, we intended to classify patients according to the percentage change in diaphragm thickness from baseline to day 4 of mechanical ventilation, reasoning that this represented the time period when early changes in diaphragm thickness attributable to mechanical ventilation occurred based on our prior observations (1). However, because a considerable subset of patients were initially extubated before day 4 (45 patients, 21% of study population), and because ultrasound measurements were not collected on Saturday or Sunday, a substantial number of day 4 measurements were unavailable (n=110 patients with unavailable measurements on day 4). Furthermore, while many subjects develop changes in diaphragm thickness before day 4 of mechanical ventilation, in some patients this change develops after day 4, and this approach to classification therefore fails to adequately select patients who develop changes in diaphragm thickness during mechanical ventilation.

We decided to classify patients according to their initial change in diaphragm thickness based on the percentage change in diaphragm thickness from baseline on the first day that this percentage change exceeded 10% (Figure E1). This 10% threshold was selected in accordance with our previous study (1) based on the measurement resolution of the ultrasound technique (15, 16) and in accordance with previous studies of myopathy in critical illness and respiratory disease (17-19).

The day on which the patient was classified was recorded. This approach was employed in order to classify patients according to the earliest changes in diaphragm thickness following initiation of ventilation while permitting some flexibility in the timing of the development of these changes. Patients were classified as “unchanged” if the diaphragm thickness did not change by more than 10% from baseline over the first week of ventilation; for these patients, the day of classification was the day on which the final measurement of diaphragm thickness was obtained before the first week of mechanical ventilation was complete (i.e. if patients were liberated or died on day 4, day 4 was the day on which the change in diaphragm thickness was classified as ‘unchanged’; if the patient was liberated on day 10, day 7 was the day on which the patient was classified). Figure E1 below illustrates this classification approach. Note that this classification system differs from the classification system employed in our previous report, where diaphragm thickness changes were classified according to the final measurement in all patients in the first week of mechanical ventilation. The classification system was revised for the present analysis in order to classify patients at the earliest stage possible. Using this classification system, there were no statistically or clinically significant differences in the timing of classification between groups of patients (see Table 1, main manuscript).

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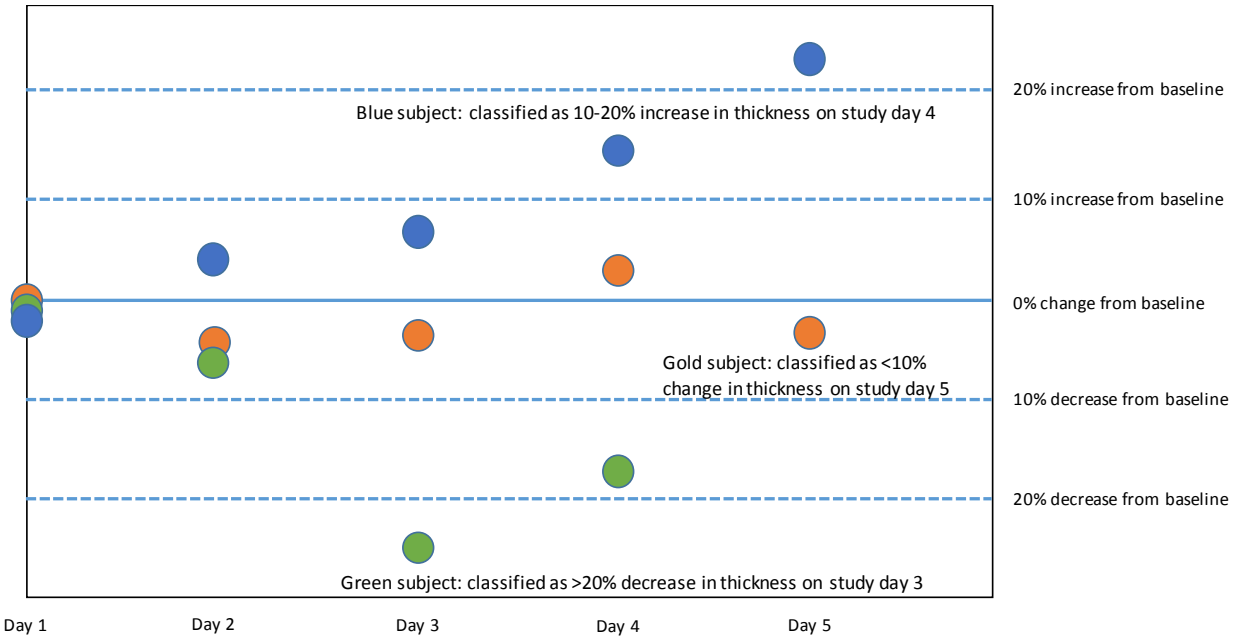

Figure E1. System employed to classify patients according to earliest significant diaphragm thickness change. Three representative subjects (gold, green, blue) illustrate potential classification results. The Gold subject never exceeds 10% change in diaphragm thickness and is classified as "<10% change" on the final measurement day in the first week of MV. The Green subject exhibits a marked drop in diaphragm thickness and crosses the 10% threshold on day 3. On that day, the decrease in diaphragm thickness is 25% and so the patient is classified accordingly. The Blue subject exceeds the 10% change threshold on day 4. On that day, the increase in diaphragm thickness is 15% so the patient is classified accordingly.

6 Statistical Analysis Plan

6.1 Primary outcome: relative hazard of liberation from mechanical ventilation

The primary preplanned analysis was designed to assess the relationship between T_{di} (expressed as a percentage of baseline T_{di}) on any given day and the hazard of liberation from mechanical ventilation on that day. Patients were deemed liberated from the ventilator once they were extubated (or completed 24 hours of tracheostomy mask breathing) without requiring reinstitution of ventilatory support during the index ICU admission.

We chose to conduct this analysis using a multivariable cause-specific Cox proportional hazards model. The model was designed based on the following considerations:

- Time to liberation from mechanical ventilation is affected by two competing processes: recovery from illness and death. We accounted for the competing risk of death by computing the cumulative incidence function each of these end-points (20, 21). Given that the cumulative incidence of death was low (<20%) and did not differ significantly between thickness changes groups, we deemed it reasonable to employ Cox proportional hazards modelling treating death as a censored event (i.e. cause-specific Cox model). Moreover, when an etiological question is under analysis as in the case of this study, a cause-specific Cox proportional hazards model is preferred over the use of cumulative risk regression models utilizing Fine-Gray subdistribution hazards (20).
- The pre-specified model covariates for multivariable adjustment included patient age, baseline SAPS II score, early SOFA score (average over first 48 hours), presence of sepsis at baseline, baseline PaO_2/FiO_2 ratio, and the presence of ≥1 comorbidity. During descriptive analysis, we found that the early changes in T_{di} were associated with the baseline T_{di} and that baseline T_{di} was inversely associated with duration of mechanical ventilation (see below). Therefore, we decided to incorporate baseline T_{di} as an important potential confounding variable in the primary model.

We modelled the exposure (T_{di} as a percentage of the baseline value) as a continuously varying time-varying covariate. Patients with only 1 measurement of Tdi were excluded from the analysis. This approach compares the hazard of liberation during intervals when diaphragm thickness is close to baseline vs. intervals when it varies more widely from baseline. We chose this approach for two reasons. First, this approach matches the real world situation in which diaphragm thickness does in fact vary over time and avoids the need to classify patients into different "T_{di} change" groups. Second, it helps to avoid systematic bias in the exposure variable (change in T_{di}) in relation to the duration of ventilation. Patients on the ventilator for longer are at higher risk of change in T_{di}, and this fact may introduce a systematic bias into the relation between change in T_{di} and time to liberation. Constructing the model with T_{di} as a time-varying covariate avoids this issue.

This time-varying covariate model was constructed by splitting the interval between each diaphragm thickness ultrasound measurement into separate intervals (n=928 intervals). The percentage change in T_{di} from baseline was computed for each interval. We then restricted the intervals under analysis to those where patients were "at risk of liberation" (i.e. where ventilator settings were low enough such that patients where the decision to extubated was at least possible: PEEP ≤ 10 cm H_2O and FiO2 ≤ 0.5), yielding 646 intervals. SOFA and sepsis were also treated as time-varying covariates in this model. Non-linearity in the relationship between change in T_{di} and time to liberation was modelled using restricted cubic splines with 3 knots.

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Model residuals were analyzed to confirm the assumption of proportional hazards. Variance inflation factors were assessed for evidence of multi-collinearity.

6.2 Sensitivity analyses of the primary model

We conducted the following sensitivity analyses on the primary model:

1. To account for potential bias arising from systematic differences in the timing of development of changes in T_{di} between patients (i.e. patients who are classified as having unchanged thickness at an early stage because they are extubated on day 3 of mechanical ventilation may not have had an opportunity to develop changes in diaphragm thickness), we conducted a sensitivity analysis of the primary model limiting patients to those with duration of ventilation of at least 7 days (landmark analysis).
2. To account for potential bias arising from the incorporation of patients from two slightly different cohorts, we conducted a sensitivity analysis of the primary model excluding patients from Cohort B.
3. Because baseline T_{di} was not a pre-specified model covariate (its role as a confounder was identified during preliminary descriptive analyses), we tested whether the results of the model were significantly different if baseline T_{di} was excluded from the model.
4. Because patients were enrolled up to 72 hours after intubation during the initial study period (first 53 patients enrolled), we conducted a sensitivity analysis excluding patients enrolled on day 3 of mechanical ventilation (n=18).

6.3 Secondary outcomes and change in diaphragm thickness

In the 'classification-based' analytical approach, the relationship between the initial changes in T_{di} and a number of secondary end-points was examined, including duration of ventilation, duration of ICU and hospital admission, ventilator-free days at day 60, the risks of death in ICU or hospital, reintubation, tracheostomy, readmission, and the composite end-point complications of acute respiratory failure (at least one of the following complications: reintubation, tracheostomy, mechanical ventilation > 14 days duration, or death). The initial change in T_{di} over the first week of ventilation was classified as ">10% decrease", "<10% change", or ">10% increase."

To estimate the absolute risk of remaining ventilator-dependent at 21 days, we computed cumulative incidence functions for liberation from mechanical ventilation and the competing risk of death (22). Differences in cumulative incidence were adjusted using competing risks regression by the method of Fine and Gray (21).

Differences between groups in ventilator-free days, duration of ventilation, duration of ICU stay, and duration of hospitalization were evaluated by bivariate Poisson regression (with quasi-Poisson distributions for over-dispersion). Differences between groups in event rates (reintubation, tracheostomy, prolonged ventilation > 14 days, or death) were evaluated by logistic regression.

Between-group differences were also adjusted for pre-specified confounders (age, SAPS II, baseline SOFA, sepsis at baseline, baseline diaphragm thickness, average Sedation Agitation Scale score over first 3 days of ventilation, exposure to neuromuscular blockade in the first week of ventilation, baseline PaO_2:FiO_2 ratio, and presence of comorbidity) in multivariable analyses.

6.4 Determinants of change in diaphragm thickness

As per our previous report (1), we employed multivariable linear mixed effects regression models to assess the effect of inspiratory effort on changes in diaphragm thickness over time

during mechanical ventilation. Inspiratory effort was quantified by the diaphragm thickening fraction measured during tidal breathing under ventilatory support. The effect of inspiratory effort on the change in thickness over time was modelled using an interaction term for the covariate of interest and time (i.e. to test whether the covariate modifies the effect of time on thickness). The models were adjusted for the following covariates: age, SAPS II, baseline SOFA, SAS, and sepsis at baseline. We computed the logarithm of diaphragm thickness values as the dependent variable for the model so that effect estimates would be indicative of relative changes in diaphragm thickness, not absolute changes.

The same model was computed using the daily average of the hourly mean diaphragm electrical activity (mean value from the 5-minute recording) as the measurement of inspiratory effort.

Finally, in similar models, we examined the effect of mode of ventilation, daily fluid balance, applied driving pressure (i.e. peak pressure – PEEP), and PEEP on changes in diaphragm thickness over time.

6.5 Sample size computation

We hypothesized that the hazard ratio for liberation from mechanical ventilation for patients with either decreased or increased diaphragm thickness compared to those with unchanged diaphragm thickness would be 0.6. This hazard ratio was selected as a clinically important difference in outcome based on the fact that a hazard ratio of this magnitude would give rise to a greater than 10% absolute difference in the cumulative incidence of liberation from mechanical ventilation after 60 days of follow-up, assuming that the cumulative incidence of liberation from mechanical ventilation is 70% and the cumulative incidence of death is 25% over that time period (and 5% of patients remain ventilator-dependent at that time point). We also hypothesized that the hazard ratio for the competing risk of death on mechanical ventilation would be 1.0 (i.e. changes in diaphragm thickness would not affect the hazard of death). On this basis, using standard formulae (23) we estimated that 120 liberation events (and therefore 175 patients, assuming that the cumulative incidence of liberation is approximately 70%) would need to be observed to detect a significant difference in the hazard of liberation (HR 0.6 or lower) with a 5% probability of a Type I error and 20% probability of a Type II error. We further expanded the target sample size from 175 patients to 200 patients to account for potential loss to follow up (i.e. a single diaphragm thickness measurement, lack of information re outcomes).

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7 CONSORT Diagram: Screening and Enrolment

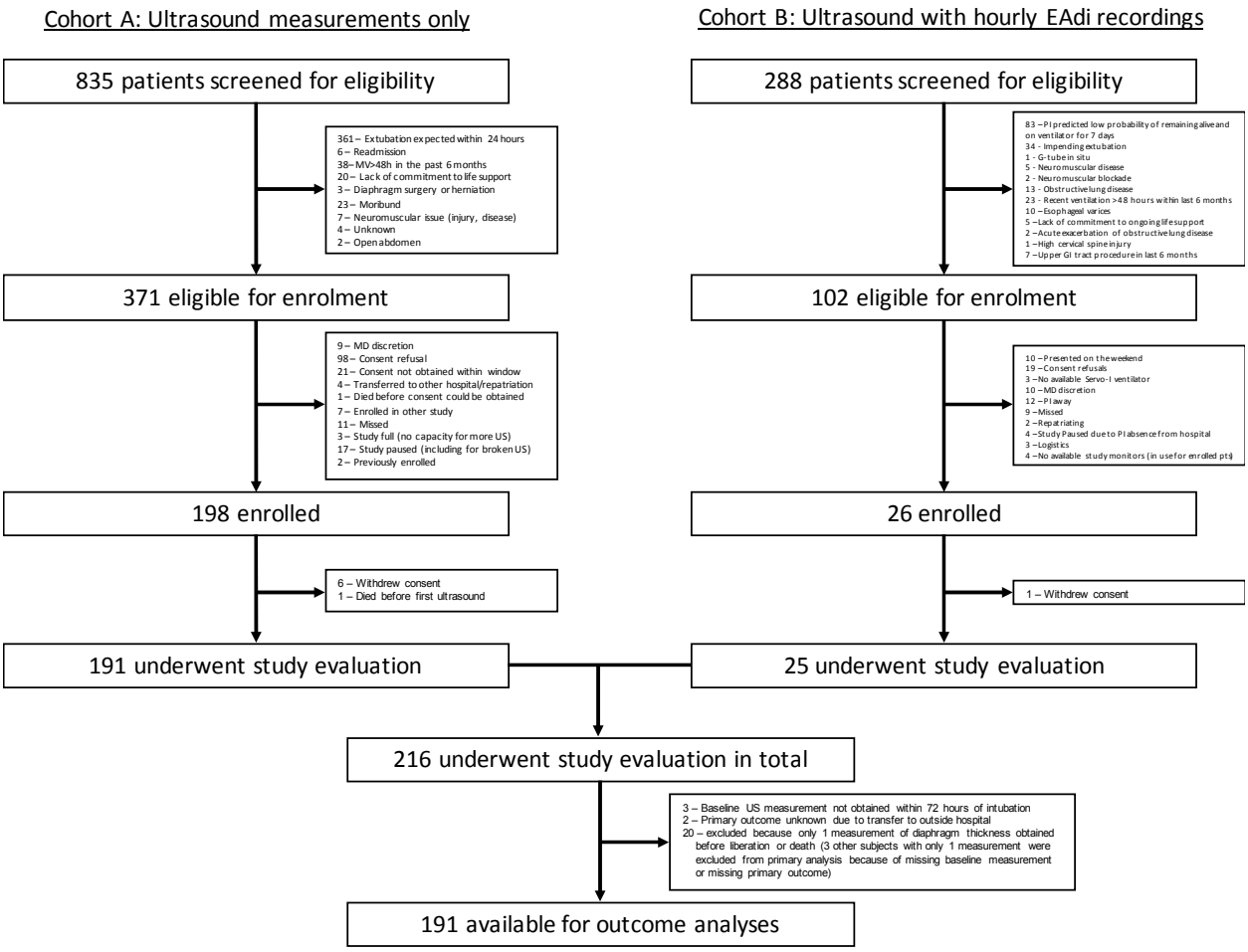

Figure E2. Screening, enrolment, evaluation and follow-up for Cohorts A and B.

8 Changes in Diaphragm Thickness and Clinical Outcomes

8.1 Primary model results

Table E3. Time-varying covariate Cox proportional hazards model results

Model Covariate	Hazard ratio for liberation from ventilation (95% CI)	p-value
>10% decrease in T_{di} below baseline vs. unchanged T_{di}*	0.69 (0.54-0.87) per 10% decrease in T_{di}	Overall association: 0.009 Test for non-linearity: p=0.007
>10% increase in T_{di} above baseline vs. unchanged T_{di}*	0.81 (0.62-1.06) per 10% increase in T_{di}	
Baseline T_{di} (per 1 mm increase)	1.69 (1.15-2.47)	0.007
Age (per decade)	0.92 (0.82-1.04)	0.19
SAPS II (per 10 point increase)	1.05 (0.93-1.19)	0.42
SOFA (per 10 point increase)*	0.43 (0.25-0.74)	0.002
Sepsis*	0.76 (0.49-1.17)	0.21
Baseline P/F ratio (per 25 mm Hg increase)	1.00 (0.96-1.05)	0.83
≥ 1 comorbidity	0.96 (0.65-1.41)	0.83
Exposure to neuromuscular blockade any time in first week of ventilation	0.72 (0.42-1.25)	0.25
Riker Sedation Agitation Scale*	1.25 (1.06-1.48)	0.009

*Modelled as time-dependent covariate

The assumption of proportional hazards was satisfied. There was no evidence of significant multi-collinearity.

8.2 Secondary analysis: sensitivity analyses on the alternate analysis framework

To assess the possibility that patients who were on the ventilator longer would have more measurements and therefore greater opportunity, even by chance, to develop changes in T_{di}, we compared the timing of classification into groups according to change in T_{di} and the number of measurements prior to classification, neither of which varied significantly across groups (Table 1, main manuscript). When the number of days prior to classification were subtracted from the duration of ventilation, the differences in duration of ventilation were similar to that reported in Table 2 of the main manuscript (<10% change: median 2 (IQR 1-4) days; >10% decrease: median 5 (IQR 2-12) days; >10% increase: median 10 (IQR 4-20) days, p<0.001 for difference between groups).

To assess whether the results were confounded by the timing of study enrolment (because measurements were not collected on weekends), we examined the relationship between the day of intubation and both the rate of the exposure and the outcome. Neither changes in T_{di} nor

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ventilator-free days were significantly associated with the day of the week on which the patient was intubated.

9 Changes in Diaphragm Thickness and Diaphragm Function

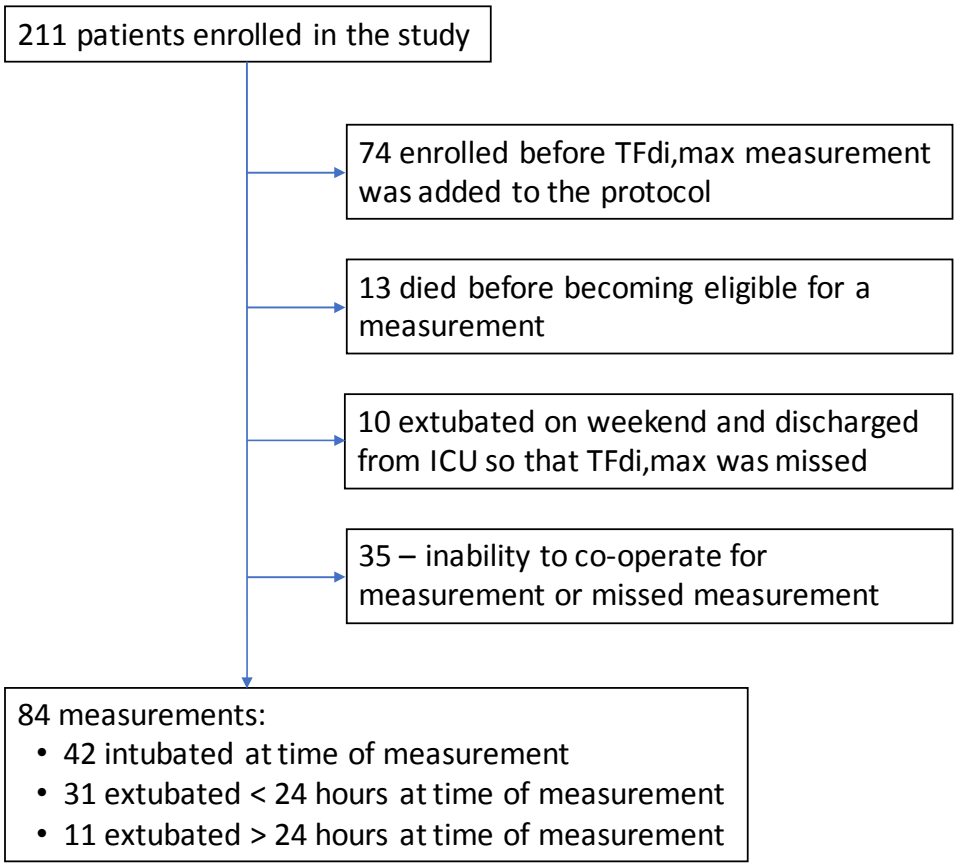

Figure E3. Reasons for missing measurements of maximal diaphragm thickening fraction.

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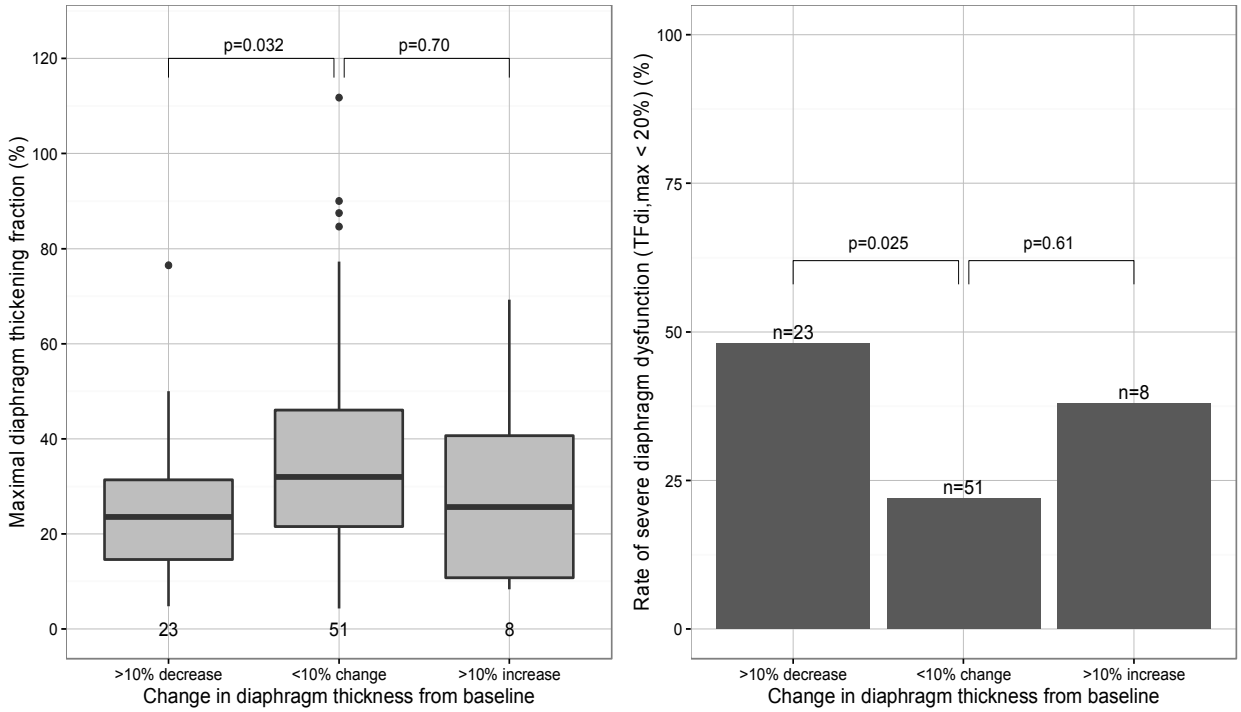

Figure E4. Relationship between diaphragm atrophy and diaphragm function. Diaphragm function, quantified by maximal thickening fraction, was lower in patients with significant diaphragm atrophy compared to patients without atrophy (Left panel, p=0.032). Similarly, severe diaphragm function (maximal thickening fraction < 20%) occurred more frequently in patients with diaphragm atrophy (Right panel, p=0.025). The association between increased diaphragm thickness and reduced maximal thickening fraction was not significant. The number of subjects in each group is shown on the plots.

10 Inspiratory Effort and Changes in Diaphragm Thickness

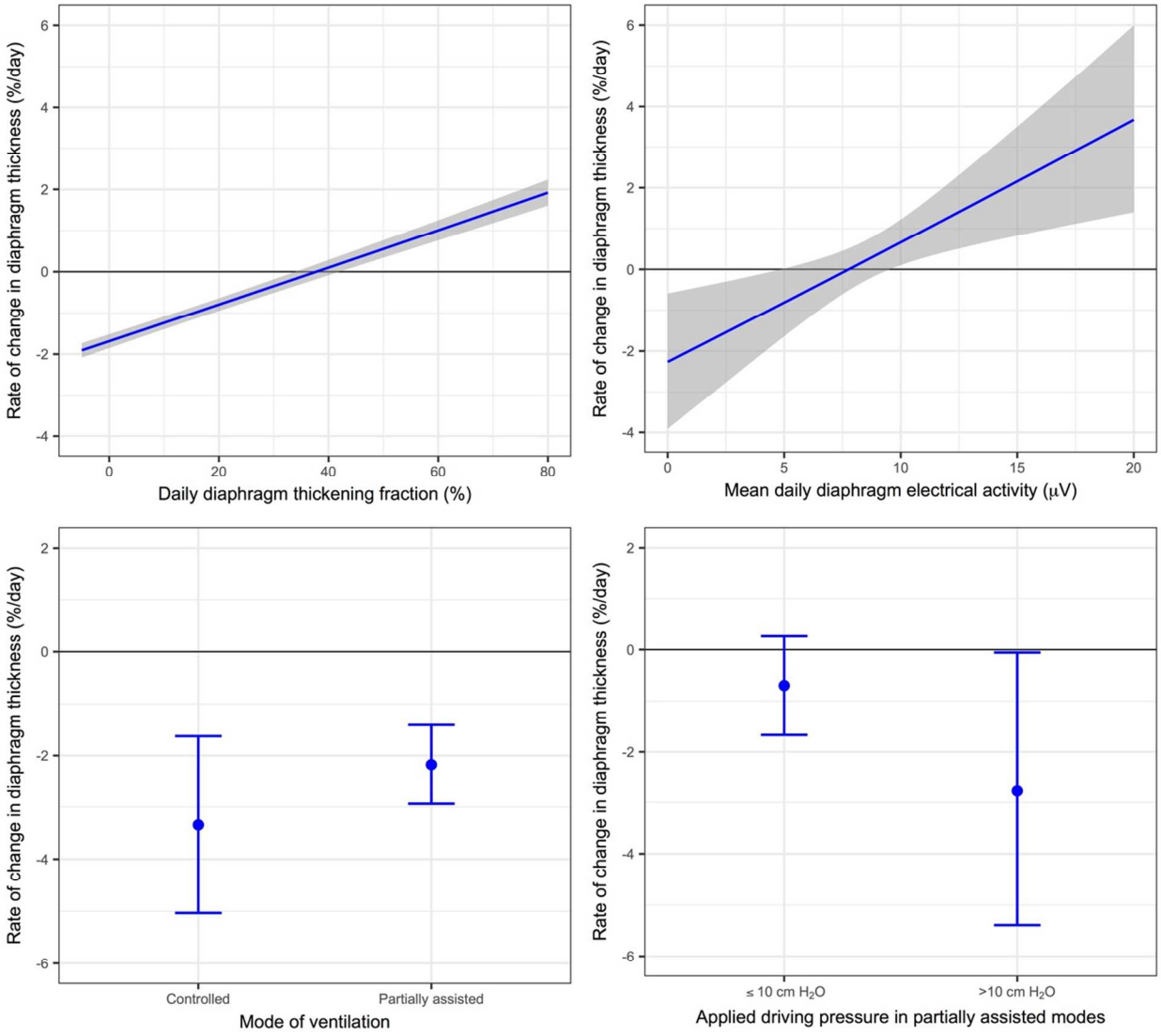

Figure E5. Determinants of the rate of change in diaphragm thickness (T_{di}) over time. Gray shaded areas and error bars represent 95% confidence intervals. **Top Left:** the rate of change in T_{di} over time varies significantly with the level of inspiratory effort quantified by diaphragm thickening fraction ($p<0.0001$ for effect modification). **Top Right:** the rate of change in T_{di} over time varies significantly with the level of inspiratory effort quantified by mean diaphragm electrical activity over 24 hours (Cohort B only) ($p=0.02$). **Bottom Left:** controlled modes of ventilation are associated with a more rapid decline in T_{di} compared to partially assisted modes of ventilation ($p=0.03$). **Bottom Right:** under partially assisted modes of ventilation, higher applied driving pressure (peak pressure – positive end-expiratory pressure) is associated with a more rapid decline in diaphragm thickness compared to lower applied driving pressure ($p=0.02$).

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11 Inspiratory Effort and Clinical Outcomes

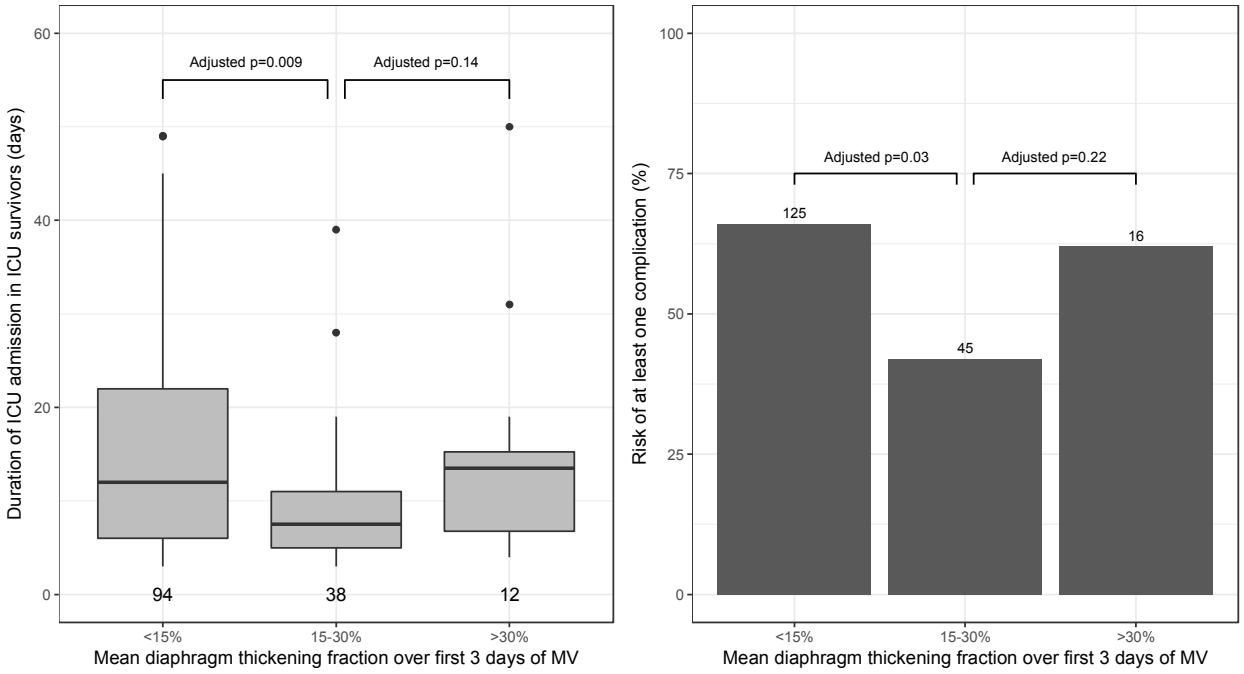

Figure E6. Inspiratory effort during the early course of mechanical ventilation is associated with clinical outcomes. The duration of ICU admission and the risk of complications of acute respiratory failure were lowest in patients with an intermediate level of inspiratory effort. Differences between intermediate and high levels of inspiratory effort did not reach statistical significance after adjusting for age, SAPS II, baseline SOFA, SAS, presence of sepsis, comorbid conditions, use of neuromuscular blockade, and baseline diaphragm thickness.

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