Diaphragm Activity and Function
During Mechanical Ventilation

by

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A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy

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2016

Abstract

Previous studies suggest that mechanical ventilation (MV) can injure the diaphragm, potentially prolonging ventilator dependence. However, there are many potential insults to diaphragm function during critical illness and the extent to which diaphragm dysfunction is attributable to ventilation remains undefined in the clinical setting. We hypothesized that diaphragm inactivity and/or injurious diaphragm loading during MV mediate diaphragm injury in the clinical setting. To test this hypothesis, we first evaluated the feasibility, validity and reproducibility of novel methods for monitoring the diaphragm in the clinical setting. We found that neuromuscular coupling (NMC), a measure of diaphragm function, is influenced by inspiratory effort, airway flow and diaphragm motion during inspiration and that NMC varies considerably over time in healthy volunteers. We also found that right hemidiaphragm thickness can be measured reproducibly by ultrasound and that increasing diaphragm thickness during inspiration reflects active contractile activity rather than passive chest wall expansion. Subsequently, in a large prospective cohort study (n=107) we demonstrated that diaphragm thickness varies considerably over time in relation to the level of inspiratory effort. Changes in muscle thickness were associated with impaired muscle function. Finally, in a preliminary clinical study (n=10) we confirmed the feasibility of selectively enrolling patients at high risk of prolonged MV and of
measuring diaphragm activity and patient-ventilator dyssynchrony on an hourly basis commencing shortly after intubation. We conclude that ultrasound is a useful clinical tool to monitor the diaphragm during MV; NMC measurement requires further standardization and validation. MV likely causes significant diaphragm injury in patients and titrating ventilatory support to maintain normal levels of inspiratory effort may protect the diaphragm from injury during MV. A detailed description of patient exposure to diaphragm inactivity and injurious loading during MV is feasible; such observations could inform the design of muscle-protective ventilation strategies.
Acknowledgements

I am deeply grateful to my supervisor, Dr. Niall Ferguson, for his patient guidance, mentorship, and support. He was the first to propose this path toward doctoral studies, and it has been my great privilege to work with a model clinician-scientist such as him over the last several years.

My thanks to the members of my supervisory committee, Dr. Brian Kavanagh, Dr. Gordon Rubenfeld, and Dr. Steffen-Sebastian Bolz. They played crucial roles in opening the way for me to pursue this work and constantly forced me to critically examine my assumptions and inferences, generously giving of their time and energy to this work.

I am deeply grateful to Dr. Laurent Brochard for his enthusiastic interest in this work and his invaluable guidance and support, especially in helping to bring parts of this work to publication. I have learned much from his brilliant mastery of the field and his genuine personal warmth.

Dr. Franco Laghi graciously hosted me in the Pulmonary Physiology Laboratory at the Veterans Affairs Hospital in Hines, IL for several months. In his lab I first experienced the excitement and challenge of physiological measurement and experimentation. Dr. Laghi exemplifies a commitment to methodological rigour and precision.

Many individuals collaborated with me in collecting the data for the studies described in this body of work. Dr. Michael Detsky and Ms. Paulina Farias generously gave of their time and energy. Special thanks to Alistair Murray, Debbie Brace, Stefannie Vorona, Ashley Lanys, Cristian Urrea and Dr. Nuttapol Rittayamai: their dedicated efforts were crucial for much of this work.

Several colleagues at the University Health Network enthusiastically collaborated in various aspects of this work. Dr. Eddy Fan served both as a collaborator and a highly valued mentor. My sincere thanks also to Dr. Margaret Herridge, Dr. Jeff Singh, Dr. Alberto Goffì and Dr. Lorenzo Del Sorbo. Dr. Christer Sinderby and Dr. Jennifer Beck, pioneers in the field of respiratory muscle physiology, were always ready to share expertise, insight, and advice.

Dr. Art Slutsky was a valuable encouragement along the way; he helped me to get started in the study of diaphragm function under mechanical ventilation in the first place.
This work was enabled by a Post-Doctoral Fellowship from the Canadian Institutes of Health Research ($275,000 over 5 years), by salary support from the Clinician-Scientist Training Program, Department of Medicine, University of Toronto ($200,000 over 4 years) and by operating grants from Physician Services Incorporated Foundation ($119,000), the University Health Network Innovation Fund ($63,000), the Ontario Thoracic Society ($50,000), and a subgrant provided by a Canadian Institutes of Health Research Operating Grant ($50,000).

I have no doubt that the curiosity and drive required to undertake a project of this magnitude (and to complete it) were inherited from my parents, Ian and Beulah Goligher. They showed me the value of diligence, perseverance, commitment, humility and joy in one’s work. They sacrificed to provide me with the very best opportunities. Under their care, I made all my most important discoveries.

Finally, and most importantly, I am indebted to my loving wife Rachel. She encouraged me to pursue this work, and she has supported me through it. She witnessed the blood, sweat and toil involved. She entered into the frustrations and shared the joys. This work would not have been possible but for her love and support.

Ewan C. Goligher
Toronto, November 2015
Table of Contents

LIST OF TABLES ................................................................................................................................. X

LIST OF FIGURES ............................................................................................................................... XI

LIST OF APPENDICES ........................................................................................................................... XV

LIST OF ABBREVIATIONS ..................................................................................................................... XVI

GENERAL INTRODUCTION .................................................................................................................. 1

1 The Human Diaphragm: Overview of Anatomy and Function ......................................................... 1

  1.1 Functional anatomy of the diaphragm in humans ................................................................. 1

  1.2 Functional characteristics of the diaphragm in humans ..................................................... 2

2 Monitoring the Diaphragm .............................................................................................................. 4

  2.1 Monitoring diaphragm structure ......................................................................................... 4

  2.2 Monitoring diaphragm function ......................................................................................... 5

  2.3 Monitoring diaphragm activity .......................................................................................... 9

  2.4 Monitoring patient-ventilator synchrony ......................................................................... 14

3 The Diaphragm in Acute Respiratory Failure: Pathophysiology and Clinical Importance ........... 15

  3.1 The Clinical Problem: Prolonged Mechanical Ventilation .............................................. 15

  3.2 Diaphragm Dysfunction During Critical Illness .............................................................. 18

4 Ventilator-induced diaphragm dysfunction (VIDD) .................................................................. 21

  4.1 Seminal clinical studies linking mechanical ventilation to diaphragm injury ................. 22

  4.2 Proposed mechanisms of VIDD ...................................................................................... 22

  4.3 VIDD: Unanswered Questions ......................................................................................... 26

5 Conclusion ...................................................................................................................................... 28

RATIONALE, HYPOTHESIS & SPECIFIC AIMS ............................................................................. 34

1 Rationale ........................................................................................................................................ 34

2 Hypothesis ...................................................................................................................................... 35

3 Specific Aims .................................................................................................................................. 35

  3.1 Chapter 3: Characterizing Neuromuscular Coupling in Healthy Volunteers and Mechanically Ventilated Patients .............................................................................................................. 35
3.2 Chapter 4: Measuring Diaphragm Thickness in Mechanically Ventilated Patients: Feasibility, Reproducibility, and Validity.................................................................................................................. 35
3.3 Chapter 5: The Evolution of Diaphragm Thickness During Mechanical Ventilation: Impact of Inspiratory Effort .......................................................................................................................... 36
3.4 Chapter 6: Monitoring Diaphragm Activity and Neuromuscular Coupling During Mechanical Ventilation: Feasibility and Preliminary Findings.................................................................................. 36

CHARACTERIZING NEUROMUSCULAR COUPLING IN HEALTHY VOLUNTEERS AND MECHANICALLY VENTILATED PATIENTS .......................................................................................................................... 38
1 Introduction.................................................................................................................................................... 38
2 Methods.......................................................................................................................................................... 39
  2.1 Experimental Subjects................................................................................................................................. 39
  2.2 Experimental Measurements........................................................................................................................ 40
  2.3 Experimental Protocol ............................................................................................................................... 41
  2.4 Signal Analysis ........................................................................................................................................... 41
  2.5 Statistical Analysis ..................................................................................................................................... 42
3 Results.......................................................................................................................................................... 43
  3.1 Relationship between ∆Pdi and ∆Edi in Healthy Volunteers........................................................................ 43
  3.2 Determinants of Relationship between ∆Pdi and ∆Edi in Healthy Volunteers.............................................. 44
  3.3 Relationship between ∆Pdi and ∆Edi in Mechanically Ventilated Patients.................................................... 44
  3.4 Stability of Neuromuscular Coupling over Time in Healthy Volunteers ..................................................... 45
  3.5 Stability of Twitch Transdiaphragmatic Pressure over Time in Healthy Volunteers ................................. 46
4 Discussion.................................................................................................................................................... 46

MEASURING DIAPHRAGM THICKNESS IN MECHANICALLY VENTILATED PATIENTS: FEASIBILITY, REPRODUCIBILITY, AND VALIDITY .............................................................................................................. 67
1 Introduction.................................................................................................................................................... 67
2 Methods.......................................................................................................................................................... 68
  2.1 Experimental Subjects................................................................................................................................. 68
  2.2 Experimental Measurements........................................................................................................................ 69
  2.3 Experimental Protocol ............................................................................................................................... 69
  2.4 Statistical Analysis ..................................................................................................................................... 70
3 Results.......................................................................................................................................................... 71
3.1 Feasibility and Reproducibility of Diaphragm Thickness Measurements in Ventilated Subjects

3.2 Validity of Inspiratory Diaphragm Thickening Fraction to Monitor Inspiratory Activity

4 Discussion

THE EVOLUTION OF DIAPHRAGM THICKNESS DURING MECHANICAL VENTILATION: IMPACT OF INSPIRATORY EFFORT

1 Introduction

2 Methods

2.1 Study Population and Setting

2.2 Diaphragm Thickness Measurements

2.3 Diaphragm Function Measurements

2.4 Clinical Risk Factors

2.5 Statistical Analysis

3 Results

3.1 Study Cohort

3.2 Time Course and Distribution of Changes in Diaphragm Thickness

3.3 Relationship between Changes in Diaphragm Thickness and Diaphragm Function

3.4 Impact of Diaphragm Contractile Activity on Diaphragmatic Thickness During Mechanical Ventilation

3.5 Determinants of Diaphragm Contractile Activity During Mechanical Ventilation

4 Discussion

MONITORING DIAPHRAGM ACTIVITY AND NEUROMUSCULAR COUPLING DURING MECHANICAL VENTILATION: FEASIBILITY AND PRELIMINARY FINDINGS
3.1 Feasibility: Enrolment and Protocol Completion ........................................................................ 121
3.2 Feasibility: Signal Acquisition and Analysis ........................................................................ 122
3.3 Preliminary Findings: Diaphragm Contractile Activity ......................................................... 122
3.4 Preliminary Findings: Patient-Ventilator Synchrony ............................................................ 122
3.5 Preliminary Findings: Neuromuscular coupling and Diaphragm Thickness ....................... 123

4 Discussion ................................................................................................................................ 123

SYNTHESIS AND FUTURE DIRECTIONS .......................................................................................... 142

1 Discussion of Key Findings ........................................................................................................ 142

1.1 Measurement Technique: Diaphragm Ultrasound ................................................................. 142
1.2 Measurement Technique: Neuromuscular Coupling ............................................................ 143
1.3 Feasibility of Clinical Investigation of Diaphragm Function During Mechanical Ventilation 145

1.4 Impact of Mechanical Ventilation on the Diaphragm in the Clinical Setting ......................... 146
1.5 Insights on the Mechanisms of Ventilator-Induced Diaphragm Dysfunction ....................... 147

2 Future Directions .......................................................................................................................... 149

2.1 Monitoring diaphragm structure ............................................................................................ 149
2.2 Measuring and applying neuromuscular coupling ................................................................. 150
2.3 Intervening to prevent diaphragm injury ................................................................................ 151
2.4 Treating established diaphragm injury .................................................................................. 152

3 Summary ..................................................................................................................................... 153

REFERENCES ................................................................................................................................... 154
List of Tables

Table 1-1. Techniques for Monitoring Diaphragm Structure, Function and Activity During Mechanical Ventilation................................................................. 29

Table 3-1. Statistical parameters characterizing the relationship between Pdi and Edi in ventilated subjects........................................................................................................... 51

Table 3-2. Bootstrap validation of correction factors for computing neuromuscular coupling.... 52

Table 3-3. Reproducibility of different computational approaches to quantifying neuromuscular coupling in healthy volunteers................................................................................ 53

Table 4-1. Clinical Characteristics of Mechanically Ventilated Subjects ......................... 76

Table 4-2. Reproducibility of Diaphragm Thickness Measurements in Mechanically Ventilated Patients..................................................................................................... 77

Table 5-1. Model parameters for linear mixed model of end-expiratory diaphragm thickness ... 98

Table 5-2. Clinical Characteristics of Study Cohort and Patient Subgroups According to Change in Diaphragm Thickness Over Time................................................................. 99

Table 5-3. Clinical characteristics of control subjects ......................................................... 100

Table 5-4. Diaphragm function measurements in mechanically ventilated patients .......... 101

Table 5-5. Model parameters for linear mixed model of diaphragm thickening fraction during mechanical ventilation ................................................................. 102

Table 6-1. Dyssynchrony classification system........................................................................ 128

Table 6-2. Clinical Characteristics and Study Protocol Outcomes of Enrolled Subjects....... 129

Table 6-3. Frequency of Diaphragm Activity and Dyssynchrony in Enrolled Subjects ....... 130
List of Figures

Figure 1-1. Schematic of costal and crural diaphragm action. ......................................................... 32

Figure 1-2. Causes and consequences of diaphragm dysfunction in the critically ill patient....... 33

Figure 3-1. The relationship between Pdi and Edi in healthy volunteers. ........................................ 54

Figure 3-2. The relationship between Pdi and Edi during inspiration under quasi–iso-lung volume conditions. ...................................................................................................................... 55

Figure 3-3. Relationship between neuromuscular coupling and inspiratory effort in healthy volunteers. ................................................................................................................................. 56

Figure 3-4. The effect of mean inspiratory flow rate on the relationship between ΔPdi and ΔEdi. .................................................................................................................................................. 57

Figure 3-5. The effect of diaphragmatic motion on the relationship between ΔPdi and ΔEdi. .... 58

Figure 3-6. Mathematical relationship between \(\int Pdi\) and \(\int Edi\) measured during airway occlusion in ventilated patients. ................................................................................................................................. 59

Figure 3-7. Relationship between neuromuscular coupling (\(\int NMC\)) and inspiratory effort level in ventilated patients. .................................................................................................................. 60

Figure 3-8. Agreement between predicted and measured ΔPdi. ......................................................... 61

Figure 3-9. Sensitivity analysis assessing impact of correcting neuromuscular coupling on accuracy of Pdi prediction. ........................................................................................................... 62

Figure 3-10. Inspiratory capacity over time in the five healthy subjects enrolled in the study... 63

Figure 3-11. Maximum ΔEdi (measured during an inspiratory capacity maneuver) varied considerably in some subjects between study days. ........................................................................ 64

Figure 3-12. Variation in neuromuscular coupling over time............................................................. 65
Figure 3-13. Variation in twitch $\Delta$Pdi over time obtained by magnetic phrenic nerve stimulation in healthy volunteers. .......................................................... 66

Figure 4-1. Typical sonographic cross-sectional image of the diaphragm. ................................. 78

Figure 4-2. Typical M-mode ultrasound image of dynamic diaphragm thickness obtained during inspiratory hold maneuver. ........................................................................................................... 79

Figure 4-3. Effect of body mass index and duration of ventilation on the variability in diaphragm thickness measurements. .................................................................................................................. 80

Figure 4-4. The relationship between end-expiratory thickness of the diaphragm and physical stature characteristics. ...................................................................................................................... 81

Figure 4-5. Inspiratory thickening fraction of the right hemidiaphragm in healthy subjects at rest and ventilated subjects under varying conditions. ................................................................. 82

Figure 4-6. The relationship between diaphragm thickening fraction and inspiratory volume. 83

Figure 4-7. Diaphragm thickening fraction is correlated with diaphragm electrical activity and transdiaphragmatic pressure during inspiratory maneuvers. ......................................................... 84

Figure 4-8. The relative contributions of inspiratory effort vs. chest wall expansion to inspiratory diaphragm thickening fraction depend on the inspiratory volume. ......................................................... 85

Figure 4-9. Active and passive changes in diaphragm thickness at varying inspiratory volumes. 86

Figure 5-1. CONSORT diagram outlining study screening and enrolment ..................................... 103

Figure 5-2. Variation in diaphragm thickness over time the first week of mechanical ventilation. ........................................................................................................................................ 104

Figure 5-3. Variation in diaphragm thickness in individual subjects over the first 14 days of mechanical ventilation. ..................................................................................................................................... 105

Figure 5-4. Changes in diaphragm thickness according to mode of mechanical ventilation. .... 106
Figure 5-5. Diaphragm thickness variation over time in non-ventilated control patients and in ventilated patients following extubation

Figure 5-6. Diaphragm thickness before and after extubation

Figure 5-7. Diaphragm function is impaired by changes in diaphragm thickness over time during mechanical ventilation

Figure 5-8. Relationship between changes in diaphragm thickness and the rate of severe diaphragm dysfunction

Figure 5-9. Diaphragm contractile activity (inspiratory effort) is associated with the rate and direction of change in diaphragm thickness during mechanical ventilation

Figure 5-10. Organ dysfunction modifies the relationship between diaphragm inactivity and changes in diaphragm thickness over time

Figure 5-11. Diaphragm contractile activity over the first three days of ventilation and changes in diaphragm thickness over the 1st week of MV

Figure 5-12. Relation between set driving pressure and change in diaphragm thickness over time

Figure 5-13. Variation in diaphragm contractile activity over during the first week of mechanical ventilation

Figure 5-14. Effect of ventilator settings on diaphragm contractile activity

Figure 6-1. Feasibility of screening and enrolment

Figure 6-2. Histogram showing the distribution of ∫Edi per breath (integrated with respect to time from onset to end-inspiration) for all neural inspiratory events recorded in the study

Figure 6-3. Variation in diaphragm electrical activity during invasive mechanical ventilation

Figure 6-4. Relationship between single daily measurements of diaphragm thickening fraction and mean Edi over 24 hours
Figure 6-5. Reverse triggering is associated with an increased risk of eccentric diaphragmatic contractions. ............................................................................................................................. 135

Figure 6-6. Variation in neuromuscular coupling of the diaphragm over time during mechanical ventilation. ........................................................................................................................................ 136

Figure 6-7. The estimated range of Pdi levels associated with Edi measurements. ..................... 137

Figure 6-8. Variation in end-expiratory diaphragm thickness over time during mechanical ventilation. ........................................................................................................................................ 138

Figure 6-9. Impact of inspiratory effort on changes in diaphragm thickness over time............. 139

Figure 6-10. Relationship between changes in diaphragm thickness and diaphragm function. . 140

Figure 6-11. Eccentric diaphragm contractions are associated with impaired diaphragm function. ........................................................................................................................................ 141
List of Appendices

APPENDIX 1: EVOLUTION OF DIAPHRAGM THICKNESS DURING MECHANICAL VENTILATION .......... 178

1 Study Methods .................................................................................................................. 178
   1.1 Study Population and Setting ...................................................................................... 178
   1.2 Diaphragm Thickness Measurements ........................................................................ 178
   1.3 Diaphragm Function Measurements .......................................................................... 179
   1.4 Risk Factors and Outcomes ....................................................................................... 179
   1.5 Statistical Analysis ................................................................................................... 180

2 Linear Mixed Model of Diaphragm Thickness ................................................................ 181
   2.1 Pre-specified Rationale for Model Variable Selection .............................................. 182

3 Mathematical Coupling: Sensitivity Analysis ................................................................. 183

4 Variability of Diaphragm Thickness over Time in Different Study Groups .................. 186

5 Linear Mixed Model of Diaphragm Thickening Fraction .............................................. 186

APPENDIX 2: PREDICTING RISK FOR PROLONGED VENTILATION .............................. 188

1 Development of Algorithm to Predict Probability of Remaining Alive and on the
   Ventilator for at least 7 days ............................................................................................ 188
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACVC</td>
<td>Assist control volume control mode</td>
</tr>
<tr>
<td>CART</td>
<td>Classification and regression tree analysis</td>
</tr>
<tr>
<td>CDAP</td>
<td>Compound diaphragm action potential</td>
</tr>
<tr>
<td>CIPMN</td>
<td>Critical illness polymyoneuropathy</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CPAP</td>
<td>Continuous positive airway pressure</td>
</tr>
<tr>
<td>Edi</td>
<td>Diaphragm electrical activity</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyogram</td>
</tr>
<tr>
<td>ETT</td>
<td>Endotracheal tube</td>
</tr>
<tr>
<td>f</td>
<td>Respiratory frequency (rate)</td>
</tr>
<tr>
<td>IC</td>
<td>Inspiratory capacity</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive care unit</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>MV</td>
<td>Mechanical ventilation</td>
</tr>
<tr>
<td>NAVA</td>
<td>Neurally adjusted ventilatory assist</td>
</tr>
<tr>
<td>NMC</td>
<td>Neuromuscular coupling</td>
</tr>
<tr>
<td>P&lt;sub&gt;0.1&lt;/sub&gt;</td>
<td>Airway occlusion pressure</td>
</tr>
<tr>
<td>Paw</td>
<td>Airway pressure</td>
</tr>
<tr>
<td>Paw,tw</td>
<td>Twitch airway pressure (measured during magnetic phrenic stimulation)</td>
</tr>
<tr>
<td>PBW</td>
<td>Predicted body weight</td>
</tr>
<tr>
<td>PCV</td>
<td>Pressure control ventilation mode</td>
</tr>
<tr>
<td>Pcw</td>
<td>Passive recoil pressure of the chest wall</td>
</tr>
<tr>
<td>Pdi</td>
<td>Transdiaphragmatic pressure (gastric pressure – esophageal pressure)</td>
</tr>
<tr>
<td>Pdi,max</td>
<td>Maximal transdiaphragmatic pressure</td>
</tr>
<tr>
<td>Pdi,tw</td>
<td>Twitch transdiaphragmatic pressure (measured during magnetic phrenic stimulation)</td>
</tr>
<tr>
<td>PEEP</td>
<td>Positive end-expiratory pressure</td>
</tr>
<tr>
<td>Pes</td>
<td>Esophageal pressure</td>
</tr>
<tr>
<td>Pes,tw</td>
<td>Twitch esophageal pressure (measured during magnetic phrenic stimulation)</td>
</tr>
<tr>
<td>Pga</td>
<td>Gastric pressure</td>
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</tbody>
</table>
Pi,max  Maximal inspiratory pressure
PMV      Prolonged mechanical ventilation
PSV      Pressure support ventilation mode
PTP      Pressure-time product
PTPmus   Pressure-time product of the respiratory muscles
PTPdi    Pressure-time product of the diaphragm
RSS      Residual sum of squares
SAS      Sedation Agitation Scale
SAPS     Severe Acute Physiology Score
SD       Standard deviation
SIRS     Systemic Inflammatory Response Syndrome
SNIP     Sniff nasal inspiratory pressure
SOFA     Severity of Organ Failure Assessment score
Tdi      Diaphragm thickness
Tdi,ee   End-expiratory diaphragm thickness
Tdi,pi   Peak-inspiratory diaphragm thickness
TFdi     Diaphragm thickening fraction
Ti       Inspiratory cycle time
TTI      Tension-time index
TTdi     Tension-time index of the diaphragm
Ttot     Total respiratory cycle time
VIDD     Ventilator-induced diaphragm dysfunction
VILI     Ventilator-induced lung injury
Vt       Tidal volume
WOB      Work of breathing
Chapter 1
General Introduction

1  The Human Diaphragm: Overview of Anatomy and Function

1.1  Functional anatomy of the diaphragm in humans

The diaphragm is a thin, dome-shaped muscular structure separating the thoracic and abdominal cavities. Together with the rib cage and abdomen it gives rise to the chest wall. It is made up of 3 separate segments: the central tendon (a non-contractile structure through which major blood vessels pass; the costal diaphragm, which has fibers projecting onto the rib cage or xiphoid process; and the crural diaphragm, the fibers of which insert into the first three lumbar vertebrae. The anatomical differences between segments are of considerable functional importance (see Section 1.2). The costal and crural portions of the diaphragm were once thought to have embryologically distinct ontogeny (1) although more recent work suggests that the crural and costal diaphragm arise from the same embryogenetic process (2). The thickness of the diaphragm is variable over its surface, with tapering from the anterior to posterior costal regions, and from its costal insertions to the central tendon (3).

Situated within the chest in the upright human, the diaphragm has a pronounced dome-like structure and the outer aspect of the costal diaphragm is apposed to the rib cage, covering approximately 1/3 of the lower rib cage surface (4). During contractile shortening, the shape of the diaphragm changes little in the coronal plane and most of the shortening is translated into axial descent (5, 6). This geometric configuration is important for the action of the diaphragm muscle during inspiration, as will be discussed below. The structural arrangement of the costal and crural diaphragm and its relations to the various components of the chest wall is summarized in Figure 1-1.

In rats, the diaphragm is composed of all four major muscle fiber types, distributed homogeneously within the diaphragm (3). Biopsies of the costal diaphragm in humans
demonstrate a relatively even distribution of slow (Type I) and fast twitch (Type IIa and IIx) fibre types (7, 8). Slow-twitch fibres are relatively fatigue resistant due to higher oxidative capacity (9) and the increase in Type I fibres observed in patients with chronic obstructive pulmonary disease (10) might account for the increased resistance to low frequency fatigue in these patients (11). Interestingly, interspecies comparisons suggest that the aerobic capacity of fibres composing the mammalian diaphragm varies with the animal’s respiratory rate (12). These observations support the concept that the normal human diaphragm is adapted in terms of fibre type and oxidative capacity to the usual physiological range of ventilatory patterning (respiratory rate and tidal volume) experienced by humans which may in turn be adapted to minimize metabolic load on the respiratory muscles (13).

The diaphragm receives its entire innervation from the phrenic nerve, originating from cervical nerve roots 3, 4, and 5. Blood is supplied to the diaphragm by a complex arterial network arising from anastomoses between the phrenic artery and internal mammary arteries and between branches of the phrenic artery and the intercostal arteries (14). The venous anatomy parallels the arterial circulation. This rich and redundant arterial supply helps to ensure that blood flow is sufficient to meet the occasionally strenuous energetic demands placed upon the muscle. Blood flow is heterogeneous throughout the muscle and directed toward regions where energetic demand is highest (15, 16).

1.2 Functional characteristics of the diaphragm in humans

Under normal conditions, the diaphragm acts within the chest much like a piston within a cylinder, generating flow as its dome descends within the thoracic cavity, displacing the abdominal contents beneath and elevating the lower thorax. During diaphragmatic contraction, the width of the zone of apposition falls as the dome descends; widening of the rib cage also contributes to a small extent to this decrease (‘piston within a widening cylinder’ model) (6). The pressure gradient generated across the dome between the thoracic and abdominal cavities, the transdiaphragmatic pressure (Pdi, quantified as the gradient between the gastric pressure, Pga, and the esophageal pressure, Pes (17)), is proportional to the tension developed within the muscle fibres and inversely proportional to the cross-sectional area of the thoracic cavity at the level of the dome (5, 18). The magnitude of the pressure swings on either side of the diaphragm depend on the volume change induced by diaphragmatic contraction and the elastance of the
thorax and abdomen, respectively. The geometric configuration of the dome of the diaphragm (i.e. its radius of curvature) exerts relatively minor influence on transdiaphragmatic pressure generation except at high lung volumes where the zone of apposition is minimal over at least part of the diaphragm (5). This appears to hold even though the diaphragm exhibits considerable flattening in the sagittal plane during contraction (6). Therefore much of the reduction in diaphragmatic force generation resulting from diaphragm shortening at higher lung volumes arises from length-tension characteristics rather than changes in radius of curvature.

As with any muscle, the tension developed by the diaphragm during contraction is influenced by the length-tension relation and the force-velocity relation. The length-tension relation, a feature of muscle function arising from myosin-actin filament interactions during myofibril excitation-contraction coupling, is characterized by the fact that as muscle is passively lengthened prior to contraction (until some optimal length is obtained), the force generated in response to a given stimulus progressively increases (18). As a consequence, the force generated by diaphragmatic activation varies considerably with the resting length of the diaphragm, which varies in turn with end-expiratory lung volume. Force generation is maximal when resting diaphragm length is approximately 25% greater than its resting length (19). Accordingly, the efficiency of diaphragmatic pressure generation was found to increase during a threshold loading protocol because concomitant activation of the abdominal muscles at end-expiration increased diaphragm length (20). Resting diaphragm length also varies with the configuration of the thorax and abdomen at any given lung volume, and this configuration influences diaphragm force generation (21).

The force-velocity relation is closely related to the length-tension relation: the more rapidly a muscle shortens during contraction (i.e. the more rapidly its length decreases), the lower the force generated during contraction (22).

Diaphragmatic contraction increases chest wall volume (generating pleural pressure and flow of air into the lungs) through two separate mechanisms: its ‘insertional’ force and its ‘appositional’ force. The insertional force serves to ‘pull’ the lower ribs upward and outward while drawing the dome of the diaphragm downward. Because this insertional force generates a negative pleural pressure, acting in isolation it draws the ribs of the upper chest wall inward; slight activity of the inspiratory intercostal muscles acts to prevent this. At the same time, the abdominal contents
resist downward displacement of the diaphragmatic dome, generating an increase in intraabdominal pressure that exerts an outward force on the rib cage within the zone of apposition (i.e. from within the diaphragm)—the ‘appositional’ force (23). The appositional force depends heavily on the size of the zone of apposition and on the elastance of the abdomen.

The costal and crural portions of the diaphragm exert differential effects on chest wall expansion (5). Because the costal diaphragm inserts into the ribs, costal diaphragmatic contraction elevates the rib cage by generating opposing force against the abdomen, and the degree of rib cage and abdominal motion depends on the relative elastance of these compartments. The crural diaphragm inserts into the lumbar spine; accordingly, it effectively ‘pulls’ the dome of the diaphragm downward, and therefore contributes to rib cage expansion through the appositional force.

This cursory overview highlights the functional interdependence between the various respiratory muscles and emphasizes that optimal diaphragmatic performance requires the co-operative assistance of other muscles of the chest wall and abdomen. This is particularly the case as ventilatory loads increase (20, 21).

2 Monitoring the Diaphragm

This body of work is concerned with the impact of mechanical ventilation on the diaphragm in the clinical setting and the potential implications of diaphragm injury for diaphragm function and for liberating patients from mechanical ventilation. Methods of monitoring diaphragm structure, function and activity are therefore of central importance to this undertaking. A focused overview of the salient techniques is warranted. This discussion is summarized in Table 1-1.

2.1 Monitoring diaphragm structure

Most studies of diaphragm structure in mechanically ventilated patients have been based on histological findings from tissue specimens (7, 24-26). This method permits detailed structural and ultrastructural evaluation of the diaphragm muscle but it has the distinct of disadvantage of being highly infeasible in living subjects. For this reason, the most human studies of diaphragm histology have studied brain dead organ donors; histological findings in diaphragm specimens obtained from living mechanically ventilated patients have only very recently been described (27).
A range of imaging techniques have been employed to evaluate changes in diaphragm structure. Computed tomography has been employed to evaluate changes in diaphragmatic volume over time (28), although the reproducibility of this technique is not known. Magnetic resonance imaging has been employed to image the diaphragm, primarily as a dynamic tool for assessing changes in shape during inspiration (6, 29). The classic tool for imaging diaphragm motion, fluoroscopy, provides no specific structural information about the diaphragm.

Ultrasound is a promising technique for non-invasive evaluation of diaphragm structure. Transthoracic ultrasound permits visualization of the diaphragm in cross-section in the zone of apposition (30). Autopsy studies confirm that diaphragm thickness measurements made by ultrasound are valid (31, 32). Ultrasound has been used to monitor changes in diaphragm thickness over time, for example in patients with idiopathic diaphragmatic paralysis (33) or during long-term diaphragm pacing (34). More recently, ultrasound has been employed to monitor changes in the character of other muscles using measurements of echogenicity (35, 36). Such changes have been shown to correlate with muscle inflammation and connective tissue deposition in acute and chronic muscle injury states (37). A method for quantifying echogenicity of the diaphragm was recently reported (36) but the physiological and clinical import of changes in echogenicity are uncertain.

Further work is required to determine the reproducibility of measurements of diaphragm thickness obtained from bedside ultrasound in mechanically ventilated patients, where chest wall edema and mechanical ventilation may introduce ‘noise’ into the measurements. The functional impact of changes in muscle thickness and echogenicity also remains to be determined.

### 2.2 Monitoring diaphragm function

There are a range of techniques for monitoring diaphragm function. Here we discuss the relevant techniques with special reference to their application in mechanically ventilated patients.

#### 2.2.1 Volitional Inspiratory pressures

The most widely employed method of assessing respiratory muscle force generation for clinical purposes is measurement of maximal inspiratory pressure (Pi,max). Pressure is measured at the airway while a subject makes maximally vigorous inspiratory efforts against a closed airway. Measuring esophageal (Pes) and gastric pressure (Pga) during this maneuver permits
measurement of maximum transdiaphragmatic pressure (Pdi,max). Combining maximum inspiratory effort against a closed airway while simultaneously contracting the abdominal muscles (the Müller-expulsive maneuver) further increases Pdi,max by lowering abdominal compliance and enhancing the diaphragm-length tension relation. This maneuver requires careful co-ordination of multiple muscle groups; consequently, it is not possible in mechanically ventilated patients. Indeed, the requirement for maximal volitional participation on the part of the subject is the main point of concern regarding the use of this measurement in ventilated patients. To address this concern, Marini and Truwit sought to maximize inspiratory effort using a 20 second airway occlusion with a one-way valve (permitting expiration but not inspiration) applied to the tip of the endotracheal tube. They showed that with this technique measured pressures corresponded very closely to pressures obtained during repeated vigorously coached inspiratory efforts (38). However, the agreement between the separate measurements depended on the level of respiratory drive at baseline as measured by the airway occlusion pressure (P0.1, details below); adequate respiratory drive is required to stimulate maximal inspiratory efforts during the brief occlusion. Some subjects find the 20-second occlusion intolerable—consequently, an element of co-operation is still required for this maneuver. In ventilated patients, considerable increases in intra-abdominal pressure and chest wall elastance may confound the relationship between airway pressure measurements and diaphragm function (39, 40). Specific assessment of diaphragm strength therefore requires direct measurement of Pdi,max as mentioned. High values of Pi,max or Pdi,max rule out respiratory muscle dysfunction; low values are more non-specific and may reflect muscle weakness, severely deranged mechanics or inadequate inspiratory effort (41).

Sniffing is an intuitive maneuver that induces reproducible maximal contractions of the diaphragm. Measurements of nasal inspiratory pressure during sniffing (SNIP) reflect respiratory muscle strength—some times obtaining values greater than Pi,max—and provide highly reproducible results (42-44). The sniff maneuver has been employed to study diaphragm function in mechanically ventilated patients (45); endotracheal intubation might render the maneuver more difficult or counterintuitive. SNIP measured at ICU discharge in patients recovering from acute respiratory failure predicts the risk of ICU readmission (46).

The ratio of ΔPga to ΔPdi during resting breathing (the Gilbert index) has been shown to be a relevant marker of diaphragm function (47). ΔPga/ΔPdi is closely related to the magnitude of
abdominal displacement during inspiration relative to total chest wall displacement (48). When the diaphragm is paralyzed, ΔPga/ΔPdi will fall below zero during resting tidal breathing (48). This parameter has been used to diagnose diaphragm dysfunction after cardiac surgery (45, 49). Because breathing pattern (i.e. the relative contributions of rib cage and diaphragm to inspiratory pressure generation) is significantly altered in many mechanically ventilated patients (50), ΔPga/ΔPdi may not be sufficiently sensitive or specific for diaphragm dysfunction to be used as a tool for clinical investigation in the intensive care unit. Expiratory activity of the respiratory muscles (which rapidly relax during the early portion of inspiration) can mask the inspiratory rise in Pga; consequently ΔPga/ΔPdi is not a valid index of diaphragm function when measured under such conditions.

### 2.2.2 Phrenic nerve stimulation

To address the challenges of obtaining consistent maximal volitional efforts for respiratory muscle testing, investigators developed techniques for phrenic nerve stimulation which standardize the ‘stimulus’ input to the diaphragm (51). The stimulus can be delivered either by electrical stimulation or magnetic stimulation—the latter is relatively painless and much more well-tolerated. The various technical approaches for phrenic nerve stimulation have been reviewed in detail (52, 53). Measurements of twitch airway pressure (Paw,tw) have been compared to twitch transdiaphragmatic pressure (Pdi,tw), as the former does not require placement of catheters for intracavitary pressure measurements. Using Paw,tw to assess respiratory muscle function has important limitations: because of the high frequency response required to transmit pleural pressure swings to the airway during the brief contraction induced by the twitch stimulus, increases in airway resistance associated with acute respiratory failure may confound the relationship between airway and pleural pressure during twitch phrenic stimulation (54). Moreover, pleural pressure swings induced by respiratory muscle activation do not entirely reflect respiratory muscle or diaphragm force generation as they do not include the force required to overcome chest wall elastance. One study in ventilated patients found that Paw,tw was on average similar to twitch esophageal pressure (Pes,tw) but limits of agreement were wide; Paw,tw was less closely related to Pdi,tw than to Pes,tw (55). Another group of investigators found that Paw,tw was correlated with Pdi,tw in ventilated patients but that limits of agreement were wide (Pdi,tw was 2.5 cm H₂O higher than Paw,tw; limits of agreement ranged from -4.4 to 9.4 cm H₂O) (54). All twitch pressure measurements exhibited strong reproducibility, suggesting
that $P_{aw,tw}$ might be useful as a surrogate for changes in diaphragm function over time (54).

Twitch airway pressures have been used to document diaphragm dysfunction in a number of cohort studies of mechanically ventilated patients (24, 56-58). Nevertheless, because of expense and the technical expertise required to obtain valid measurements, this technique is unlikely to become a widely available clinical test of diaphragm function in mechanically ventilated patients. Great care must be taken to ensure supramaximal stimulation of the phrenic nerve and to avoid the confounding effects of twitch potentiation. As with any measure of diaphragm function, twitch pressures are affected by resting diaphragm length and thoracoabdominal configuration. In at least one clinical study, the technique proved to be poorly tolerated by mechanically ventilated patients (57).

2.2.3 Neuromuscular Coupling

An index of the global performance of the diaphragm may be computed from the ratio of diaphragm pressure generation ($P_{di}$) to the neural stimulus activating the diaphragm (quantified by the rectified diaphragm electromyogram, $E_{di}$) (41)—neuromuscular coupling (NMC). Recent advances in monitoring crural diaphragm EMG with the use of Neurally Adjusted Ventilatory Assist (NAVA) developed by Sinderby et al. (59, 60) have greatly facilitated $E_{di}$ monitoring in the clinical setting. NMC is a novel and potentially feasible and reliable method of monitoring respiratory muscle function. Because it takes the neural stimulus to the diaphragm into account, NMC may be independent of volitional inspiratory effort, potentially overcoming a key limitation of standard methods of assessing diaphragm function as discussed above. Changes in the performance of the respiratory muscles can be detected by changes in NMC, as shown in one study of levosimendan administered to healthy volunteers (61). NMC was recently shown to be a useful predictor of extubation success or failure when measured during a trial of spontaneous breathing (62), lending important clinical validity to this measure. The validity of NMC as a measure of diaphragm function—and its independence from volition—is predicated on the assumption that $P_{di}$ and $E_{di}$ are linearly related. In a number of early studies, the relationship between $P_{di}$ and $E_{di}$ was found to be curvilinear or quasilinear (21, 63). This assumption requires further examination in mechanically ventilated patients, as studies in ventilated patients have obtained varying results (64, 65); efforts to standardize measurement conditions may successfully address potential sources of non-linearity (i.e. length-tension relation, force-velocity relation). As with twitch pressure measurement, this measure is also vulnerable to variation in
resting diaphragm length over time, apart from any changes in muscular contractile function. Furthermore, because ‘normal’ resting Edi varies considerably between individuals, NMC will primarily be useful to detect differences in diaphragm performance over time within individuals, rather than between individuals.

### 2.2.4 Ultrasound

Bedside ultrasound is employed increasingly frequently as a method for assessing diaphragm function. Two sonographic approaches have been described. Using a low frequency phased array transducer positioned under the costal margin at the mid-clavicular line, the dome of the diaphragm can be visualized in B mode, and its excursion can be quantified in M mode (30, 66). Normal values of diaphragm excursion measured by ultrasound for various inspiratory maneuvers have been published (67) and the reproducibility of this technique is excellent (67). Diminished diaphragm excursion has been shown to be diagnostic of diaphragm dysfunction (49). In mechanically ventilated patients, decreased diaphragm excursion is associated with failure of spontaneous breathing trials and prolonged duration of ventilation (68).

A second approach for diagnosing diaphragm dysfunction is to measure diaphragm thickness and thickening during inspiration using a high frequency linear array transducer positioned in the 8th or 9th intercostal space between the mid- and anterior axillary lines (as described above). This technique has been used to diagnose diaphragm paralysis (69) and to monitor recovery from paralysis (33). The percentage increase in thickness during inspiration—the diaphragm thickening fraction, TFdi—is related to shortening of the diaphragm during inspiration (70). TFdi therefore provides a surrogate estimate of diaphragm shortening during inspiratory effort. Maximal TFdi (measured during a maximal inspiratory effort) is correlated with diaphragm function when measured under both under quasi-static conditions (i.e. no inspiratory flow) (71, 72) and under dynamic conditions (i.e. inspiratory flow present) (32, 73). Maximal TFdi predicts the risk of weaning and extubation failure (73, 74). The reproducibility of these measurements has been examined in healthy subjects (75) and in one small study of non-invasively ventilated patients (76). Further work is required to confirm the reproducibility of this technique in ventilated patients and its relation to diaphragm function as measured by gold standard techniques.

### 2.3 Monitoring diaphragm activity
Diaphragm injury may result from either excessive or insufficient (or mistimed) diaphragm contractile activity during ventilation (see below for discussion). Here we briefly consider the various techniques available for monitoring diaphragm activity (inspiratory effort) under mechanical ventilation.

2.3.1 Intrathoracic pressure measurements

2.3.1.1 Respiratory muscle pressure

The pressure generated by all the respiratory muscles is termed the respiratory muscle pressure (Pmus). Pmus is given by the equation of motion (77). The pressure developed by the respiratory muscles can be integrated with respect to inspired volume (work of breathing, WOB) or with respect to time (the pressure-time product, PTP). PTP is preferred over WOB as a measure of inspiratory muscle effort because WOB is strongly confounded by mechanical conditions—for example, WOB is totally insensitive to respiratory muscle effort under iso-lung volume conditions. PTP measurements are more closely related to muscle oxygenation consumption than WOB (78, 79).

The total respiratory muscle pressure (muscle pressure-time product, PTPmus) can be measured directly from the area subtended by the esophageal pressure swing and the pressure-time curve describing the pressure generated by inflation of the chest wall during inspiration (Pcw). Since the elastance of the chest wall is linear over most of the range of chest wall expansion (41), Pcw can be computed at any given time from the product of the instantaneous inflation volume and the chest wall elastance. Chest wall elastance can be measured directly from the rise in Pes during passive inflation of the chest or may be estimated from published predicted values (although these values have not been validated in critically ill patients) (80). Measurements of PTPmus must take into account the potential load due to intrinsic PEEP by marking the onset of effort from the initial drop in Pes rather than from the onset of inspiratory flow (77). Care must be taken to exclude the confounding effect of pre-inspiratory abdominal muscle relaxation on the initial drop in Pes during inspiration (81).

Jubran and Tobin partitioned PTPmus into the various mechanical loads applied to the respiratory muscles during spontaneous breathing in a small cohort of mechanically ventilated patients with chronic obstructive pulmonary disease subjected to a trial of spontaneous breathing
They accounted for the potential effects of abdominal muscle relaxation on PTPmus by computing a lower bound for PTPmus (computing PTPmus from the onset of inspiratory flow) and an upper bound for PTPmus (computing PTPmus from the onset of Pes swing). They found that in the patients under study airway resistance and intrinsic PEEP accounted for a substantial majority of the load on the respiratory muscles. In patients who passed the trial of spontaneous breathing, upper bound PTPmus was approximately 200 cm H₂O-seconds/minute (compared to 388 cm H₂O-seconds/minute in patients who failed an SBT), suggesting a possible upper limit for tolerable inspiratory effort during mechanical ventilation.

Tension-time index (TTI) of the respiratory muscles is computed by taking the ratio of Pmus over Pi,max multiplied by the respiratory duty cycle (ratio of inspiratory time, Ti, over total cycle time, Ttot). This unitless quantity permits evaluation of the balance of load and capacity of the respiratory system. TTI takes into account both the pressure load on the respiratory muscles as a proportion of the maximum force that can be generated, as well as the time available for blood flow to the muscles (which occurs predominantly during muscle relaxation under high inspiratory loads) (83).

2.3.1.2 Transdiaphragmatic pressure

Pdi represents the pressure generated by diaphragmatic contraction. As discussed above it is a function of the tension generated by contraction and the cross-sectional area of the dome of the diaphragm. At high lung volumes where the zone of apposition is markedly reduced it also depends on the various radii of curvature across different aspects of the diaphragm surface (5). Because it takes into account pressure development on both sides of the diaphragm, Pdi is a very reliable measure of diaphragm contractile activity and is not confounded by pre-inspiratory abdominal muscle relaxation. In rare situations, a rise in Pdi may be produced by contraction of the abdominal muscles pressing the abdominal contents into the diaphragm at its elastic limit (84). Transdiaphragmatic pressure-time product (PTPdi) is closely related to diaphragm energy consumption (79). Transdiaphragmatic tension-time index (TTdi) is strongly related to the duration of time that a subject can tolerate a given inspiratory load before developing respiratory failure (85). Loads resulting in a TTdi below 0.15 can be sustained indefinitely, while loads above 0.15 eventually result in task failure. Bellemare and colleagues demonstrated that TTdi has an inverse logarithmic relationship with time to task failure for a given inspiratory load,
suggesting that it is a valid measure of respiratory load-capacity balance (85). Elevated TTdi is associated with a higher risk of spontaneous breathing trial failure in mechanically ventilated patients (86, 87).

2.3.1.3 Airway occlusion pressure

The airway occlusion pressure (P_{0.1}) is a noninvasive technique for monitoring respiratory muscle activity. The P_{0.1} measures the airway pressure developed against a closed valve 100 milliseconds after the initiation of inspiration by the patient. The drop in airway pressure closely parallels the drop in pleural pressure because the airway is occluded. Because the patient does not perceive the presence of the closed airway valve within this short time, the patient’s inspiratory effort is not modified by the brief occlusion. As a result, the measured pressure provides a reliable indicator of the rate of rise of inspiratory effort – a measure of neural respiratory drive (41, 88).

P_{0.1} correlates with the work of breathing and varies inversely with the level of ventilatory support (89, 90). Consequently, it could guide the titration of ventilatory support to some optimal level of respiratory drive that is neither inadequate nor excessive (91). There is considerable breath-to-breath variability in this measurement (92) and the relationship between respiratory drive and airway occlusion pressure may be confounded by changes in end-expiratory lung volume and diaphragm configuration, especially in patients with elevated airways resistance (88).

2.3.2 Electromyography

While diaphragm electromyography (EMG) has been employed to monitor inspiratory effort in the physiology laboratory for a number of decades (93), significant advances in the last 15 years have made diaphragm EMG a feasible monitoring modality for clinical use. This technique has been implemented as the control signal for Neurally Adjusted Ventilatory Assist (NAVA), developed by Sinderby and Beck (59). In the setting of critical illness, EMG signals are usually obtained from intraesophageal electrodes located near the crural diaphragm because of the widespread use of nasogastric tubes and the ease with which stable signals may be obtained. Signals obtained from a multiple array electrode are analyzed using a cross-correlation algorithm to continuously identify the location of maximal EMG signal (as the diaphragm descends and
relaxes) and this signal is filtered to remove ECG artefact. Average values for 50 millisecond windows of the root mean square EMG are computed as the diaphragm electrical activity (Edi). This signal is displayed in real time on the Servo-i ventilator (Maquet, Sweden) and can be used for clinical monitoring or to control mechanical ventilation. Techniques for monitoring EMG signals from the costal diaphragm are also well-described (94, 95). Previous work suggests that the costal and crural diaphragm regions are activated homogeneously during inspiration (63, 96).

Edi has been shown to correlate with inspiratory effort at varying lung volumes and under varying inspiratory loads (63, 97). Indeed, insofar as Edi quantifies the neural stimulus to the diaphragm, it is in theory a more valid reflection of inspiratory effort than pressure-based measurements. Because the measured Edi exhibits considerable intersubject variability (possibly related to differences between electrode-muscle distance due to anatomic variation), between-subject comparisons require normalization for maximum Edi obtained during inhalation to total lung capacity (63, 97). However, it is often difficult to obtain maximal volitional inspiratory efforts in mechanically ventilated patients, precluding Edi normalization. Moreover, because changes within the diaphragm over time during mechanical ventilation (i.e. atrophy, discussed below) may affect the magnitude of the EMG signal (98), maximal Edi may vary considerably from day to day in critically ill patients. One study found that normalizing maximal Edi against the ECG artefact reduces day-to-day variation in Edi measurements (99).

Bellani and colleagues described an approach to predicting Pmus from the Edi signal based on measurements of neuromuscular coupling (64). As discussed earlier, the validity of this approach is predicated on a linear relation between Pmus and Edi, and most previous studies suggest that the relation is substantially curvilinear even under static conditions. Further work is required to demonstrate how the Edi signal may be employed to titrate mechanical ventilation (100).

2.3.3 Ultrasound: inspiratory thickening of the diaphragm

As the diaphragm shortens during inspiratory contractions, its thickness increases due to the fixed volume of the muscle and the recruitment of fibres from the dome of the diaphragm into the zone of apposition (70). Thickening of the diaphragm in the zone of apposition during an inspiratory effort can be visualized and quantified by surface ultrasound (31, 101). Diaphragm thickening correlates with inspiratory pressure development (71) and inspiratory volume (32). Inter-rater reliability in non-ventilated subjects is excellent (102). During non-invasive and
invasive ventilation, diaphragm thickening correlates well with the work of breathing (76, 103). Therefore, monitoring diaphragm thickening represents a potential means for noninvasive assessment of respiratory muscle activity.

Important questions remain: (1) How reproducible are diaphragm thickening measurements between observers in the general population of mechanically ventilated patients? (2) How easily can inexperienced observers be trained to measure thickening reliably? (3) Could diaphragm thickening reflect a passive change in configuration of the muscle as lung volume increases during mechanical insufflation or does it specifically represent contractile shortening of the muscle? These questions will be addressed as part of this body of work.

2.4 Monitoring patient-ventilator synchrony

Synchronization between patient and ventilator is assessed in terms of whether the timing of inspiratory and expiratory ventilator cycling is closely matched to the timing components of the patient’s respiratory cycle (104, 105). Consequently, monitoring synchrony requires knowledge of both the timing of ventilator cycling (readily available for the ventilator monitor) and the timing of the patient’s neural cycling (more challenging to assess). The components of the neural respiratory cycle times are conventionally assessed by monitoring inspiratory and expiratory flow but this technique cannot be relied upon in mechanically ventilated patients where flow is determined largely by mechanical cycling and the presence of intrinsic PEEP or ineffective triggering may confound the relationship between flow and neural cycle times. Careful inspection of airway pressure waveforms on the ventilator screen can aid detection of patient-ventilator synchrony but even expert clinicians fail to identify many dyssynchronous events with this approach (106). Consequently, alternate methods for identifying the onset and offset of neural inspiration are required.

Several of the techniques for monitoring diaphragm activity already discussed in section 2.3 may be employed to assess patient-ventilator synchrony. Monitoring Pes allows reasonably accurate identification of the onset of inspiration based on the onset of the negative pressure swing (if present) on the esophageal pressure tracing. Edi constitutes the gold standard method of determining the onset of neural inspiration (apart from monitoring phrenic nerve firing activity, a measurement not feasible in the clinical setting). Neural expiration is believed to occur when Edi declines to 70% of the inspiratory peak value (107). Both Pes and Edi monitoring have been
employed to readily detect a range of dyssynchronous events in clinical studies (77, 108-110). More recently, some have proposed diaphragm ultrasound as a means of monitoring patient-ventilator synchrony (30) but this technique has not been evaluated for this purpose in clinical studies as yet. Automated real-time analysis of airway pressure waveforms by computerized algorithms may also reliably detect certain types of dyssynchronous events (111) but the infrastructure and software to implement this approach are not yet widely available.

3 The Diaphragm in Acute Respiratory Failure: Pathophysiology and Clinical Importance

We now discuss the clinical significance of diaphragm function in the context of mechanical ventilation by considering the clinical and economic impact of prolonged ventilator-dependence and the physiological issues that account for failed liberation from mechanical ventilation with particular reference to diaphragm dysfunction. We will examine the prevalence and mechanisms of diaphragm dysfunction in this population. Key concepts in the following discussion are summarized in Figure 1-2.

3.1 The Clinical Problem: Prolonged Mechanical Ventilation

3.1.1 Prevalence, Outcomes and Costs

Most patients receiving invasive ventilatory support for acute respiratory failure are readily liberated from the ventilator upon recovery from their acute illness. For a significant minority of patients, however, the transition from mechanical ventilatory support to unassisted breathing proves challenging. Once ventilated patients demonstrate evidence of initial recovery from the cause of acute respiratory failure, they are subjected to a daily trial of spontaneous breathing (112, 113). Difficult weaning is diagnosed by failure of a trial of spontaneous breathing. A consensus panel of experts defined difficult weaning as failure of up to 2 trials of spontaneous breathing, and prolonged weaning as failure of 3 or more trials (114). Using these definitions, estimates of the prevalence of difficult weaning range between 25-40% and 6-20% of patients meet criteria for prolonged weaning (115, 116).

The outcomes of patients who prove difficult to wean from mechanical ventilation are much worse than those of patients who are readily liberated from ventilation. Prolonged mechanical ventilation (PMV) is associated with weakness acquired in the intensive care unit (ICU) (117)
and there is mounting evidence that long-term functional prognosis is poor in this population (118, 119). The mortality rate is higher than predicted (115) and survivors experience very poor long-term functional outcomes. In one study, just under 10% of PMV patients (PMV defined as requirement for MV for at least 21 days) were alive with no functional dependency one year after ICU admission (120). Most survivors had considerable functional dependency and 66% of patients remained in a hospital or nursing home.

Although they constitute a small proportion of all ventilated patients, PMV patients occupy over 20% of intensive care unit (ICU) bed-days (121) and account for nearly two-thirds of all healthcare costs related to mechanical ventilation (122). A recent study found that chronic critical illness (of which prolonged acute mechanical ventilation is the most common cause) occurred in 34.4/100,000 individuals and accounts for up to $26 billion in healthcare costs in the United States (123). A recent Canadian survey suggested that patients requiring PMV (defined as receiving at least 21 days of ventilation) occupied 11% of Canadian ICU beds (124).

Projections based on current demographic trends suggest that the population of patients requiring prolonged mechanical ventilation will grow by approximately 50% over the next decade (125). Given its impact on clinical outcomes, the pressures it places on the healthcare system, and the projected growth in population with the aging population, prolonged mechanical ventilation constitutes a pressing public health concern.

### 3.1.2 Pathophysiologic Mechanisms of Difficult Weaning From Mechanical Ventilation

Only a few studies have reported rigorous measurements of respiratory mechanics and respiratory muscle function in patients failing a trial of spontaneous breathing. Nevertheless, these studies provide profoundly important insights as to why patients fail efforts at weaning from mechanical ventilation. Three separate groups of investigators demonstrated that the key determinants of the outcome of a trial of spontaneous breathing are the tension-time index and frequency-tidal volume ratio (82, 86, 126). These findings suggest that ventilator-dependence results both from an imbalance between the load and capacity of the respiratory muscle pump and from the breathing pattern adopted by the patient’s respiratory controller in response to the load/capacity imbalance. While many clinicians assume that the frequency-tidal volume ratio (f/Vt) reflects load-capacity imbalance, f/Vt was in fact independent of TTI in both these studies,
suggesting that the breathing pattern adopted during spontaneous breathing is indicative of an independently determined response to respiratory loading arising from the brainstem respiratory centers, likely with input from higher cortical and subcortical centers. This hypothesis is further supported by the observation that rapid shallow breathing develops during a failed trial of spontaneous breathing before there is any evidence of respiratory muscle fatigue (127).

Patients who fail a trial of spontaneous breathing commonly exhibit excess respiratory loads secondary to weaning-induced pulmonary edema (128, 129), atelectasis (130), or dynamic hyperinflation (82). Importantly, these loads seem to be produced by the act of spontaneous breathing, as elastance and resistance (measured before a trial of spontaneous breathing) are very similar between patients who successfully complete a spontaneous breathing trial compared to those who fail (131). By contrast, at the termination of a spontaneous breathing trial the total load on the respiratory muscles (pressure-time product) is markedly higher in patients who fail the trial compared to those who successfully pass the trial (82, 86). Increases in intrinsic positive end-expiratory pressure (PEEP) and airway resistance accounted for most of the increase in load. In these studies, hypercapnia developed in weaning failure patients due to an alteration in breathing pattern rather than a change in pulmonary dead space or the rate of CO₂ production. These findings suggest that (a) the development of a rapid shallow breathing pattern in response to a given load is a significant determinant of the outcome of a spontaneous breathing trial as it causes hypercapnia and probably contributes to the rise in intrinsic PEEP; and (b) the increase in inspiratory effort during a trial of spontaneous breathing precipitates physiological changes that cause a deterioration in respiratory mechanics, principally airway resistance (and, in consequence, worsened intrinsic PEEP). Diaphragm contraction increases venous return by virtue of its opposing effects on intraabdominal pressure (increase) and intrathoracic pressure (decrease) (132), particularly in volume overloaded subjects (129). Accordingly, the increase in diaphragm contractile activity during spontaneous breathing may precipitate acute pulmonary edema (128) which could explain the rise in airway resistance during spontaneous breathing trials.

At the same time, many ventilator-dependent patients exhibit striking loss of respiratory pump capacity. Diaphragm dysfunction is an important risk factor for failing weaning attempts: Pdi,max and Pi,max are significantly lower in patients who fail a trial of spontaneous breathing compared to those who succeeded (86, 126). Diaphragm dysfunction ascertained by ultrasound
techniques is also associated with weaning failure (68, 73, 74) and recovery of respiratory muscle strength is associated with successful weaning after prolonged mechanical ventilation (133). Consequently, maintaining and restoring respiratory muscle function is a priority for the prevention and treatment of prolonged ventilator-dependence.

3.2 Diaphragm Dysfunction During Critical Illness

3.2.1 Prevalence and Outcomes

Patients requiring prolonged mechanical ventilation exhibit a striking loss of respiratory muscle capacity over the course of their critical illness. Dramatic reductions in respiratory muscle strength have been documented suggesting that the patient’s capacity to tolerate even relatively minor increases in work of breathing is greatly compromised (55). Twitch transdiaphragmatic and airway pressures have been measured by several studies of ventilated patients. In one study of 57 mechanically ventilated patients, Pdi, tw averaged 7.9 cm H₂O (SD 0.6 cm H₂O, lower limit of normal = 28 cm H₂O), consistent with profound diaphragm weakness (58). Several studies have documented Pdi, tw in a similarly low range (54, 55, 57). Low Pdi, tw is associated with an increased risk of mortality and a prolonged duration of mechanical ventilation (57, 58).

Demoule and colleagues measured Paw, tw on days 1 and 3 of mechanical ventilation in 85 patients; median Paw, tw was 8.2 cm H₂O (IQR 5.9-12.3) on day 1. Using a cut-off of 11 cm H₂O for the lower limit of normal (based on measurements in control subjects), they found that 64% of ventilated patients had significant respiratory muscle weakness at admission. There was no significant difference between Paw, tw on day 1 and day 3. Notably, the early respiratory muscle weakness documented in this study was associated with mortality but not with duration of mechanical ventilation, suggesting that early respiratory muscle weakness may be a marker of illness severity rather than a mediator of clinical weaning outcomes.

Kim and colleagues ascertained diaphragm function during the first spontaneous breathing trial by measuring diaphragm excursion during unassisted tidal breathing (68). In their study, 29% of patients met criteria for diaphragm dysfunction (excursion < 1 cm); diaphragm dysfunction was associated with a significantly higher risk of difficult weaning and longer ICU and hospital stay.

In summary, diaphragm dysfunction is very common in the setting of mechanical ventilation and is associated with difficult weaning and excess mortality. The vast majority of mechanically
ventilated patients have no antecedent history of neuromuscular disease (134). The relatively high frequency of diaphragm dysfunction in mechanically ventilated therefore likely results from factors associated with critical illness and acute respiratory failure.

### 3.2.2 Structural abnormalities of the human diaphragm in critical illness

A number of studies have characterized structural changes in the diaphragm during critical illness by studying diaphragm tissue obtained from brain dead organ donors after relatively brief periods of brain death (1-3 days). Collectively, these studies have demonstrated (1) dramatically reduced cross-sectional area of both slow-twitch and fast-twitch fibers compared to control samples obtained from thoracic surgery controls (7, 24); (2) evidence of oxidative stress and nutrient excess (7, 25, 135); a significant increase in the activity of proteolytic pathways (calcium-dependent proteases, autophagy, ubiquitin-proteasome system) (7, 24, 135); (3) ultrastructural injury characterized by myofibrillar disarray, and (4) impaired mitochondrial biogenesis and metabolism (25). Of note, the degree of myofibrillar atrophy and the magnitude of ultrastructural injury were not correlated, suggesting that separate insults may be responsible for these phenomena (24).

Diaphragm myofibrillar abnormalities in living mechanically ventilated patients have only recently been described. Diaphragm muscle biopsies from 22 patients undergoing mechanical ventilation for varying durations of time demonstrated a 25% reduction in cross-sectional area with increased activation of the ubiquitin-proteasome proteolytic pathway, similar to the findings from brain dead organ donors (136). Diaphragm tissue staining revealed infiltration with neutrophils and macrophages, suggesting an inflammatory response within the diaphragm. Myofibrillar disarray was also documented in some parts of the tissue.

### 3.2.3 Functional abnormalities of the human diaphragm in critical illness

A detailed examination of the mechanisms of contractile weakness at the myofibrillar level in tissue from the human diaphragm in live patients revealed that both fiber atrophy and dysfunction of sarcomeric proteins (specifically through a reduction in the number of attached cross-bridges during activation due to myofibrillar damage) contributed to loss of force-generating capacity in individual myofibers (136). The observed degree of loss of force-
generating capacity at the myofibrillar level in that study was sufficient to explain the reductions in Pdi, tw typically reported in mechanically ventilated patients, suggesting that these molecular changes account for the majority of clinical diaphragm dysfunction (136).

3.2.4 Mechanisms of Diaphragm Injury and Weakness in Critically Ill Patients

3.2.4.1 Systemic factors

A range of systemic factors associated with critical illness can impair diaphragm function. Sepsis is associated with marked acute reductions in diaphragm strength (56, 58, 137) mediated by proinflammatory cytokines (138), oxidative stress (139) and activation of proteolytic pathways (140, 141). One study found that sepsis is associated with an accelerated rate of muscle atrophy (preferentially affecting the diaphragm) based on serial computed tomography scans (28). Sepsis also renders the diaphragm more sensitive to load-related injury, possibly by increasing sarcolemmal membrane fragility (142). Many medications employed in the care of critically ill patients have myopathic effects, including neuromuscular blockade (which may be slow to resolve after discontinuation of an agent), corticosteroids and aminoglycosides (143). Metabolic derangements including acidosis and hypokalemia contribute (144). Pre-existing systemic conditions such as malnutrition or endocrinopathies may also be responsible for diaphragm dysfunction in the critically ill (143).

3.2.4.2 Neuromuscular factors

Deleterious changes in diaphragm length or geometry due to thoracic hyperinflation are probably reasonably common in the setting of acute respiratory failure due to the effects of mechanical ventilation or an increase in airway resistance (and hence, intrinsic PEEP) associated with many acute respiratory pathologies. Critical illness polymyoneuropathy (CIPMN) may contribute to significant diaphragm dysfunction. In patients receiving ventilation for seven or more days, MIP was found to be correlated with the degree of peripheral neuromuscular weakness as measured by the Medical Research Council score, suggesting that CIPMN affected the phrenic nerve or diaphragm as well as the limb muscles (145). Critical illness polyneuropathy is associated with prolonged ventilation (146) and one study documented diaphragm denervation in almost 25% of difficult-to-wean patients (147).
Given that the severity of myofibre contractile dysfunction resulting from the ultrastructural changes outlined above is sufficient to account for the degree of diaphragm weakness documented in clinical studies, and because such changes have been demonstrated in both brain dead organ donors and live patients during mechanical ventilation, these myofibrillar derangements provide the most likely explanation of muscle weakness in the clinical setting. The features typical of critical illness myopathy (selective loss of thick filaments, patchy necrosis and regeneration and atrophy of Type I and II fibers) have not been demonstrated in the diaphragm of ventilated patients to this point, suggesting that mechanisms other than those responsible for CIPMN may be at play. Moreover, the injury has been documented to occur very early after initiating mechanical ventilation. A growing body of evidence suggests that mechanical ventilation itself may be responsible for the structural injury and dysfunction observed in the diaphragm in critically ill patients.

4 Ventilator-induced diaphragm dysfunction (VIDD)

The dangers to the lung associated with mechanical ventilation (ventilator-induced lung injury, VILI) have been recognized and investigated for decades (148). From the study of VILI we have learned that careful application of mechanical ventilation may prevent morbidity and mortality in patients with acute respiratory failure. However, the field has only recently begun to recognize that mechanical ventilation can acutely injure the diaphragm. This possibility raises the spectre of a vicious cycle of ventilator-dependence: acute respiratory failure renders patients ventilator-dependent, and injurious mechanical ventilation weakens the respiratory muscles which perpetuates ventilator-dependence. Just as the widely recognized phenomenon of ventilator-induced lung injury seriously worsens the outcomes of acute lung injury patients, ventilator-induced diaphragm dysfunction (VIDD) may put all patients with acute respiratory failure at increased risk of persistent ventilator dependence attended by excess morbidity and mortality.

The evidence implicating mechanical ventilation in diaphragm injury can be summarized as follows: (1) mechanical ventilation is strongly associated with structural and functional abnormalities of the diaphragm and (2) there are several putative mechanisms with strong experimental support by which mechanical ventilation could cause diaphragm injury. Nevertheless, as will be discussed below, the extent to which these mechanisms are responsible for diaphragm injury in the clinical setting is largely unknown.
4.1 Seminal clinical studies linking mechanical ventilation to diaphragm injury

Diaphragm atrophy following mechanical ventilation was originally described in premature neonates subjected to prolonged mechanical ventilation (149). The authors attributed the abnormally thin diaphragm observed at autopsy to disuse atrophy or failure of normal growth processes and speculated that this phenomenon could contribute to difficult weaning from ventilator support.

Several studies comparing diaphragm biopsy specimens from brain dead organ donors to specimens obtained from patients undergoing relatively short thoracic surgical procedures have provided important evidence linking mechanical ventilation to diaphragm injury in the clinical setting. Significant atrophy of both slow and fast twitch fibres was documented in brain dead subjects after a short period of brain death and hence total diaphragmatic quiescence (between 18 and 69 hours, the duration of ventilation prior to brain death was not reported) using specimens from control patients (mechanical ventilation for only 2-3 hours during thoracic surgery) for comparison (7). By contrast, there were no differences in the pectoralis major muscle cross-sectional area between the two groups, suggesting that the injury was specific to the diaphragm. A second study of similar design demonstrated that the degree of diaphragm atrophy and the magnitude of ultrastructural injury was correlated with the duration of mechanical ventilation (24). Furthermore, daily measurements of Paw,tw demonstrated progressively diminishing respiratory muscle strength over the first 5-6 days of mechanical ventilation (24). Finally, diaphragm thickness measured by ultrasound was shown to progressively decrease over the first several days of mechanical ventilation in a small case series (150). Taken together, these studies provide important—albeit circumstantial—evidence that mechanical ventilation may cause diaphragm injury (the VIDD hypothesis).

4.2 Proposed mechanisms of VIDD

4.2.1 Diaphragm inactivity

The earliest experimental evidence in support of the VIDD hypothesis was reported by Anzueto and colleagues who subjected baboons to 11 days of controlled mechanical ventilation: they found that lung function and hemodynamics remained stable over that period but diaphragm strength decreased by 25% and endurance decreased by 36% (151). In 2002, three separate
groups independently reported the following key findings from carefully controlled experiments in different animal models of VIDD: (1) controlled mechanical ventilation results in diaphragm atrophy and loss of force generating capacity \textit{in vitro}; (2) the atrophy and weakness occurred rapidly, beginning within 12 hours of commencing controlled mechanical ventilation; (3) the muscle atrophy was specific to the diaphragm – peripheral skeletal muscle was unaffected; (4) anaesthetized but spontaneously breathing control animals with only continuous positive airway pressure (CPAP) applied did not show any evidence of deteriorating muscle structure or function (152-154). These key findings have been replicated many times over by subsequent experiments (155).

These data suggest that diaphragm contractile inactivity induced by hyperventilation during mechanical ventilation causes rapid disuse atrophy of the diaphragm. The importance of diaphragm inactivity is further supported by experimental observations that diaphragm atrophy and dysfunction during mechanical ventilation can be attenuated by maintaining some level of respiratory muscle activity during ventilation. VIDD was less severe in animals receiving assisted mechanical ventilation where animals triggered ventilator breaths compared to controlled mechanical ventilation with no triggered breaths (156). In contrast to controlled mechanical ventilation, pressure support ventilation (PSV) was not associated with an altered balance between proteolysis and protein synthesis (157). The use of adaptive support ventilation prevented diaphragm atrophy and weakness in piglets (158). Another group found that intermittent periods of spontaneous breathing during controlled ventilation significantly reduced the severity of VIDD (159). While these data seem to suggest that partial support modes of breathing will effectively prevent VIDD, a recent study found that high levels of pressure support produced significant suppression of respiratory muscle activity, atrophy and muscle weakness – nearly identical to the effects of controlled ventilation (160). In contrast, lower levels of pressure support did not result in VIDD. These authors documented significant suppression of respiratory muscle activity in the high PSV rats. Collectively, these studies suggest that suppressing respiratory muscle activity injures the diaphragm irrespective of the mode of mechanical ventilation \textit{per se}.

Considerable progress has been made in delineating the molecular pathways linking diaphragm inactivity during mechanical ventilation to diaphragm injury and dysfunction. Postulated mechanisms have been reviewed in detail elsewhere (161). Briefly, diaphragm inactivity during
mechanical ventilation results in the production of reactive oxygen species (25, 162) possibly as a consequence of mitochondrial dysfunction (25). This oxidative stress activates several proteolytic pathways (calcium-dependent proteolytic enzymes (160), ubiquitin-proteasome pathway (163), and autophagy (164)). The resulting imbalance between proteolysis and protein synthesis leads to progressive myofibrillar atrophy and may be responsible for myofibrillar disarray. The critical role of oxidative stress is supported by the observation that treatment with various antioxidant agents mitigates experimental VIDD (165-168).

One fascinating experiment observation raises the possibility that diaphragm inactivity may mediate contractile dysfunction via changes in diaphragm vascular function. Davis and colleagues found that 6 hours of mechanical ventilation resulted in a progressive decline in diaphragm tissue PO$_2$ and a dramatic reduction in diaphragm blood flow (169). Following the period of inactivity, blood flow augmentation with stimulated diaphragm contractions was markedly attenuated, suggesting a dysfunctional vascular response to metabolic demands (normally very tightly coupled to contractile activity in the diaphragm and other muscle tissues). This observation suggests that vascular dysfunction could also contribute to diaphragm dysfunction when the muscle is reloaded during weaning from mechanical ventilation in the clinical setting (170).

4.2.2 Excess diaphragm loading

While the potentially injurious effects of diaphragmatic quiescence during mechanical ventilation have been the subject of active investigation, less attention has been paid to the potential deleterious effects of insufficient unloading of the diaphragm during mechanical ventilation. There is strong evidence that excess respiratory muscle loads can cause diaphragm injury and respiratory failure. Tracheal banding experiments in hamsters (resistance load applied to increase inspiratory muscle effort to 20% of Pi$_{max}$) demonstrated sarcomeric disruption and muscular inflammation in response to chronic low level loading (171). Respiratory failure ensued over a period of several days. Acute inspiratory resistive loading can cause diaphragm inflammation, injury and dysfunction when sufficiently high, although the contractile weakness is often delayed by up to 24-48 hours (172, 173). Muscle inflammation following inspiratory resistive loading results from endothelial expression of adhesion molecules which assist granulocytes and macrophage infiltration of muscle tissue (174).
Diaphragm injury due to both chronic and acute inspiratory loading has been documented in humans. Histologic examination of diaphragm specimens obtained from both healthy subjects and COPD patients (who are subjected to chronically elevated resistive loads) reveals sarcomere disruption, worse in patients with COPD (175). Following an inspiratory resistive loading protocol, the degree of sarcomeric disruption was increased in both healthy subjects and COPD patients (175).

These observations raise the possibility that insufficient respiratory support during mechanical ventilation for acute respiratory failure could cause diaphragm injury and dysfunction. However, it is unclear to what extent patients may be exposed to injurious loads during mechanical ventilation given the careful attention paid to patients in the intensive care setting. The load required for injury is surprisingly low in some studies (less than 20% of Pi,max). Loads exceeding the fatigue threshold have been documented during weaning trials in at least one study (127). There was no acute change in diaphragm force generating capacity in that study (Pdi,tw measured immediately following resumption of mechanical ventilatory support), but some have suggested that diaphragm weakness could have developed in the ensuing 48 hours (176). Further data is required on the frequency, duration, magnitude and mechanisms of excess inspiratory effort during mechanical ventilation in the clinical setting.

4.2.3 Eccentric contractions

The potential for diaphragm injury from a given inspiratory load heavily depends on the timing of load application. It is well-established that eccentric muscle contractions (contractile activation during muscle lengthening) are far more injurious than concentric contractions (contractile activation during muscle shortening) (177). Eccentric contractile conditions increase the tension applied to the respiratory muscles (178). Although low levels of eccentric contractile activity are common during resting tidal ventilation in humans where the inspiratory and expiratory muscle groups may be simultaneously activated to control air flow to a fine degree (9), eccentric contractions can cause significant acute diaphragm injury as demonstrated in experimental rat and canine models (179, 180).

Diaphragm injury from eccentric contractions has not yet been documented in humans. In mechanically ventilated patients, the diaphragm may be exposed to eccentric contractile injury when the ventilator is poorly synchronous with the depth and timing of the patient’s inspiratory
effort. One common type of patient-ventilator dyssynchrony, ineffective efforts, manifests as diaphragm contraction during mechanical expiration (108). Because lung volume is falling as the diaphragm contracts, such contractions may occasionally be eccentric and diaphragm injury may result. Reverse triggering dyssynchrony, where mechanical insufflation induces neural inspiration, can result in diaphragm contractile activation while the ventilator cycles from inspiration to expiration (and lung volume precipitously falls) as clearly documented by Akoumianaki and colleagues in their original description of this phenomenon (110). Eccentric diaphragm contractions may also occur in the context of expiratory braking, where the diaphragm is activated to prevent excessive loss of lung volume and development of atelectasis (181, 182).

This discussion is largely theoretical as the frequency with which the diaphragm is exposed to eccentric contractile conditions remains largely unknown. Systematic clinical observation is required to clarify whether eccentric contractions constitute an important mechanism of diaphragm injury in the clinical setting.

4.3 VIDD: Unanswered Questions

4.3.1 Is VIDD a clinically significant phenomenon?

While laboratory evidence strongly suggests that mechanical ventilation has the potential to injure the diaphragm in the clinical setting, there is significant uncertainty about the importance of this phenomenon in the clinical setting. This uncertainty presents an important barrier to the design, evaluation and implementation of interventions to prevent diaphragm injury and to accelerate liberation from mechanical ventilation. There are several reasons for this uncertainty.

First, as discussed earlier, diaphragm function is affected by many systemic and neuromuscular factors commonly associated with critical illness. The specific contribution of VIDD to the high prevalence of impaired diaphragm function in this population is therefore uncertain. Interventions to prevent VIDD are unlikely to improve important clinical outcomes unless VIDD accounts for a significant proportion of diaphragm injury and dysfunction occurring under mechanical ventilation. To date there is no information on the incidence of VIDD in the general population of mechanically ventilated patients and its impact on physiological function and clinical outcomes.
Second, the extent to which critically ill patients are exposed to the various putative mechanisms of VIDD is unknown. While patients subjected to neuromuscular blockade clearly have a significant period of inactivity, this accounts for a very small proportion of patients and a relatively short duration of ventilatory support. The magnitude and duration of diaphragm inactivity or excess inspiratory effort, and the frequency of eccentric contractions, constitute key unknowns that make it difficult to assess the clinical impact of VIDD.

Third, a direct link from diaphragm inactivity (or excess activity) and/or patient-ventilator dyssynchrony to diaphragm injury and dysfunction has not been established in the clinical setting. Claims about the harmful effects of ventilation-induced diaphragm inactivity in patients are based on extrapolations from laboratory models. These putative mechanisms require clinical confirmation.

4.3.2 How can we monitor for the development of VIDD in the clinical setting?

As detailed earlier, monitoring diaphragm function in the clinical setting is challenging. While twitch stimulation has been employed to measure changes in respiratory muscle strength in some cohort studies, such measurements do not specifically reflect the effect of mechanical ventilation on the diaphragm but are also affected by sepsis and systemic inflammation, pharmacologic exposures and metabolic derangements. Twitch phrenic nerve stimulation is labour-intensive and technically challenging, requiring considerable operator experience. Alternative non-volitional measurements might be more feasible to study changes in diaphragm function longitudinal in sizable cohort studies. It is unknown whether changes in neuromuscular coupling might supply useful insights in this regard.

Given the anatomical location of the diaphragm, it is not feasible to obtain serial biopsies to describe changes over time in diaphragm structure and function or to obtain a sufficient quantity of observations to be able to link such changes to the manner in which ventilation is applied.

Ultrasound represents a potential monitoring technique to evaluate changes in muscle thickness and preliminary observations suggest that important changes in diaphragm thickness can be detected by ultrasound (150). At present there is very little data on the histopathological changes associated with changes in diaphragm thickness over time (183), so that the precise structural
changes responsible for sonographic changes, if any, are unclear. Ultrasound might also permit assessments of the level of inspiratory effort on mechanical ventilation. However, the reproducibility and validity of this modality to monitor changes in diaphragm contractile activity or changes in diaphragm structure in mechanically ventilated patients have not been confirmed. Finally, it is uncertain whether a single daily measurement with ultrasound is representative of the patient’s average inspiratory effort level over a 24 hour period.

5 Conclusion

Respiratory muscle weakness, especially weakness of the diaphragm, is a major cause of prolonged ventilator dependence. Mechanical ventilation may be a major cause of diaphragm dysfunction. If this is in fact the case, muscle-protective ventilatory strategies that maintain adequate diaphragm activity during ventilation could prevent prolonged ventilator dependence. To develop muscle-protective ventilation strategies, better methods for measuring both diaphragm activity and function are required.
Table 1-1. Techniques for Monitoring Diaphragm Structure, Function and Activity During Mechanical Ventilation

<table>
<thead>
<tr>
<th>Monitoring Goal</th>
<th>Monitoring Technique</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>Histopathology</td>
<td>Precise structural information permitting mechanistic insights</td>
<td>Very difficult to obtain diaphragm biopsies, especially to ascertain serial changes over time</td>
</tr>
<tr>
<td></td>
<td>Computed tomography</td>
<td>Permits precise measurements of diaphragm volume</td>
<td>Reproducibility not established</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not feasible to obtain serial CTs of the diaphragm outside research setting</td>
</tr>
<tr>
<td></td>
<td>Magnetic resonance imaging</td>
<td>Permits very accurate information about diaphragm geometry</td>
<td>Expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not feasible to obtain serial MRIs of the diaphragm outside research setting</td>
</tr>
<tr>
<td></td>
<td>Ultrasound</td>
<td>Highly feasible to obtain measurements at bedside</td>
<td>Reproducibility uncertain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Provides insights on changes in muscle thickness and inflammation</td>
<td>Limited location for assessment (lateral costal regions of each hemidiaphragm)</td>
</tr>
<tr>
<td>Function</td>
<td>Maximal inspiratory pressure</td>
<td>Simple maneuver and easy to measure at bedside</td>
<td>Can be difficult to obtain full cooperation of critically ill patient for maximal volitional inspiratory effort</td>
</tr>
<tr>
<td></td>
<td>Sniff inspiratory pressure</td>
<td>Simple intuitive maneuver and easy to measure at bedside</td>
<td>Uncertain whether ventilated patients find ‘sniffing’ intuitive and whether this generates the same effort as a maximal Müller maneuver in ventilated patients</td>
</tr>
<tr>
<td></td>
<td>ΔPga/ΔPdi (Gilbert index)</td>
<td>Simple maneuver and easy to interpret</td>
<td>Diagnostic sensitivity and specificity uncertain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Requires insertion of esophageal and gastric balloon catheters</td>
</tr>
<tr>
<td></td>
<td>Phrenic nerve stimulation</td>
<td>Standardized stimulus to diaphragm overcomes difficulties with variable volition in mechanically ventilated patients</td>
<td>Paw,tw does not reflect Pdi,tw, so balloon catheters required for accurate diaphragm assessment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Requires considerable operator expertise</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Equipment is expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serial twitches may not be well-tolerated in critically patients</td>
</tr>
<tr>
<td>Monitoring Goal</td>
<td>Monitoring Technique</td>
<td>Pros</td>
<td>Cons</td>
</tr>
<tr>
<td>----------------</td>
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<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Neuromuscular coupling</td>
<td>Relatively easy to ascertain</td>
<td>Requires insertion of nasogastric catheter and specialized equipment for acquiring and processing EMG signals</td>
<td>Must monitor for confounding effects (twitch potentiation, supramaximality)</td>
</tr>
<tr>
<td>Ultrasound: excursion</td>
<td>Highly reproducible measurement</td>
<td>May be affected by respiratory mechanics as excursion reflects expansion of chest wall rather than effort <em>per se</em></td>
<td>Requires patient volitional effort (depending on measurement)</td>
</tr>
<tr>
<td>Ultrasound: maximal thickening fraction</td>
<td>Highly feasible to obtain at bedside</td>
<td>Reproducibility uncertain</td>
<td>Requires patient volitional effort (depending on measurement)</td>
</tr>
<tr>
<td>Activity</td>
<td>Respiratory muscle pressure</td>
<td>Valid assessment of global inspiratory effort</td>
<td>Requires esophageal balloon catheter</td>
</tr>
<tr>
<td>Work of breathing</td>
<td>Valid assessment of mechanical work done by the respiratory muscles</td>
<td>Reflects work done and so is confounded by mechanical conditions (i.e. effort may be high while work may below)</td>
<td>Requires knowledge of passive chest wall elastance (unclear if predicted elastance matches actual elastance in critically ill patients) Care must be taken to correct for intrinsic PEEP and pre-inspiratory abdominal muscle relaxation Could be confounded by changes in diaphragm geometry due to changes in thoracic volume/configuration</td>
</tr>
<tr>
<td>Tension-time index</td>
<td>Reflects load/capacity balance of the respiratory system</td>
<td>Requires esophageal balloon catheter to measure Pmus or Pdi, knowledge of chest wall elastance, and valid measurement of Pi,max</td>
<td></td>
</tr>
<tr>
<td>Transdiaphragmatic pressure</td>
<td>Specific measure of force generation by the diaphragm and avoids confounding from abdominal muscle activity</td>
<td>Requires two balloons for measurement of Pes and Pga and suitable 2 channel signal acquisition system</td>
<td>Could be confounded by changes in diaphragm geometry due to changes in thoracic volume/configuration</td>
</tr>
<tr>
<td>Airway occlusion pressure</td>
<td>Non-volitional measure of neural respiratory drive</td>
<td>Not implemented on all ventilators</td>
<td></td>
</tr>
<tr>
<td>Monitoring Goal</td>
<td>Monitoring Technique</td>
<td>Pros</td>
<td>Cons</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Electromyography</td>
<td>Easy to obtain at the bedside</td>
<td>Reproducibility and validity of the measurements has not been widely evaluated in the clinical setting</td>
</tr>
<tr>
<td></td>
<td>Ultrasound: excursion</td>
<td>Highly specific reflection of neural stimulus to muscle independent of lung volume</td>
<td>Requires esophageal electrode catheter and specialized equipment for signal acquisition and processing</td>
</tr>
<tr>
<td></td>
<td>Ultrasound: thickening</td>
<td>Highly feasible to measure at the bedside</td>
<td>Reflects chest wall expansion from both mechanical insufflation and diaphragm contraction (therefore only valid when ventilator support is discontinued)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Highly feasible to measure at the bedside</td>
<td>Reproducibility unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Relationship to diaphragm pressure generation and diaphragm electrical activity not firmly established</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uncertain whether passive chest wall expansion from mechanical insufflation affects diaphragm thickening</td>
</tr>
</tbody>
</table>
Figure 1-1. Schematic of costal and crural diaphragm action.

This Figure highlights the functional arrangement of the costal and crural diaphragm with respect to their various interactions with the different components of the chest wall. Because costal diaphragm inserts onto the rib cage, its action can elevate the lower ribs, while the action of the crural diaphragm, which inserts onto the lumbar spine, acts only to lower the dome of the diaphragm. Figure taken from reference (5).
A range of factors impair diaphragm function in the critically ill patient. Of special interest to this body of work are the proposed deleterious effects of mechanical ventilation. As outlined in the text, the putative effects of mechanical ventilation on the diaphragm have not been demonstrated conclusively in the clinical setting.
Chapter 2
Rationale, Hypothesis & Specific Aims

1 Rationale

As outlined in Chapter 1, diaphragm dysfunction is an important obstacle to liberation from mechanical ventilation. Diaphragm dysfunction frequently arises during mechanical ventilation and may directly result from the effects of mechanical ventilation on the respiratory system. Experimental evidence from animals suggests that disuse atrophy occurs rapidly after the institution of mechanical ventilation and that maintaining diaphragm activity during mechanical ventilation may mitigate atrophy.

Although autopsy studies suggest that the human diaphragm atrophies rapidly during mechanical ventilation, the rate and magnitude of atrophy and dysfunction has not been directly linked to diaphragm inactivity in the clinical setting. Other clinical factors may account for this atrophy (comorbidities, critical illness, medications). The aim of this research program is to establish whether diaphragm inactivity and/or injurious loading modulates the rate and magnitude of diaphragm atrophy and dysfunction in the clinical setting. If clinically significant diaphragm dysfunction arises from diaphragm inactivity (or diaphragm over-loading), then identifying and maintaining optimal levels of diaphragm activity during mechanical ventilation may prevent diaphragm dysfunction and accelerate liberation from mechanical ventilation.

A key barrier to studying the impact of ventilation on diaphragm function in the clinical setting is the lack of measurement modalities to feasibly assess diaphragm structure and function at the bedside. Previous studies suggest that measurements of diaphragm thickness in the zone of apposition by ultrasound and measurements of diaphragm performance by neuromuscular coupling may provide feasible and valid techniques for bedside monitoring of diaphragm activity and function. However, these techniques require rigorous evaluation in mechanically ventilated patients.
2 Hypothesis

We hypothesize that diaphragm inactivity and/or injurious diaphragm loading during mechanical ventilation mediates diaphragm injury and dysfunction in patients with acute respiratory failure and that this injury and dysfunction can be ascertained and characterized using transthoracic ultrasound and longitudinal monitoring of diaphragm electrical activity and neuromuscular coupling.

3 Specific Aims

3.1 Chapter 3: Characterizing Neuromuscular Coupling in Healthy Volunteers and Mechanically Ventilated Patients

We hypothesize (1) that transdiaphragmatic pressure varies linearly with diaphragm electrical activity, (2) that the relationship between transdiaphragmatic pressure and diaphragm electrical activity is modified by the inspiratory flow rate and the pattern of diaphragmatic motion during inspiration, and (3) that neuromuscular coupling (the ratio of transdiaphragmatic pressure to diaphragm electrical activity) is sufficiently reproducible over time in healthy subjects with stable diaphragm function so as to sensitively detect clinically significant changes in diaphragm function (i.e. reproducibility coefficients less than 20% of mean value of measurement).

The specific aims of this study are:

1. To characterize the nature and determinants of the relationship between transdiaphragmatic pressure and diaphragm electrical activity
2. To ascertain the normal range of variation in neuromuscular coupling over time and compare this variation to the gold standard technique for measuring diaphragm function (magnetic phrenic nerve stimulation)
3. To compare the reproducibility of different methods of quantifying neuromuscular coupling

3.2 Chapter 4: Measuring Diaphragm Thickness in Mechanically Ventilated Patients: Feasibility, Reproducibility, and Validity

We hypothesize (1) that the reproducibility of diaphragm thickness and inspiratory thickening fraction measurements in mechanically ventilated patients is sufficiently acceptable to detect
significant changes in diaphragm thickness over time (reproducibility coefficients less than 20% of mean measurement value) and (2) that, in mechanically ventilated patients, the diaphragm thickening fraction measured on ultrasound reflects active contractions of the diaphragm rather than passive expansion of the chest wall by the ventilator.

The specific aims of this study are:

1. To establish the feasibility and reproducibility of diaphragm thickness and thickening fraction measurements in mechanically ventilated patients
2. To quantify the relative contributions of active diaphragm contraction and passive thoracic expansion to diaphragm thickening visualized by ultrasound during inspiration.

3.3 Chapter 5: The Evolution of Diaphragm Thickness During Mechanical Ventilation: Impact of Inspiratory Effort

We hypothesize (1) that the level of diaphragm contractile activity during mechanical ventilation modifies diaphragm thickness and function in critically ill patients in a dose-response relationship and (2) that changes in diaphragm thickness are associated with impaired diaphragm function.

The specific aims of this study are:

1. To describe the distribution, rate and magnitude of changes in diaphragm thickness over time in a representative cohort of mechanically ventilated patients.
2. To determine whether the level of diaphragm contractile activity affects the rate and direction of change in diaphragm thickness
3. To evaluate the impact of ventilator settings (mode of ventilation, level of inspiratory support) on diaphragm contractile activity
4. To ascertain the impact of changes in diaphragm thickness over time on diaphragm function

3.4 Chapter 6: Monitoring Diaphragm Activity and Neuromuscular Coupling During Mechanical Ventilation: Feasibility and Preliminary Findings
We hypothesize that it is feasible to conduct a study to ascertain the hour-by-hour variation in diaphragm contractile activity and patient-ventilator dyssynchrony and the day-to-day variation in neuromuscular coupling and diaphragm thickness in mechanically ventilated patients commencing within 24 hours of intubation and continuing for 7 days of ventilation.

The specific aims of this study are:

1. To determine the feasibility of identifying patients at high risk of remaining alive and ventilator-dependent for at least 7 days and enrolling them into the study within 24 hours
2. To determine the feasibility of obtaining hourly automated recordings of diaphragm electrical activity and ventilator flow and pressures in terms of the number of missed recordings and the quality of the recorded signals
3. To obtain preliminary data on the degree of diaphragm inactivity during ventilation and the rates of different types of patient-ventilator dyssynchrony and eccentric diaphragmatic contractions.
Chapter 3
Characterizing Neuromuscular Coupling in Healthy Volunteers and Mechanically Ventilated Patients

1 Introduction

Mechanical ventilation is associated with injury to the diaphragm (26) and many mechanically ventilated patients exhibit marked diaphragm dysfunction (54, 56, 58, 152, 153). Acute respiratory failure and critical illness are associated with a range of insults to diaphragm function (e.g. sepsis, neuromuscular blocking agents, critical illness polymyoneuropathy) (143) and mechanical ventilation can itself cause diaphragm injury (153, 160). Diaphragm inactivity resulting from ventilator support results in rapid, progressive diaphragm atrophy and weakness (7), a phenomenon well-documented in both the laboratory and clinical settings (24, 184). On the other hand, excess inspiratory loads due to inadequate ventilator support may cause diaphragm injury (180, 185, 186). Eccentric diaphragm contractions resulting from either premature ventilator cycling or expiratory braking to maintain end-expiratory lung volume are thought to be particularly injurious (177, 180). Due to the range of potential causes of diaphragm injury, it is not surprising that diaphragm dysfunction is common in patients with acute respiratory failure.

Given the considerable prevalence of diaphragm dysfunction and the crucial role diaphragm function plays in successful liberation from mechanical ventilation (68, 86, 87), reliable techniques for evaluating diaphragm function are required for both clinical evaluation of difficult weaning and clinical investigation of the mechanisms and impact of diaphragm dysfunction (47). Traditional methods of assessing respiratory muscle function such as measurement of maximal inspiratory pressure generation during a maximal volitional inspiratory effort are limited by difficulties in achieving consistent maximal volitional efforts in mechanically ventilated, critically ill patients (38, 41). The current gold standard technique, measurement of transdiaphragmatic pressure (Pdi) in response to magnetic phrenic nerve twitch stimulation, overcomes this limitation but the technique requires considerable technical expertise and the magnets employed for stimulation are not widely available (52-54). While single twitch stimulations are generally well-tolerated, seriously ill patients may not find repeated stimulations...
acceptable, raising concerns about the feasibility of detecting changes in twitch pressure over time in sizable longitudinal cohort studies.

Neuromuscular coupling (NMC), the ratio of Pdi to the root mean square of the crural diaphragm electromyogram (diaphragm electrical activity, Edi), may provide another means of quantifying diaphragm function (20, 61). Because the magnitude of pressure generation is adjusted for the level of diaphragm electrical activation, NMC may be independent of the level of inspiratory effort and therefore provide a non-volitional estimate of diaphragm function. NMC is independent of volition only insofar as Pdi and Edi are linearly related. Importantly, previous studies differ on this point (21, 64, 65, 100, 187). Furthermore, the variability in NMC between patients may be considerable because Edi depends heavily on the anatomic arrangement of the esophagus and diaphragmatic crura and the thickness of the diaphragm muscle (63). Finally, the variability of this measurement within patients over time has not been established.

The objectives of the present study were to determine the reproducibility of NMC measurements in comparison to twitch transdiaphragmatic pressure and to ascertain whether NMC is affected by the magnitude of patient effort, inspiratory flow, inspiratory volume, and diaphragmatic motion during inspiration.

2 Methods

This study was conducted in the outpatient Pulmonary Research Laboratory of Edward Hines Jr. VAH (Hines IL, USA) and in two medical-surgical intensive care units at University Health Network, Toronto, Canada. All subjects (or, in the case of mechanically ventilated patients, their substitute decision makers) provided written informed consent. The study was approved by the Institutional Review Board of both participating institutions and was therefore performed in accordance with the ethical standards laid down in the 2008 Declaration of Helsinki.

2.1 Experimental Subjects

We enrolled five healthy adult volunteers with no history antecedent neuromuscular or cardiopulmonary disease and ten mechanically ventilated patients. Subjects were excluded from the healthy volunteer study if they had any implanted cardiac devices or other metal devices presenting a contraindication to magnetic phrenic nerve stimulation.
We also enrolled adult critically ill patients within 24 hours of initiating invasive mechanical ventilation for moderate or severe acute respiratory distress syndrome (188), septic shock (189), or severe acute brain injury (defined as traumatic brain injury or intracranial hemorrhage causing coma with Glasgow Coma Scale score below 9 prior to intubation). Patients were excluded if they were deemed unlikely to remain on the ventilator for 7 days, if they had a high spinal cord injury or neuromuscular condition interfering with diaphragm function, if they had esophageal varices or recent upper gastrointestinal tract surgery (such that esophageal catheter placement was unsafe), or if there was any history of obstructive lung disease.

2.2 Experimental Measurements

In healthy volunteers, we placed an 8 French nasogastric catheter fitted with polyurethane esophageal and gastric balloons and multiple-array electrodes for crural diaphragm electromyography (NeuroVent Inc., Toronto Canada). Subjects breathed through a mouth piece connected to a pneumotachograph (Hans Rudolph, Kansas City, MO) connected to a differential pressure transducer (MPX2010DP, Freescale Semiconductor Inc., Tempe AZ) to record inspiratory flow and the signal was integrated to obtain inspiratory volume. Esophageal pressure (Pes) and gastric pressure (Pga) were acquired by differential pressure transducers (MPX2050DP) coupled to the esophageal and gastric balloons. Correct position of the esophageal and gastric balloons was confirmed by the occlusion technique (190), by the presence of Pga fluctuations with gentle manual pressure on the subject’s abdomen, and the presence of esophageal peristaltic artefact on the Pes tracing while absent on the Pga tracing (191). Transdiaphragmatic pressure (Pdi) was obtained by digital subtraction of Pes from Pga. Electromyography (EMG) signals were band pass filtered at 10-1000 Hz and processed to remove cardiac electrical artefact from the root mean square (hereafter referred to as the diaphragm electrical activity - Edi). Bilateral surface electrodes recorded compound diaphragmatic action potentials (CDAPs) elicited by phrenic nerve stimulation (MagStim, UK). Supramaximality of phrenic nerve stimulation was confirmed by inspecting CDAP potentials for a plateau during progressive increase of twitch stimulation from 60% to 100%. All signals were acquired at a sampling rate of 2,000 Hz and recorded on a personal computer using a digital acquisition system (NeuroVent Inc., Toronto, Canada).
In mechanically ventilated patients, we placed a 14 French multifunction nasogastric catheter (Neurovent Inc., Toronto, Canada). The catheter is fitted with balloons to measure esophageal and gastric pressure, multiple-array electrodes to acquire crural diaphragm electromyogram (EMG), and a catheter lumen for enteral nutrition. EMG signals were acquired, filtered and processed to obtain the diaphragm EMG root mean square (diaphragm electrical activity, Edi) according to previously published methods (59) by the Servo-I mechanical ventilator (Maquet, Solna, Sweden). Paw, flow and Edi 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). Esophageal and gastric balloons (Pes and Pga, respectively) were coupled to pressure transducers (MPX2050DP, Freescale Semiconductor, Inc., Tempe, AZ) and pressures were acquired at a sampling frequency of 62.5 Hz. Pdi was obtained by digital subtraction of Pes from Pga. Study catheter positioning was confirmed by the EMG signals (59) and esophageal pressure validity was assessed by the occlusion technique (190). Positioning was reconfirmed on a daily basis for the duration of the study. NMC was measured by recording Pdi and Edi during transient airway occlusions (duration = T_{tot}) applied intermittently at 20-30 random intervals over a 10-minute period. During airway occlusions, patients were advised that they might feel some difficulty breathing, but were not coached to increase inspiratory efforts.

2.3 Experimental Protocol

Healthy volunteers performed a series of inspiratory maneuvers (resting tidal breathing, threshold loading with a 20 cm H$_2$O threshold load and inspiratory capacity maneuvers) on three separate occasions separated by at least 48 hours. One volunteer also performed maximal Müller maneuvers on one occasion. Phrenic nerve stimulation was performed to measure Pdi, tw on each occasion.

In mechanically ventilated patients, NMC was measured on a daily basis for the first seven days of mechanical ventilation (or until death or extubation, if earlier). No measurements were obtained if respiratory effort was completely absent.

2.4 Signal Analysis

The maximal inspiratory swing in Pdi, Paw, and Edi was recorded as ΔPdi, ΔPaw and ΔEdi, respectively. Pdi, Paw, and Edi were integrated from the onset of inspiration to three different
intrabreath time points (standard volume, peak of Pdi, or end-inspiration—70% of peak Edi). Unless otherwise specified, \( \int \text{Pdi} \), \( \int \text{Paw} \) and \( \int \text{Edi} \) refer to the integral of these parameters from onset to peak of Pdi. Pdi and Edi were also measured at a standard inspiratory volume specified for each healthy volunteer; this volume was chosen so as to fall below the subject’s resting tidal volume (it was either 200 cc or 300 cc). Neuromuscular coupling was computed as either the ratio of \( \Delta \text{Pdi}/\Delta \text{Edi} \) (\( \Delta \text{NMC} \)) or the ratio of \( \int \text{Pdi}/\int \text{Edi} \) (\( \int \text{NMC} \)).

### 2.5 Statistical Analysis

The linearity of the relationship between Pdi and Edi was assessed by fitting linear and non-linear models (pre-specified as \( \Delta \text{Pdi} = a*(\Delta \text{Edi})^b \)). Goodness of fit was compared between models by comparing differences in the residual sum of squares (RSS). Model intercepts were set to arise at the origin since this was known to be the case based on the signal analytic approach. Transformations performed on \( \Delta \text{Edi} \) to render \( \Delta \text{Pdi} \) and \( \Delta \text{Edi} \) relations linear (‘corrected’ Edi) were derived from the exponents of the fitted non-linear models. The validity of using this computational approach was assessed by (1) examining whether correcting NMC rendered NMC stable across the range of inspiratory effort levels during each recording; and (2) simulating the effect of correction on the relationship between actual and predicted values of Pdi and Paw using a bootstrap resampling technique. In the bootstrap procedure, NMC parameters (computed with and without the correction factor), Edi and Pdi were independently randomly sampled from individual hourly recordings from mechanically ventilated patients (10 measurements sampled during each of 1000 repetitions). \( \Delta \text{Pdi} \) computed from NMC and \( \Delta \text{Edi} \) (predicted \( \Delta \text{Pdi} \)) was compared to randomly sampled measured Pdi recordings. The effect of the correction factor was evaluated based on differences in agreement (bias and variance) between actual and predicted \( \Delta \text{Pdi} \).

The effects of mean inspiratory flow and inspiratory diaphragm motion on the relationship between \( \Delta \text{Pdi} \) and \( \Delta \text{Edi} \) and the effect of inspiratory effort (quantified by \( \Delta \text{Edi} \)) on NMC were evaluated in linear mixed effects models to account for repeated measures within subjects and within study days. The linear transformation of \( \int \text{NMC} \) was employed as the dependent variable in these models.

To evaluate the reproducibility of NMC and Pdi,tw, random effects models (taking subject and study day as random effects) were computed to quantify the variance in NMC and Pdi,tw over
time and to quantify measurement variability (quantified by the intraclass correlation, ICC). The coefficient of repeatability was computed as 2.77*(within-subjects measurement standard deviation) (192). To permit comparisons in repeatability between different parameters, the repeatability coefficient was normalized against the mean value of the measured parameter yielding a quantity we termed the “maximum variability over time.”

All statistical analyses were carried out in R software 3.0.0 (www.r-project.org).

3 Results

3.1 Relationship between ∆Pdi and ∆Edi in Healthy Volunteers

During a series of varying inspiratory maneuvers (without airway occlusion), ∆Pdi exhibited an overall curvilinear relationship with ∆Edi during inspiration (Figure 3-1). Similarly ∫Pdi (integrated from onset to peak Pdi) exhibited a curvilinear relationship with ∫Edi during inspiration (Figure 3-1). These curves were best described by non-linear exponential models with fitted exponents ranging between 0.5 to 0.7. Similar results were obtained when graphical and statistical analysis was limited to measurements obtained during resting tidal breathing.

In one subject, ∆Pdi and ∆Edi were measured at multiple time points during maximal inspiratory efforts against an occluded airway (Figure 3-2). Under these conditions, ∆Pdi exhibited a quasi-linear relationship with ∆Edi and fitting a non-linear curve (model exponent 0.84) obtained a 14% reduction in residual sum of squares compared to a linear fit.

The non-linearity in the ∆Pdi-∆Edi relation (and ∫Pdi-∫Edi relation) could be largely corrected by recomputing ∆Edi as ∆Edi^{1/2} or ∫Edi as (∫Edi)^{2/3} (Figure 3-1); these exponent values were initially selected based on the non-linear fit parameters obtained in mechanically ventilated patients (see discussion below). Computing ∆Edi^{1/3} ‘overcorrected’ the nonlinearity of the Pdi-Edi relation (converted from convex to concave curve); computing ∆Edi^{1/2} achieved acceptable linearity. After this transformation of Edi, goodness of fit between linear and non-linear models of Pdi vs Edi was similar (less than 10% difference in residual sum of squares).

Whereas NMC decreased significantly with increasing ∆Edi before transformation, NMC computed from transformed values of ∆Edi did not vary with ∆Edi on average across all subjects (Figure 3-3).
3.2 Determinants of Relationship between $\Delta$Pdi and $\Delta$Edi in Healthy Volunteers

Mean inspiratory flow significantly modified the relationship between $\Delta$Pdi and $\Delta$Edi. At lower inspiratory flow rates, the slope of the $\Delta$Pdi-$\Delta$Edi relation was higher, while at higher inspiratory flow rates, the slope of the $\Delta$Pdi-$\Delta$Edi relation was lower (Figure 3-4, p<0.001 for effect of inspiratory flow). The effect of inspiratory flow was independent of the inspiratory volume (p=0.38 for interaction). The slope of the $\Delta$Pdi-$\Delta$Edi relation also varied with inspiratory volume (p=0.08 for interaction) but this effect did not persist after adjusting for mean inspiratory flow. Diaphragm motion, estimated by the ratio of $\Delta$Pga/$\Delta$Pdi, significantly influenced the $\Delta$Pdi-$\Delta$Edi relation. Higher values of $\Delta$Pga/$\Delta$Pdi were associated with a decreased slope of the $\Delta$Pdi-$\Delta$Edi relation (Figure 3-5, p<0.001 for interaction). There was no correlation between mean inspiratory flow and $\Delta$Pga/$\Delta$Pdi ($R^2=0.00$, beta=-0.002, p=0.06).

3.3 Relationship between $\Delta$Pdi and $\Delta$Edi in Mechanically Ventilated Patients

We enrolled 10 mechanically ventilated patients. Thirty five recordings of $\Delta$Pdi and $\Delta$Edi during airway occlusion were obtained on different days in 9 patients (1 patient died before exhibiting inspiratory activity so NMC could not be measured). Moderate linear fit was observed between $\Delta$Pdi and $\Delta$Edi in most recordings; goodness of fit was much stronger for $\int$Pdi vs $\int$Edi compared to $\Delta$Pdi vs $\Delta$Edi (Table 3-1) and for $\hat{\int}$Pdi and $\hat{\int}$Edi integrated from onset to peak inspiration vs. end-inspiration. However, the y-intercept for the Pdi-Edi relation was significantly greater than 0 cm H$_2$O in most recordings; when the intercept was forced to the origin, the linear models exhibited considerably poorer fit (Figure 3-6) as indicated by a significant increase in the residual sum of squares (Table 3-1). A non-linear fit (modeled as an exponential relationship between Pdi and Edi) with the intercept at the origin improved fit considerably as indicated by a significant decrease in the residual sum of squares (Table 3-1). A similar pattern was observed with the relationship between Paw and Edi (Table 3-1).

We examined whether computing NMC using an exponentiation of Edi based on the model parameters derived from the non-linear fit between Pdi and Edi (and Paw and Edi) could correct for non-linearity. Corrected $\hat{\int}$NMC was computed from the ratio of $\hat{\int}$Pdi and ($\hat{\int}$Edi)$^{2/3}$. Corrected $\Delta$NMC was computed from the ratio of $\Delta$Pdi and $\Delta$Edi$^{1/3}$. Using this computational approach...
significantly enhanced the linearity of the relationship between Pdi and Edi (Figure 3-6) as indicated by a smaller change in slope in linear fit after forcing the intercept of the linear fit to the origin (Table 3-1) and by the relatively small decrease in residual sum of squares using non-linear fit vs corrected linear fit (Table 3-1). Furthermore, uncorrected $\dot{J}\text{NMC}$ tended to decrease with higher levels of inspiratory effort ($\beta = -0.025 \text{ cm H}_2\text{O}/\mu\text{V}^2$, $p<0.001$) whereas corrected $\dot{J}\text{NMC}$ was stable across the range of inspiratory effort on average across all patients ($\beta = 0.001 \text{ cm H}_2\text{O}/\mu\text{V}^2$, $p=0.79$) (Figure 3-7).

The accuracy of using uncorrected and corrected NMC parameters to predict Pdi and Paw during airway occlusion were compared using a bootstrap resampling technique. Pdi predicted based on corrected NMC exhibited better agreement (lower bias, narrower limits of agreement) with measured Pdi compared to Pdi predicted using uncorrected NMC (Figure 3-8, Table 3-2). In a sensitivity analysis using NMC computed during low inspiratory efforts (Edi < median value in a given recording) to predict Pdi measured during large inspiratory efforts (Edi > median value in a given recording) (i.e. to maximize the effect of varying inspiratory effort level on the difference between predicted and measured Pdi), predicted Pdi computed from the corrected NMC value exhibited close linear agreement to measured Pdi, whereas predicted Pdi computed from uncorrected NMC values exhibited considerable non-linear bias (Figure 3-9).

### 3.4 Stability of Neuromuscular Coupling over Time in Healthy Volunteers

Inspiratory capacity was stable over time in these subjects over the study period (Figure 3-10), supporting the assumption of unchanging diaphragm function, but maximum $\Delta$Edi varied considerably from day to day (Figure 3-11). The reproducibility parameters of various measures of NMC are displayed in Table 3-3. The within-day variability of $\dot{J}\text{NMC}$ was much lower compared to $\Delta$NMC (ICC 0.77 vs 0.31); within-day variability was similar between uncorrected and corrected $\dot{J}\text{NMC}$ values (ICC 0.82 vs 0.77). Within-day variability was similar computing corrected $\dot{J}\text{NMC}$ at different intra-breath time points and during different inspiratory maneuvers (Table 3-3).

Both uncorrected and corrected $\dot{J}\text{NMC}$ (measured during resting tidal breathing) varied considerably within and between subjects across the 3 study days (Figure 3-12). The maximum variability over time (ratio of coefficient of repeatability to the mean parameter value) was lower
for ∫NMC than for ΔNMC. The lowest maximum variability over time was obtained from corrected ∫NMC measured from onset to the subject-specific standard inspiratory volume during resting tidal breathing, from onset to peak Edi during threshold loading, and from onset to peak Edi during an inspiratory capacity maneuver (Table 3-3). The between-days standard deviation of corrected ∫NMC measured from the onset of inspiration to the peak of Edi was 0.2 cm H$_2$O/µV in healthy volunteers compared to 0.7 cm H$_2$O/µV in mechanically ventilated patients.

Because of the influence of ΔPga/ΔPdi on the Pdi-Edi relation, corrected ∫NMC tended to be lower at higher values of ΔPga/ΔPdi (beta = -0.40, p<0.001). Correcting for the effect of ΔPga/ΔPdi on ∫NMC improved reproducibility (as indicated by lower maximum variability over time, Table 3-3).

### 3.5 Stability of Twitch Transdiaphragmatic Pressure over Time in Healthy Volunteers

The mean twitch Pdi was 27.4 cm H$_2$O (between-subjects SD 6.2 cm H$_2$O). The stability of twitch Pdi is compared to that of NMC in Table 3-3. The reproducibility of twitch Pdi was very high in healthy volunteers (ICC = 0.90) and twitch Pdi was very stable over time (Figure 3-13): the between-days SD was 2.5 cm H$_2$O and the calculated maximum variability over time was approximately 25% of the mean twitch Pdi.

### 4 Discussion

In this study, we found that the Pdi-Edi relation is curvilinear and that it is modified by both the inspiratory flow rate and diaphragmatic motion during inspiration. We also found that neuromuscular coupling (the ratio of Pdi to Edi) was much more variable over time than twitch transdiaphragmatic pressure measurements. Transforming Edi by a fractional exponent obtained a linear relation between Pdi and Edi and may therefore render neuromuscular coupling independent of the level of Edi, although further validation is required. We conclude that (1) without further standardization, the validity of neuromuscular coupling to monitor diaphragm function is questionable; (2) neuromuscular coupling should be evaluated while the airway is occluded to minimize the confounding effects of flow and diaphragm motion; and (3) transforming Edi to linearize the Pdi-Edi relationship might render neuromuscular coupling a more valid estimate of diaphragm function.
Previous descriptions of the relationship between Pdi and Edi report variable findings. In a classic description of the Pdi-Edi relation, Goldman et al. reported a quasilinear curve, the slope of which varied with the rate of inspiratory flow (21). Beck and colleagues reported a slight curvilinear relationship between Pdi and Edi during a series of isovolume inspiratory maneuvers in a study of six patients (63). Importantly, these studies were carefully conducted in the respiratory physiology laboratory where rib cage and abdominal wall motion was carefully monitored and controlled. In a study of mechanically ventilated patients, Bellani and colleagues reported a linear relationship between Pmus and Edi under both occluded and unoccluded airway conditions (64)—although visual inspection of some the curves displayed in their paper suggests that the y-intercept exceeds 0 cm H₂O in some cases. They did not report a comparison of non-linear vs. linear (with intercept forced to origin) fits, as described in the present study. Notably, they also reported that the Pdi-Edi relation differed significantly when measured at different time points during single breaths, suggesting that the relationship was in fact non-linear within individual breaths. By contrast, Akoumianaki et al. found no correlation between $\dot{\text{Pdi}}$ and $\dot{\text{Edi}}$ during ventilation under neurally adjusted ventilatory assist (NAVA) mode (65) and Carteaux et al. recently reported that the relationship between Pmus and Edi varies significantly at different levels of NAVA support, with Pmus/Edi progressively decreasing at higher levels of ventilator support (100).

This brief survey of the literature, together with the findings of the present investigation in both healthy subjects and mechanically ventilated patients, leads us to conclude that, in general, Pdi does not vary linearly Edi. It has been convincingly demonstrated that the configuration of the rib cage and abdomen determine the extent to which diaphragm electrical activation (Edi) generates mechanical pressure (Pdi) (187) and that changes in thoracoabdominal configuration during inspiration, in combination with the force-length and force-velocity relations characteristic of muscle performance, modify the Pdi-Edi relation over the course of a given inspiration (21). Accordingly, we found that the Pdi-Edi relation varied significantly with the ratio of $\Delta\text{Pga}/\Delta\text{Pdi}$—a surrogate for the relative contribution of abdominal motion to inspired volume (48)—likely due to the magnitude of change in thoracoabdominal configuration and the diaphragm length-tension relationship. Furthermore, the Pdi-Edi relation varied with mean inspiratory flow—likely due to the rate of change in thoracoabdominal configuration and the
diaphragm force-velocity relationship. In support of this inference, the Pdi-Edi relation was nearly linear when evaluated under quasi-iso-lung volume conditions in one healthy volunteer.

To mitigate the effects of the force-velocity relation and changing thoracoabdominal configuration on Pdi-Edi, we measured Pdi and Edi during airway occlusion maneuvers in mechanically ventilated patients. Nevertheless, the relationship between Pdi-Edi remained significantly curvilinear during inspiration under these ‘isovolume’ conditions. Goldman et al. demonstrated that inspiration along an ‘isofunction’ line on the Konno-Mead (K-M) diagram—where slight inward abdominal displacement exactly maintains diaphragm length and curvature in the face of increasing rib cage expansion—achieved a Pdi-Edi relation of near maximal linearity identical to that obtaining under static conditions (21). Because the K-M isofunction line differs from the K-M isovolume line, applying an airway occlusion may not necessarily prevent changes in thoracoabdominal configuration during inspiration, and the Pdi-Edi relation remains curvilinear—although to an attenuated extent.

The curvilinearity of the Pdi-Edi relation renders NMC effort-dependent, limiting the utility of this parameter for day-to-day comparisons of diaphragm function. We attempted to correct for the curvilinearity using an empirically derived transformation of Edi. Computing Edi to the 2/3 power consistently yielded a reasonably linear ∫Pdi-∫Edi relation in both the healthy volunteers and in the mechanically ventilated patients. In ventilated patients, ∫NMC computed on this basis was stable over the range of inspiratory effort and reasonable agreement was obtained between measured Pdi and predicted Pdi (estimated by a bootstrap technique). The ‘2/3’ exponent was selected based on the distribution of fitted exponential model parameters in mechanically ventilated patients—but notably it also proved effective when applied in the healthy subjects. Because this computational approach was derived on an empiric basis rather than from first principles, it requires further validation in an independent cohort and if successful, could aid monitoring of respiratory muscle effort based on Edi and would render NMC a more valid method of monitoring changes in diaphragmatic performance over time. An alternate approach (not evaluated in the present study) would be to estimate NMC using measurements of Pdi obtained at a standardized Edi level, which might also address the variability in NMC due to curvilinearity in the Pdi-Edi relation.
We evaluated the day-to-day variability in NMC to establish the maximum normal physiological variability in this measure over change as a reference standard. NMC was computed in a number of different ways to determine the most stable estimate of NMC. We found that ∫NMC was more stable than ∆NMC, and the most stable estimates of ∫NMC were obtained when measured at a standard inspiratory volume (between 200-300 cc, selected for each patient based on their tidal volume), or under threshold loading conditions (20 cm H2O threshold load). We hypothesize that day-to-day variability was lowest with these measures because standardizing the timing of the measurement or applying a threshold load may reduce breath-to-breath (and hence day-to-day) variation in the curvilinearity of Pdi-Edi. Importantly, the apparatus used to measure diaphragm EMG in healthy subjects quantifies the EMG root mean square in arbitrary units whereas the Servo-I ventilator calibrates the measurement to µV (personal correspondence, C. Sinderby). Consequently, Edi measurements in healthy subjects were on a very different scale compared to measurements obtained in ventilated subjects. Further work is required to determine whether measuring NMC during inspiratory efforts against an occluded airway with the Servo-I ventilator would obtain lower day-to-day variability than that observed in healthy subjects in the present study. Notably, the variability of ∫NMC over time was significantly greater in the mechanically ventilated patients compared to healthy subjects, suggesting that despite its suboptimal variability over time ∫NMC may nevertheless permit detection of important differences in diaphragm performance over time in the clinical setting.

The distribution and variability of twitch Pdi observed in our study were very similar to the values reported in a previous study in 32 healthy subjects (193). Both studies suggest that changes in twitch Pdi of more than 7 cm H2O signify true differences in diaphragm function. The day-to-day variability of twitch Pdi was much lower than that of NMC, suggesting that a considerable portion of NMC measurement variability is unrelated to diaphragm performance and further standardization in NMC measurement technique as discussed above may therefore significantly improve our performance.

The principle limitations of this study are as follows. First, we did not assess the reproducibility of NMC over time measured during complete airway occlusion in healthy subjects; consequently it is difficult to obtain a true comparison with the variability of NMC over time in ventilated patients. Second, the computational method used to render the Pdi-Edi relation linear for NMC computation was validated in the derivation dataset by bootstrap resampling. Further study is
required to determine whether this method can be generalized to all ventilated patients. Third, the experimental apparatus employed to measure Edi in healthy subjects provides very different values of Edi from the Servo-I ventilator; consequently we cannot be confident that the range of NMC values computed for the healthy subjects in our study is the same as that which would be measured by the Servo-I. Fourth, because NMC measurements vary considerably between subjects, it is difficult to establish normal values for diaphragm function for this measurement.

In summary, we found that Pdi varies with Edi in a curvilinear relation dependent on the mean inspiratory flow and the magnitude of diaphragm motion. Transforming Edi based on an exponential function mitigated the non-linearity and rendered NMC stable across the range of inspiratory effort. NMC computed on this basis may permit reliable predictions of Pdi from Edi but this approach requires independent validation. NMC exhibits much greater variability over time compared to twitch Pdi; further standardization of NMC measurement and computation technique may reduce this variability.
Table 3-1. Statistical parameters characterizing the relationship between Pdi and Edi in ventilated subjects

<table>
<thead>
<tr>
<th>Fit type</th>
<th>Parameter (range)</th>
<th>Distribution of y-intercepts</th>
<th>% of significant y-intercepts</th>
<th>Distribution of R² values</th>
<th>ΔRSS¹</th>
<th>% change in slope</th>
<th>ΔRSS²</th>
<th>Model parameter: slope</th>
<th>Model parameter: exponent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>ΔP_{di}/ΔE_{di}</td>
<td>4.8 (1.8-9.4)</td>
<td>69%</td>
<td>0.12 (0.05-0.27)</td>
<td>39%</td>
<td>50% (-109%-157%)</td>
<td>-28%</td>
<td>3.27 (1.66-8.23)</td>
<td>0.32 (-0.06-0.55)</td>
</tr>
<tr>
<td>Linear (forced intercept at origin)</td>
<td>ΔP_{di} / (ΔE_{di})^{1/3}</td>
<td>-0.1 (-6.0 - 3.5)</td>
<td>29%</td>
<td>0.12 (0.05-0.28)</td>
<td>7%</td>
<td>-46% (-105% - 3%)</td>
<td>-6%</td>
<td>3.27 (1.66-8.23)</td>
<td>0.96 (-0.19-1.64)</td>
</tr>
<tr>
<td>Non-linear (forced intercept at origin)</td>
<td>ΔP_{aw} / (ΔE_{aw})^{1/3}</td>
<td>-0.2 (-6.2 - 3.8)</td>
<td>20%</td>
<td>0.19 (0.06-0.34)</td>
<td>8%</td>
<td>-15% (-51% - 99%)</td>
<td>-7%</td>
<td>-5.3 (-10.8 - 2.7)</td>
<td>0.93 (0.26 - 1.26)</td>
</tr>
<tr>
<td>Non-linear (forced intercept at origin)</td>
<td>ΔP_{aw} / (ΔE_{aw})^{1/3}</td>
<td>-1.14 (-3.47 - 0.49)</td>
<td>60%</td>
<td>0.61 (0.48-0.77)</td>
<td>30%</td>
<td>42% (14%-78%)</td>
<td>-28%</td>
<td>-1.89 (-2.85 - 1.18)</td>
<td>0.67 (0.52-0.88)</td>
</tr>
<tr>
<td>Non-linear (forced intercept at origin)</td>
<td>ΔP_{aw} / (ΔE_{aw})^{1/3}</td>
<td>0.15 (-0.68 - 1.29)</td>
<td>31%</td>
<td>0.63 (0.49-0.76)</td>
<td>8%</td>
<td>-2% (-23% - 26%)</td>
<td>-8%</td>
<td>-1.89 (-2.86 - 1.18)</td>
<td>1.00 (0.79 - 1.31)</td>
</tr>
</tbody>
</table>

¹Percentage change in residual sum of squares (RSS) following conversion from linear model to linear model with intercept at origin
²Percentage change in residual sum of squares (RSS) following conversion from linear model (with intercept at origin) to non-linear model
Table 3-2. Bootstrap validation of correction factors for computing neuromuscular coupling

<table>
<thead>
<tr>
<th>Predicted parameter</th>
<th>NMC parameter used for prediction</th>
<th>Actual parameter value</th>
<th>Predicted – actual parameter value</th>
<th>Limits of agreement</th>
<th>p-value for difference in variance between corrected and uncorrected NMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta P_{di}$</td>
<td>$\frac{\Delta P_{di}}{\Delta E_{di}}$</td>
<td>10.7 (9.0)</td>
<td>-0.4 (2.2)</td>
<td>-4.8 – 4.0</td>
<td>p&lt;0.001</td>
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<td></td>
<td>$\Delta P_{di} / \left(\Delta E_{di}\right)^{1/3}$</td>
<td>10.5 (8.7)</td>
<td>0.0 (1.8)</td>
<td>-3.5 – 3.6</td>
<td></td>
</tr>
<tr>
<td>$\int_{\text{onset}}^{\text{peak}} P_{di}$</td>
<td>$\frac{\int_{\text{onset}}^{\text{peak}} P_{di}}{\int_{\text{onset}}^{\text{peak}} E_{di}}$</td>
<td>4.3 (3.9)</td>
<td>-0.2 (1.0)</td>
<td>-2.2 – 1.8</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$\frac{\int_{\text{onset}}^{\text{peak}} P_{di}}{\left(\int_{\text{onset}}^{\text{peak}} E_{di}\right)^{2/3}}$</td>
<td>4.3 (3.8)</td>
<td>0.0 (0.8)</td>
<td>-1.5 – 1.5</td>
<td></td>
</tr>
<tr>
<td>$\Delta P_{aw}$</td>
<td>$\frac{\Delta P_{aw}}{\Delta E_{di}}$</td>
<td>-15.5 (10.9)</td>
<td>0.6 (2.2)</td>
<td>-3.8 – 5.0</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$\Delta P_{aw} / \left(\Delta E_{di}\right)^{1/3}$</td>
<td>-15.2 (10.7)</td>
<td>0.0 (1.6)</td>
<td>-3.4 – 3.4</td>
<td></td>
</tr>
<tr>
<td>$\int_{\text{onset}}^{\text{peak}} P_{aw}$</td>
<td>$\frac{\int_{\text{onset}}^{\text{peak}} P_{aw}}{\int_{\text{onset}}^{\text{peak}} E_{di}}$</td>
<td>-7.4 (6.1)</td>
<td>0.3 (1.0)</td>
<td>-1.7 – 2.3</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$\frac{\int_{\text{onset}}^{\text{peak}} P_{aw}}{\left(\int_{\text{onset}}^{\text{peak}} E_{di}\right)^{2/3}}$</td>
<td>-7.3 (6.0)</td>
<td>0.0 (0.7)</td>
<td>-1.5 – 1.5</td>
<td></td>
</tr>
</tbody>
</table>
Table 3-3. Reproducibility of different computational approaches to quantifying neuromuscular coupling in healthy volunteers

<table>
<thead>
<tr>
<th>Measurement parameter</th>
<th>Measurement condition</th>
<th>Measurement timing</th>
<th>Mean</th>
<th>ICC</th>
<th>Between-subjects SD</th>
<th>Between-days SD</th>
<th>Coefficient of repeatability</th>
<th>Maximum variability over time**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncorrected ΔNMC</td>
<td>Resting breathing</td>
<td>Onset to peak Edi</td>
<td>0.16</td>
<td>0.58</td>
<td>0.08</td>
<td>0.07</td>
<td>0.20</td>
<td>121%</td>
</tr>
<tr>
<td>Corrected ΔNMC</td>
<td>Resting breathing</td>
<td>Onset to peak Edi</td>
<td>0.89</td>
<td>0.41</td>
<td>0.34</td>
<td>0.17</td>
<td>0.47</td>
<td>53%</td>
</tr>
<tr>
<td>Uncorrected ∫NMC</td>
<td>Resting breathing</td>
<td>Onset to peak Edi</td>
<td>0.30</td>
<td>0.82</td>
<td>0.10</td>
<td>0.06</td>
<td>0.15</td>
<td>50%</td>
</tr>
<tr>
<td>Corrected ∫NMC</td>
<td>Resting breathing</td>
<td>Onset to peak Edi</td>
<td>1.07</td>
<td>0.77</td>
<td>0.28</td>
<td>0.24</td>
<td>0.66</td>
<td>62%</td>
</tr>
<tr>
<td>Corrected ∫NMC (with correction for ΔPga/ΔPdi)</td>
<td>Resting breathing</td>
<td>Onset to peak Edi</td>
<td>1.27</td>
<td>0.67</td>
<td>0.29</td>
<td>0.22</td>
<td>0.60</td>
<td>47%</td>
</tr>
<tr>
<td>Corrected ∫NMC</td>
<td>Resting breathing</td>
<td>Onset to standard inspiratory volume*</td>
<td>0.94</td>
<td>0.66</td>
<td>0.22</td>
<td>0.15</td>
<td>0.43</td>
<td>46%</td>
</tr>
<tr>
<td>Corrected ∫NMC</td>
<td>Resting breathing</td>
<td>Onset to end inspiration</td>
<td>0.96</td>
<td>0.86</td>
<td>0.41</td>
<td>0.29</td>
<td>0.79</td>
<td>82%</td>
</tr>
<tr>
<td>Corrected ∫NMC</td>
<td>Threshold loading</td>
<td>Onset to peak Edi</td>
<td>1.22</td>
<td>0.81</td>
<td>0.36</td>
<td>0.17</td>
<td>0.46</td>
<td>38%</td>
</tr>
<tr>
<td>Corrected ∫NMC</td>
<td>Inspiratory capacity</td>
<td>Onset to peak Edi</td>
<td>0.89</td>
<td>0.88</td>
<td>0.16</td>
<td>0.19</td>
<td>0.51</td>
<td>57%</td>
</tr>
<tr>
<td>Twitch Pdi</td>
<td>Relaxed with airway occluded</td>
<td>Onset to peak Pdi</td>
<td>27.4 cm H₂O</td>
<td>0.90</td>
<td>6.2 cm H₂O</td>
<td>2.5 cm H₂O</td>
<td>6.9 cm H₂O</td>
<td>25%</td>
</tr>
</tbody>
</table>

*The standard volume was either 200 cc or 300 cc depending the individual subject’s tidal volume (same for all study days for each subject)

**Maximum variability over time was computed as the ratio of the coefficient of repeatability to the mean value of the parameter
Figure 3-1. The relationship between Pdi and Edi in healthy volunteers.

The relationship between ΔPdi and ΔEdi (Panel A) and ∫Pdi and ∫Edi (Panel C) is curvilinear during inspiration in healthy subjects. These relations were close to linear after recomputing ΔEdi and ∫Edi to the 2/3 power (Panels B and D).
Figure 3-2. The relationship between Pdi and Edi during inspiration under quasi-iso-lung volume conditions.

The relationship between $\Delta$Pdi and $\Delta$Edi measured at different time points (at Pdi = 20 cm H$_2$O, 33% of peak Pdi, 66% of peak Pdi and 100% of peak Pdi) during repeated maximal Müller maneuvers. Fitting a non-linear curve reduced residual sum of squares by 14% compared to a linear fit.
Figure 3-3. Relationship between neuromuscular coupling and inspiratory effort in healthy volunteers.

Before correction for nonlinearity, neuromuscular coupling was lower at higher levels of inspiratory effort (p<0.0001) but after correction, neuromuscular coupling did not vary significantly with inspiratory effort (p=0.11). Colours represent individual subjects. Dashed line indicates slope of linear mixed effects regression model adjusting for repeated measures within subjects and study days.
Figure 3-4. The effect of mean inspiratory flow rate on the relationship between ΔPdi and ΔEdi.

The curvilinearity and slope of Pdi vs Edi varies significantly with the mean inspiratory flow rate (p<0.0001 for interaction).
Figure 3-5. The effect of diaphragmatic motion on the relationship between $\Delta P_{di}$ and $\Delta E_{di}$.

Diaphragm motion (as estimated using the Gilbert index - $\Delta P_{ga}/\Delta P_{di}$) significantly modifies the relationship between $\Delta P_{di}$ and $\Delta E_{di}$ during inspiration ($p<0.0001$ for interaction).
Figure 3-6. Mathematical relationship between $\int P_{di}$ and $\int E_{di}$ measured during airway occlusion in ventilated patients.

Preliminary linear regression (blue line) exhibited a y-intercept greater than 0; forcing the linear fit to have its intercept at the origin reduced the goodness of fit (dashed blue line). A non-linear exponential fit with intercept at the origin restored the goodness of fit (black line). Adjusting $\int E_{di}$ by the correction factor ($\int E_{di}^{2/3}$) obtained acceptable linear fit (red line) similar to linear fit with intercept at origin (dashed red line).
Figure 3-7. Relationship between neuromuscular coupling (\(\hat{J}\)NMC) and inspiratory effort level in ventilated patients.

Each point represents the mean \(\hat{J}\)NMC level measured at each quantile of Edi; points are grouped according individual recordings (n=35 recordings from 9 subjects). Colours represent individual subjects. Before correction (left panel), \(\hat{J}\)NMC tended to be lower with increasing levels of effort due to the non-linear relationship between \(\hat{J}\)Pdi and \(\hat{J}\)Edi (beta = -0.025 cm H\(_2\)O/\(\mu\)V\(^2\), p<0.001). After adjusting \(\hat{J}\)NMC using the proposed correction factor (right panel), \(\hat{J}\)NMC did not vary significantly with inspiratory effort level (beta = 0.001 cm H\(_2\)O/\(\mu\)V\(^2\), p=0.79).
Figure 3-8. Agreement between predicted and measured $\Delta P_{di}$.

Computing predicted $\Delta P_{di}$ from corrected NMC (red points) achieved less bias and narrower limits of agreement (red lines) compared to predicted $\Delta P_{di}$ obtained from uncorrected NMC (black points, black lines).
Figure 3.9. Sensitivity analysis assessing impact of correcting neuromuscular coupling on accuracy of Pdi prediction.

Predicted ΔPdi was more closely aligned with measured ΔPdi when computed using corrected NMC (red line, fitted by LOESS) than predicted ΔPdi obtained from uncorrected NMC (blue line, fitted by LOESS). Black dashed line indicates line of unity.
Figure 3-10. Inspiratory capacity over time in the five healthy subjects enrolled in the study.

There was no evidence of clinically important changes in diaphragm function during the study period.
Figure 3-11. Maximum $\Delta E_{di}$ (measured during an inspiratory capacity maneuver) varied considerably in some subjects between study days.
Figure 3-12. Variation in neuromuscular coupling over time.

Neuromuscular coupling (uncorrected $\int\text{NMC}$ – left panel; corrected $\int\text{NMC}$ – right panel) varied over time during resting breathing in healthy subjects (points represent mean value for the subject, error bars indicate standard deviation). Corrected $\int\text{NMC}$ is obtained by computing $\int\text{Edi}$ to the 2/3 power.
Figure 3-13. Variation in twitch ∆Pdi over time obtained by magnetic phrenic nerve stimulation in healthy volunteers.
Chapter 4
Measuring Diaphragm Thickness in Mechanically Ventilated Patients: Feasibility, Reproducibility, and Validity

The contents of this chapter have undergone peer review and are published in *Intensive Care Medicine* 2015 (194).

1 Introduction

Respiratory muscle dysfunction is now recognized as an important complication of critical illness (144, 145). In patients receiving invasive mechanical ventilation for acute respiratory failure, diaphragm dysfunction has been linked to increased mortality (56) and difficulty weaning from mechanical ventilation (195, 196). Mechanically ventilated patients may develop diaphragm dysfunction from a range of factors (143, 144) including disuse atrophy from mechanical ventilation (24, 152, 153, 197). Assessing and monitoring diaphragm contractile activity, strength and structural dimensions during mechanical ventilation is therefore an increasingly important clinical and research priority.

Bedside assessment of diaphragm activity and function is challenging during mechanical ventilation. Monitoring diaphragm activity ordinarily requires measurement of esophageal and gastric pressures or crural electromyographic (EMG) signals (as in Neurally Adjusted Ventilatory Assist - NAVA) (47). Diaphragm function is traditionally measured as the transdiaphragmatic pressure generated in response to volitional stimuli or from magnetic twitch stimulation of the phrenic nerve (41, 47, 53, 54). These techniques require nasogastric catheterization, considerable technical and physiological expertise, and a degree of patient cooperation often not possible in the critically ill (54). Moreover, these measurements are not designed to detect changes in diaphragmatic structure (such as atrophy) that may be triggered by mechanical ventilation (24, 27, 151, 197).

Transthoracic diaphragm ultrasound imaging represents an important potential advance in diaphragm monitoring during mechanical ventilation (30). This technique provides a non-
invasive, feasible measure of diaphragm thickness and inspiratory thickening previously validated in healthy subjects (32, 71, 101, 102, 198, 199). Increases in diaphragm thickness with inspiration—quantifiable by ultrasound—are correlated with inspiratory effort (32, 76). Accordingly, transthoracic ultrasound represents a potential modality for evaluating diaphragm activity, function and dimensions during mechanical ventilation.

The feasibility and precision of diaphragm thickness measurements have not yet been established in mechanically ventilated patients. Moreover, it is unknown whether inspiratory thickening of the diaphragm primarily reflects diaphragm contractile activity or passive expansion of the thorax per se (which could critically confound the validity of diaphragm inspiratory thickening as a measure of inspiratory effort as these two factors are uncoupled during mechanical ventilation). Accordingly, we aimed to test the feasibility and reproducibility of these measurements in ventilated subjects and to quantify for the first time the independent contributions of muscle contraction and passive expansion of the chest wall to inspiratory diaphragm thickening on ultrasound.

2 Methods

This study consisted of two parts: (1) a clinical study to evaluate the feasibility and reproducibility of diaphragm thickness measurements in mechanically ventilated patients (conducted in the two medical-surgical intensive care units at University Health Network, Toronto ON) and (2) a physiological study to evaluate the validity of measuring diaphragm thickening to assess inspiratory effort (conducted in the outpatient Pulmonary Research Laboratory of Edward Hines Jr. VAH, Hines IL). All subjects (or, in the case of mechanically ventilated patients, their substitute decision makers) provided written informed consent. The study was approved by the Institutional Review Boards at both participating institutions and have therefore been performed in accordance with the ethical standards laid down in the 2008 Declaration of Helsinki.

2.1 Experimental Subjects

In the reproducibility study, we enrolled adult critically ill patients receiving invasive mechanical ventilation. Patients were excluded from the study if they had a previously diagnosed neuromuscular disorder, had active hemodynamic or respiratory instability at the time of
assessment, or exhibited behavioural agitation. In the validation study, we enrolled healthy adult subjects without prior history of cardiopulmonary or neuromuscular disease.

### 2.2 Experimental Measurements

Diaphragm thickness was measured by ultrasound as previously described (30, 32, 76). Briefly, a 13 MHz linear array transducer (HFL-38xe, FUJIFILM Sonosite Inc., Bothell WA) was placed in the ninth or tenth intercostal space near the mid-axillary line and angled perpendicular to the chest wall (30). In this location, the diaphragm is identified as a three-layered structure just superficial to the liver, consisting of a relatively non-echogenic muscular layer bounded by the echogenic membranes of the diaphragmatic pleura and peritoneum (101) (Figure 4-1).

Diaphragmatic thickness was measured at end-expiration (Tdi,ee) and peak inspiration (Tdi,pi – i.e. peak thickness value during inspiration) as the distance between the diaphragmatic pleura and the peritoneum using M-mode (69, 72). Measurements of Tdi,ee and Tdi,pi were always made on two breaths visualized in a single M-mode image. Diaphragm thickening during inspiration ($\Delta$Tdi) was taken as the difference between Tdi,pi and Tdi,ee. Diaphragm thickening fraction (TFdi) was defined as the percentage change in diaphragm thickness during inspiration (computed from the quotient of $\Delta$Tdi and Tdi,ee).

In the validation substudy, subjects underwent nasogastric catheterization for esophageal and gastric manometry and diaphragmatic electromyography (as per methods described in Chapter 3, Section 2.2) (20). Inspiratory volumes were measured using a Wright spirometer. Tdi,ee and Tdi,pi were measured as described above.

### 2.3 Experimental Protocol

In the reproducibility study, three observers (in random order) measured Tdi,ee and Tdi,pi in mechanically ventilated patients in a single study session. Observers were blinded to each other’s findings. The three observers included an intensivist experienced in the performance of diaphragm ultrasound (EG), an intensivist with experience in general critical care ultrasound but no prior experience with diaphragm ultrasound (MD) and a research co-ordinator with no prior experience in diaphragm ultrasound (PF). Observers were required to demonstrate competence with the technique on six patients before commencing the study. To assess intra-observer repeatability, one of the observers (EG) repeated the measurement 5 minutes after the initial
measurement. During the study it proved difficult to consistently visualize the left hemidiaphragm, so measurements of left hemidiaphragm thickness were discontinued.

Upon reviewing the preliminary results of the reproducibility study, it was felt that some excess measurement variability may arise from inadequate observer training and from variation in the precise location of the ultrasound probe as the thickness of the diaphragm is heterogeneous across its surface (3). Therefore we repeated the reproducibility study with the following modifications: (1) observers had to demonstrate competency on 15 ultrasound examinations before beginning study data collection and (2) the probe placement site was marked by the first observer. Two other observers (AM and DB – both previously untrained research assistants) were then trained after these modifications. One of the observers (AM) obtained two repeated measurements in a larger convenience sample of patients to assess measurement repeatability; in a subset of these patients, two observers (AM and DB) independently obtained two repeated measurements to assess measurement reproducibility.

In the validation study (healthy subjects), right hemidiaphragm thickness was continuously recorded using M-mode ultrasound while each subject inhaled from functional residual capacity to a series of target inspiratory volumes: 25%, 50%, 75% and 100% of inspiratory capacity (IC). Upon reaching the target volume, subjects were instructed to close the glottis (so as to maintain the end-inspiratory lung volume) and relax the respiratory muscles (so as to remove the effect of diaphragmatic contraction on diaphragm thickness). T_{di} was recorded at end-expiration, peak inspiration (maximal effort condition determined from peak thickness), and end-inspiration (relaxed condition) (Figure 4-2). At least two such maneuvers were conducted at each target inspiratory volume. End-inspiratory (relaxed condition) EMG signals were examined to ensure relaxation of the diaphragm.

2.4 Statistical Analysis

Descriptive statistics were expressed as means and standard deviations where appropriate. The relationships between physiological variables and TF_{di} were evaluated using linear mixed effects models to account for repeated measures.

Interobserver repeatability and intraobserver reproducibility of ultrasound measurements were assessed by computing repeatability and reproducibility coefficients (192, 200, 201) from the
variance components of random effects models of repeated measurements of diaphragm thickness (192). These coefficients indicate the 95\textsuperscript{th} percentile for the range of variation in repeated measurements either within observers (repeatability) or between observers (reproducibility).

The sample size of the initial reproducibility study was calculated to achieve 95\% confidence intervals of \pm 0.1 mm for the reproducibility limits assuming a standard deviation of repeated measurements between-observers of 0.2 mm, which yielded a required sample of 30 patients (201). The sample size of the second reproducibility study was calculated assuming a standard deviation of repeated measurements between observers of 0.1 mm (based on the observed standard deviation within observers in the initial study), which yielded a required sample size of 15 patients to achieve confidence intervals of \pm 0.1 mm for the reproducibility limits. In order to enhance clinical generalizability of the repeatability estimate and avoid spectrum bias, we enrolled a convenience sample of an additional 51 subjects to assess measurement repeatability. The sample size of the validation study was initially estimated based on physiological judgment regarding the number subjects required for a generalizable inference.

3 Results

3.1 Feasibility and Reproducibility of Diaphragm Thickness Measurements in Ventilated Subjects

Thirty mechanically ventilated patients were enrolled in the initial reproducibility study. After modifying the measurement protocol (see Methods) we enrolled an additional 66 ventilated patients; of these, 51 underwent ultrasound examination by a single observer and 15 underwent separate ultrasound examinations by two independent observers. Clinical characteristics of the ventilated patients are shown in Table 4-1.

Right hemidiaphragm Tdi,ee and Tdi,pi measurements were obtained on 96\% and 95\% of a total of 542 attempts in ventilated patients, respectively. Right hemidiaphragm measurements were generally only missing for one observer and there were no missing measurements in the second reproducibility study. Left hemidiaphragm Tdi,ee and Tdi,pi measurements were obtained on 85\% and 79\% of a total of 104 attempts in ventilated patients, respectively.
The distributions of Tdi,ee, Tdi,pi, ΔTdi and TFdi and the coefficients of repeatability and reproducibility for these measurements are reported in Table 4-2. There was no difference between the right hemidiaphragm and the left hemidiaphragm for both Tdi,ee (mean difference 0.0 mm, 95% CI -0.2 - 0.2 mm) and Tdi,pi (mean difference 0.0 mm, 95% CI -0.2 - 0.2 mm). Reproducibility of right hemidiaphragm measurements improved considerably when the site of probe placement was routinely marked. Using this technique, repeatability and reproducibility of Tdi,ee and Tdi,pi measurements were excellent given the observed distributions of those measurements. However, the repeatability and reproducibility of ΔTdi and TFdi were only moderately acceptable given their observed distributions. Tdi,ee and TFdi measurement variability did not vary with body mass index or duration of mechanical ventilation (Figure 4-3) and Tdi,ee was not significantly correlated with height, weight or body mass index (Figure 4-4).

### 3.2 Validity of Inspiratory Diaphragm Thickening Fraction to Monitor Inspiratory Activity

Compared to the healthy subjects, TFdi was considerably lower in ventilated patients and nearly absent in patients subjected to neuromuscular blockade (in the reproducibility study cohort) (Figure 4-5, p<0.0001 for difference across groups). TFdi was not significantly different between patients in controlled and partially assisted modes of ventilation (mean difference 3%, 95% CI -2% - 9%), and patients in both groups exhibited a wide range of TFdi values.

Nine healthy subjects participated in the validation study. Transdiaphragmatic pressure and diaphragm electrical activity measurements were obtained in five of the participants. In the series of inspiratory maneuvers to varying inspiratory volumes, TFdi was correlated with inspiratory volume (Figure 4-6, R²=0.32, p<0.0001), and with both diaphragm electrical activity (Edi) and transdiaphragmatic pressure (Pdi) (Figure 4-7, R²=0.32 and 0.28, respectively, p<0.01).

When subjects inhaled from functional residual capacity (FRC) to volumes below 50% of inspiratory capacity, inspiratory increases in diaphragm thickness were almost entirely related to muscular contraction as end-inspiratory diaphragm thickness (passive condition) was very similar to end-expiratory thickness (Figure 4-8). By contrast, when subjects inhaled from FRC to inspiratory volumes above 50% of inspiratory capacity, inspiratory increases in diaphragm thickness resulted both from muscle contraction and increased thoracic volume as end-inspiratory thickness (passive condition) was considerably higher than end-expiratory thickness.
The contribution of increased thoracic volume to diaphragm thickening fraction was not statistically significant when inspiratory volumes were approximately less than 1500 cc (Figure 4-9).

4 Discussion

This is the first report of the feasibility and reproducibility of sonographic measurements of diaphragm thickness and inspiratory thickening in a sizable cohort of mechanically ventilated patients. Our findings suggest that sonographic measurements of diaphragm thickness provide a useful non-invasive tool to assess and monitor for diaphragm atrophy and inactivity during mechanical ventilation.

A number of recent studies have employed ultrasound to measure diaphragm thickness and inspiratory thickening in ventilated patients. Grosu and colleagues found evidence of a progressive decrease in diaphragm thickness in a small series of ventilated patients (150). TFdi has been employed to estimate of inspiratory effort during partially assisted ventilation (76) and to predict the probability of successful liberation from ventilation (73, 74). While Vivier et al. reported intra-analyzer reproducibility in a small number of ventilated subjects (76), prior to the present study the precision of these measurements was not well established in a broad population of critically patients, limiting the inferences that may be drawn using this technique.

Our study demonstrates that measurement of right hemidiaphragm thickness is feasible and highly reproducible in a representative cohort of mechanically ventilated patients. By contrast, we found it challenging to consistently visualize and measure the thickness of the left hemidiaphragm. In the 13 patients with bilateral measurements, right and left hemidiaphragm Tdi,ee and TFdi were similar. Left hemidiaphragm measurements may therefore be unnecessary unless there is some specific clinical reason to suspect unilateral muscle injury or dysfunction (i.e. phrenic nerve injury).

Anatomical studies have shown that the thickness of the diaphragm is heterogeneous across its surface (3); accordingly placement of the probe must be standardized to minimize to measurement variability. We initially sought to standardize probe placement using anatomical landmarks; however, we found that marking the site significantly improves measurement reproducibility, particularly between observers. This limits comparisons of diaphragm thickness
between patients but changes in diaphragm thickness as small as 0.2 mm over time can be reliably detected within patients by this technique.

The reproducibility of the inspiratory thickening fraction (TFdi) was less satisfactory: measurements may vary between or within observers by as much as 16%-17%, obscuring important differences in inspiratory muscle activity (given that during resting tidal breathing diaphragm thickening fraction in healthy subjects is 30-40%). In fact, the repeatability coefficient for absolute value of the change in diaphragm thickness during inspiration (ΔTdi) was very similar to the repeatability coefficient for end-expiratory diaphragm thickness (0.3 mm vs. 0.2 mm). Because TFdi incorporates the measurement error of both ΔTdi and Tdi,ee it is unlikely that further improvements in the reproducibility of TFdi can be obtained. Of interest, our repeatability and reproducibility coefficients correspond closely with those previously reported in healthy volunteers (102) and in ventilated subjects (76).

While inspiratory volume and muscle pressure generation are entirely collinear in spontaneously breathing subjects, they are variably dissociated during mechanical ventilation. If passive inflation of the chest could cause apparent tidal diaphragm thickening, TFdi would not be a valid measure of diaphragm contractile activity in these patients. Based on our findings, we conclude that at relatively low inspiratory volumes (<50% of inspiratory capacity) thickening of the diaphragm during inspiration arises from muscular contraction rather than increasing thoracic volume per se (Figure 4-8). Significant thickening of the diaphragm under passive conditions occurred only at high inspiratory volumes (>50% of inspiratory capacity). Supporting this conclusion, we found that subjects under neuromuscular blockade exhibited only minimal levels of diaphragm thickening.

The degree of diaphragm thickening for a given level of inspiratory effort varied considerably between subjects (Figure 4-7). Accordingly, a given inspiratory TFdi value does not imply the same transdiaphragmatic pressure swing across different subjects, limiting the utility of this measurement for precise interpatient comparison. TFdi is therefore best suited to qualitative comparisons of inspiratory muscle activity between subjects and to quantitative comparisons of changes in inspiratory muscle activity within subjects.

Our study is subject to a number of limitations. First, it is not entirely clear whether improvements in measurement reproducibility observed in the second study phase are
attributable to marking the probe location or more rigorous training. We reason that the first explanation is most likely because after marking the site the between-observer reproducibility improved considerably more than within-observer reproducibility. Second, the need to mark the location of the probe to ensure adequate measurement reproducibility greatly limits the utility of this technique for comparisons between subjects. Third, we assessed the validity of TFdi measurements in healthy subjects and we did not measure diaphragm electrical activity in mechanically ventilated patients. However, our conclusion that diaphragm thickening fraction is a valid marker of inspiratory effort is corroborated by the low levels of thickening observed in ventilated patients subjected to neuromuscular blockade and by a recent description of the relationship between diaphragm thickening fraction and inspiratory effort during mechanical ventilation (76).

In summary, we conclude that ultrasound of the diaphragm can reproducibly measure variation in right hemidiaphragm thickness within mechanically ventilated patients over time. We also conclude that inspiratory thickening of the diaphragm reflects the degree of contractile activation of the muscle during partially assisted mechanical ventilatory support. This technique may therefore be employed in ventilated patients to reliably detect diaphragmatic atrophy over time and assess diaphragm contractile activity. Future research is required to confirm the utility of this technique to assess muscle function in response to a maximal volitional inspiratory effort and to diagnose patient-ventilator dyssynchrony.
Table 4-1. Clinical Characteristics of Mechanically Ventilated Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Reproducibility Study Part 1</th>
<th>Reproducibility Study Part 2</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>30</td>
<td>66</td>
<td>96</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 (15)</td>
<td>59 (14)</td>
<td>59 (14)</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>53%</td>
<td>36%</td>
<td>42%</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>27.1 (7.4)</td>
<td>26.2 (5.9)</td>
<td>26.5 (6.4)</td>
</tr>
<tr>
<td>Admission Diagnosis Category</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>0 (0%)</td>
<td>4 (6%)</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Gastrointestinal/hepatic</td>
<td>1 (3%)</td>
<td>5 (8%)</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>7 (23%)</td>
<td>4 (6%)</td>
<td>11 (11%)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (3%)</td>
<td>7 (11%)</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>Post-operative</td>
<td>0</td>
<td>8 (12%)</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>13 (43%)</td>
<td>7 (11%)</td>
<td>20 (21%)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>2 (7%)</td>
<td>19 (29%)</td>
<td>21 (22%)</td>
</tr>
<tr>
<td>Transplant</td>
<td>6 (20%)</td>
<td>12 (18%)</td>
<td>18 (19%)</td>
</tr>
<tr>
<td>Duration of ventilation (days)</td>
<td>10 (3-15)</td>
<td>2 (2-3)</td>
<td>2 (2-7)</td>
</tr>
<tr>
<td>Mode of ventilation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume control</td>
<td>4 (13%)</td>
<td>3 (5%)</td>
<td>7 (7%)</td>
</tr>
<tr>
<td>Pressure control</td>
<td>8 (27%)</td>
<td>39 (59%)</td>
<td>47 (49%)</td>
</tr>
<tr>
<td>High frequency oscillation</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Pressure support</td>
<td>17 (57%)</td>
<td>23 (35%)</td>
<td>40 (42%)</td>
</tr>
<tr>
<td>Proportional assist</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
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Table 4-2. Reproducibility of Diaphragm Thickness Measurements in Mechanically Ventilated Patients

<table>
<thead>
<tr>
<th>Measurement (units)</th>
<th>Side</th>
<th>Site marked</th>
<th>N subjects</th>
<th>Mean (SD)</th>
<th>Repeatability Coefficient§</th>
<th>Reproducibility Coefficient§</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{di,ee} (mm)</td>
<td>Left</td>
<td>No</td>
<td>13</td>
<td>2.1 (0.8)</td>
<td>1.9</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>No</td>
<td>30</td>
<td>2.4 (0.8)</td>
<td>0.4</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Yes</td>
<td>66</td>
<td>2.4 (0.8)</td>
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<td>0.4</td>
</tr>
<tr>
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<tr>
<td></td>
<td>Right</td>
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<td>0.5</td>
<td>2.2</td>
</tr>
<tr>
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<td>0.6</td>
</tr>
<tr>
<td>ΔT_{di} (mm)</td>
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<td>13</td>
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<td>Right</td>
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<td>0.6</td>
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<tr>
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<td>66</td>
<td>0.3 (0.3)</td>
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</tr>
<tr>
<td>T_{Fdi} (%)</td>
<td>Left</td>
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<td>13</td>
<td>7% (2%-19%)*</td>
<td>13%</td>
<td>39%</td>
</tr>
<tr>
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<td>30</td>
<td>11% (3%-17%)*</td>
<td>19%</td>
<td>27%</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Yes</td>
<td>66</td>
<td>11% (3%-17%)*</td>
<td>17%</td>
<td>16%</td>
</tr>
</tbody>
</table>

§95% of differences in repeated measurements by the same observer (repeatability) or by two separate observers (reproducibility) will be equal to or less than this coefficient. *Values are expressed as the median (interquartile range). †Data on interobserver agreement available for 15 of the 66 subjects. T_{di,ee} = end-expiratory diaphragm thickness; T_{di,pi} = peak inspiratory diaphragm thickness; ΔT_{di} = absolute value of difference between peak inspiratory and end-expiratory diaphragm thickness; T_{Fdi} = fractional (percentage) change in diaphragm thickness between end-expiration and peak-inspiration.
Figure 4-1. Typical sonographic cross-sectional image of the diaphragm.

The probe is positioned between the ribs over the lateral chest wall. B-mode imaging reveals the diaphragm is a three-layered structure just superficial to the liver bounded by the parietal pleura and peritoneum. M-mode imaging reveals the variation in diaphragm thickness over time at the point-indicated by the blue line.
Figure 4-2. Typical M-mode ultrasound image of dynamic diaphragm thickness obtained during inspiratory hold maneuver.

M-mode image of diaphragm thickness at end-expiration (point A) following by inspiratory effort (peak inspiratory muscle contraction at point B) targeting an pre-specified inspiratory volume (in this case 75% of inspiratory capacity) followed by an end-inspiratory hold (glottis closed to maintain lung volume) with relaxed inspiratory muscles (point C).
Figure 4-3. Effect of body mass index and duration of ventilation on the variability in diaphragm thickness measurements.

Measurement variability does not vary significantly with duration of ventilation and body mass index (p>0.4 for all significance tests of difference of regression line slope from zero). Duration of ventilation is plotted using data from the first reproducibility study, as most patients in the second reproducibility study were enrolled at an early stage (day 2-3 of mechanical ventilation).
Figure 4-4. The relationship between end-expiratory thickness of the diaphragm and physical stature characteristics.

None of the correlations were statistically significant (p>0.1).
Inspiratory thickening fraction of the right hemidiaphragm in healthy subjects at rest and ventilated subjects under varying conditions.

Inspiratory thickening is observed at very low levels in patients subjected to neuromuscular blockade, suggesting that thickening mainly reflects diaphragm contractile activation rather than increases in thoracic volume *per se.*
Figure 4-6. The relationship between diaphragm thickening fraction and inspiratory volume.

Diaphragm thickening fraction was correlated with inspiratory volume ($R^2=0.28$, $p<0.0001$).
Figure 4-7. Diaphragm thickening fraction is correlated with diaphragm electrical activity and transdiaphragmatic pressure during inspiratory maneuvers.

$E_A_{di} = \text{diaphragm electrical activity. } P_{di} = \text{transdiaphragmatic pressure.}$
Figure 4-8. The relative contributions of inspiratory effort vs. chest wall expansion to inspiratory diaphragm thickening fraction depend on the inspiratory volume.

At lower inspiratory volumes (i.e. below 50% IC), inspiratory changes in diaphragm thickness are almost entirely attributable to inspiratory effort (i.e. diaphragm thickness returns nearly to end-expiratory levels during the end-inspiratory hold - “Hold”). At higher inspiratory volumes, inspiratory changes in thickness are due to both inspiratory effort and increased thoracic volume (diaphragm thickness observed during the end-inspiratory hold remains considerably higher than end-expiratory levels). IC = inspiratory capacity.
Figure 4-9. Active and passive changes in diaphragm thickness at varying inspiratory volumes.

The relationship between inspiratory volume and diaphragm thickness differs between peak inspiratory condition (blue line) and passive inflation condition (cyan line). The gray shading indicates 95% confidence intervals for the regression lines. The passive inflation condition was obtained by an end-inspiratory hold (see text for details). At lower inspiratory volumes, passive inflation exerts negligible effects on diaphragm thickness (as a proportion of the effect of active inspiration on diaphragm thickness). Passive increases in diaphragm thickness were statistically significant at inspiratory volumes of 1300 cc or higher.
Chapter 5
The Evolution of Diaphragm Thickness During Mechanical Ventilation: Impact of Inspiratory Effort

The contents of this chapter have undergone peer review and are published in the American Journal of Respiratory and Critical Care Medicine 2015 (202).

1 Introduction

Many patients simply need liberation from the ventilator as they recover from an episode of acute respiratory failure, but a substantial proportion have difficult or prolonged weaning (114, 115). Diaphragm function is an important determinant of successful liberation from ventilation and recovery from critical illness (46, 68, 86). Both animal models and autopsy studies of brain-dead organ donors (24, 25, 152, 156, 184, 197) suggest that mechanical ventilation can cause diaphragm myofiber atrophy, sarcomeric disruption, intracellular lipid accumulation and mitochondrial dysfunction (ventilator-induced diaphragm dysfunction, VIDD).

The clinical significance of VIDD remains uncertain for a number of reasons. First, although diaphragm atrophy has been demonstrated in small studies of highly selected live mechanically ventilated patients (27, 28, 136, 150), the prevalence and severity of changes in diaphragm thickness across the general population of mechanically ventilated patients are unknown. Second, it is unclear how the depth and duration of inactivity in living mechanically ventilated patients compares to the complete diaphragm inactivity seen following brain death. Third, given the widespread use of partially assisted modes of ventilation—which ameliorate diaphragm atrophy in animal models (158, 160)—it is unknown whether disuse contributes significantly to diaphragm atrophy in the clinical setting (203). Finally, there are myriad mechanisms of muscle injury and dysfunction in critical illness and the specific contribution of mechanical ventilation is unknown in this context (143).

Diaphragm ultrasound is a promising new method for evaluating the diaphragm during mechanical ventilation, where loss of diaphragm thickness over time can indicate atrophy (69,
The thickness of the right hemidiaphragm can be feasibly and reproducibly measured in the zone of apposition in mechanically ventilated patients (204). In addition, tidal diaphragm thickening during inspiration visualized by ultrasound provides a noninvasive means of quantifying inspiratory effort (diaphragm contractile activity) (30, 76, 103, 204) and maximal diaphragm thickening (during a maximal inspiratory effort) can be used to assess diaphragm function (69, 71, 72, 74).

We set out to determine whether the level of inspiratory effort during mechanical ventilation modifies diaphragm thickness and function over time in adult critically ill patients, accounting for the severity of illness, sepsis and multi-organ failure. We hypothesized that there is a ‘dose-response’ relationship between inspiratory effort and changes in diaphragm thickness over time during ventilation. We also aimed to ascertain the effect of changes in diaphragm thickness on diaphragm function in mechanically ventilated patients.

2 Methods

2.1 Study Population and Setting

The study was conducted at three tertiary academic intensive care units in Toronto, Canada. The study was conducted in two separate epochs (epoch 1: May-August 2013 – results previously presented in abstract form (24); epoch 2: May 2014-January 2015). The Research Ethics Boards at the University Health Network and St. Michael’s Hospital approved the study protocol and informed consent was obtained from patients or their substitute decision makers prior to enrolment.

We identified eligible patients by regular screening (Monday-Thursday) in the ICU. Patients were eligible for enrolment if they had received invasive mechanical ventilation for acute respiratory failure for fewer than 72 hours, and after the first 53 subjects, enrolment was restricted to patients receiving mechanical ventilation for fewer than 36 hours. Patients were excluded if they were expected to be liberated from mechanical ventilation within 24 hours of screening or if they had received invasive mechanical ventilation for greater than 48 hours in the previous six months. We also enrolled a control group consisting of non-ventilated patients admitted to the ICU for any reason. Patients were enrolled within 36 hours of ICU admission.
2.2 Diaphragm Thickness Measurements

The thickness of the right hemidiaphragm at end-expiration (‘diaphragm thickness’) was measured using techniques we have previously shown to be reliable (see Chapter 4, Section 2.2 for details) (204). Briefly, we measured thickness 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 (32). Inspiratory thickening fraction (a measure of diaphragm contractile activity) was quantified by the percentage change in right hemidiaphragm thickness from end-expiration to peak inspiration during tidal breathing under mechanical ventilation. These measurements were made daily from Monday to Friday until extubation or until day 14 of ventilation, whichever came first.

In non-ventilated controls, we measured diaphragm thickness measurements daily for up to 7 days. In a convenience sample of ventilated patients enrolled later in the study, we also collected thickness measurements over the first 4 days following extubation as an additional internal control.

2.3 Diaphragm Function Measurements

At a later stage in the study, we assessed diaphragm function in study participants. Diaphragm function was assessed after 1 week of mechanical ventilation (once the patient was awake and breathing spontaneously) by measuring maximal diaphragm thickening fraction during coached maximal inspiratory efforts (69, 71, 74) and maximal inspiratory sniff maneuvers (43, 46) while in CPAP mode. The observer was blinded to the change in diaphragm thickness over time. If the patient was extubated before 1 week of ventilation was completed, diaphragm function was assessed 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 maximal inspiratory efforts (38). The highest value obtained for thickening fraction during repeated inspiratory efforts was taken as the measurement of muscle function.

2.4 Clinical Risk Factors

Demographic data, comorbidities, admission diagnosis, and severity of illness (Severe Acute Physiology Score [SAPS] II) were collected at baseline. Ventilator settings, arterial blood gas
tensions, diagnosis of sepsis, and Severity of Organ Failure Assessment (SOFA) scores were ascertained on a daily basis for the duration of the study.

2.5 Statistical Analysis

Data were expressed as mean (SD), median (interquartile range), and absolute and relative frequencies, as indicated. ANOVA or Kruskal-Wallis tests were used to compare continuous variables and chi-square tests were used for categorical variables. Missing data for SOFA score (5% missing) were imputed from the median patient value. The amount of missing data was not significant for other variables (See Table 5-1).

The study population was divided into three groups based on the overall change in diaphragm thickness from the baseline measurement to the last measurement obtained during the first week of mechanical ventilation (MV) using a 10% cutoff value selected *a priori* to define clinically relevant decreases or increases in diaphragm thickness. Cut-off selection was based on the measurement resolution of the ultrasound technique (199, 204) and in accordance with previous studies of myopathy in critical illness and respiratory disease (205-207). Clinical characteristics and changes in diaphragm function were compared between patients in these categories using ANOVA or chi-square tests as appropriate.

We used linear mixed effects regression models to evaluate the effect of diaphragm contractile activity on changes in diaphragm thickness over time (primary analysis) and to identify key determinants of diaphragm contractile activity. This modeling strategy was selected for the primary analysis because it efficiently incorporates all repeated daily measurements from all patients. A series of secondary analyses were conducted to corroborate the primary analysis. The effect of theoretical mathematical coupling between diaphragm thickness and thickening fraction was explored using simulation (see Appendix 1 for details).

We planned to enroll 120 mechanically ventilated patients and 10 control patients (see Online Supplement for sample size considerations). All statistical analyses were conducted using R software, version 3.0.2 (www.r-project.org).

3 Results

3.1 Study Cohort
128 mechanically ventilated patients (54 in study epoch 1 and 74 in epoch 2) and 10 control patients were enrolled in the study (see enrolment flow diagram in Figure 5-1). Two patients withdrew consent and baseline measurements were not obtained in 4 patients. A further 15 patients were extubated or died before a second thickness measurement was obtained and were excluded from the study cohort, leaving 107 ventilated patients in whom changes in diaphragm thickness over time were ascertained. Of these, 23 (21%) were enrolled on day 1 of mechanical ventilation, 72 (67%) on day 2, and 12 (11%) on day 3. Patients were enrolled in the study for a median of 7 days (IQR 5 – 11). Diaphragm thickness measurements were obtained on a total of 527 patient-days during mechanical ventilation (median 4 measurements per subject, IQR 3 – 7).

Demographic and clinical characteristics of the study population are shown in Table 5-2. Most patients (71%) were initially ventilated in a controlled mode of mechanical ventilation. Clinical characteristics of control subjects are provided in Table 5-3.

### 3.2 Time Course and Distribution of Changes in Diaphragm Thickness

The magnitude and rate of change in diaphragm thickness over time varied widely: over the first week of ventilation, diaphragm thickness remained unchanged in 47 subjects (44%), decreased by more than 10% in 47 subjects (44%), and increased by more than 10% in 13 subjects (12%) (Figure 5-2 and Figure 5-3). There were no significant differences in clinical, physiological, or management characteristics between these three groups (Table 5-2). Changes in diaphragm thickness (both increases and decreases) occurred predominantly during the early course of ventilation (Figure 5-3, p<0.0001 for heterogeneity in rates of change between week 1 and week 2) and rapid early decreases in diaphragm thickness were observed during both controlled ventilation and partially assisted ventilation (Figure 5-4).

Diaphragm thickness was comparatively stable over time in non-ventilated control subjects (N=10; Figure 5-5 Panel A, see Appendix 1, Table E1) and in patients following extubation (N=29, Figure 5-5 Panel B). In some patients, diaphragm thickness tended to return toward baseline following extubation (Figure 5-6).

### 3.3 Relationship between Changes in Diaphragm Thickness and Diaphragm Function
Maximal diaphragm thickening fraction (a marker of diaphragm function) during maximal inspiratory efforts was ascertained in 20 ventilated subjects (Table 5-4) and 4 control subjects. Maximal diaphragm thickening fraction was lower in patients with both significant decreases and increases in diaphragm thickness over time compared to patients with unchanged thickness (Figure 5-7, p=0.04). Maximal thickening fraction was significantly higher in controls compared to patients with decreased thickness (p=0.01) and increased thickness (p=0.01); maximal thickening fraction in patients with near baseline thickness was not significantly different from controls (p=0.14). Using a previously published cut-off value of 20% for maximal thickening fraction (69), diaphragm dysfunction was more frequent in patients with decreased or increased diaphragm thickness compared to patients in whom thickness was unchanged (Figure 5-8, p=0.06).

3.4 Impact of Diaphragm Contractile Activity on Diaphragmatic Thickness During Mechanical Ventilation

The rate and direction of change in diaphragm thickness over time was significantly influenced by inspiratory thickening fraction (Figure 5-9, p=0.002 for effect modification), even adjusting for age, sex, severity of illness, organ failures, and presence of sepsis (Table 5-1). Lower inspiratory thickening fraction was associated with decreasing thickness over time whereas higher inspiratory thickening fraction was associated with increasing diaphragm thickness over time (Figure 5-9). The association between inspiratory thickening fraction and changes in thickness over time was most pronounced during the first week of mechanical ventilation (adjusted interaction beta=0.063, 95% CI 0.012-0.114, p=0.03 for heterogeneity between the first and second weeks of ventilation).

Higher daily SOFA scores were associated with increasing diaphragm thickness (adjusted interaction beta=0.002, 95% CI 0.001-0.002); diaphragm contractile activity and SOFA had additive effects on the rate of change in thickness (Table 5-1, Figure 5-10). Sepsis did not significantly modify the rate of change in diaphragm thickness over time (adjusted interaction beta=0.001, 95% CI -0.006-0.008). The effect of diaphragm contractile activity on the rate and direction of change in diaphragm thickness was consistent across study epochs (p=0.16 for heterogeneity). Model effects were robust to removal of two potentially influential observations.
In accordance with the results of the primary model, the change in diaphragm thickness over the first week of ventilation was correlated with diaphragm contractile activity as assessed by the average diaphragm thickening fraction during the first 3 days of ventilation (p<0.01, $R^2=0.05$, Figure 5-11). The change in diaphragm thickness over the first week of ventilation was inversely correlated with the average ventilator driving pressure over the first 72 hours of ventilation (Figure 5-12, p=0.04 after removal of a single highly influential outlier).

### 3.5 Determinants of Diaphragm Contractile Activity During Mechanical Ventilation

Diaphragm contractile activity varied widely between and within patients over the first week of ventilation and tended to increase over time from relatively low baseline levels (Figure 5-13, median baseline thickening fraction 12%, IQR 5-14%, reference range in healthy subjects during resting tidal breathing is 25-40% (102, 204)). The use of lower ventilator driving pressures and partially assisted modes of ventilation was associated with higher thickening fraction (p=0.01 and p=0.02 respectively, Figure 5-14, Table 5-5), although there was considerable overlap in thickening fraction across levels of exposure to controlled ventilation and across levels of ventilator driving pressure (Figure 5-14). Thickening fraction was only slightly higher when patients in controlled modes of ventilation were triggering the ventilator above the set rate (14% vs. 10%, p=0.008). Contractile activity tended to be lower with higher SOFA scores (p=0.01, Table 5-5) but arterial pH, $P_aCO_2$, sedation level (SAS sore), tidal volume, respiratory frequency and PEEP were not independently associated with contractile activity (Table 5-5).

### 4 Discussion

In this study we found that changes in diaphragm thickness are common in mechanically ventilated patients, occur early in the course of ventilation, and seem to be modulated by the intensity of respiratory muscle work done by the patient, even under partially assisted modes of ventilation. We also observed that diaphragm thickness increased in some patients and that both decreased and increased diaphragm thickness were associated with significant diaphragm dysfunction. Our findings raise the possibility that titrating ventilatory support to maintain adequate (but not excessive) levels of inspiratory effort might prevent changes in diaphragm configuration during mechanical ventilation.
Disuse atrophy of the diaphragm has been demonstrated repeatedly in animal models (152, 153, 184) but the significance of disuse atrophy in the clinical setting has been unclear because partially assisted modes of ventilation are commonly employed (159, 160). Decreases in diaphragm thickness observed in this study likely correspond to diaphragm myofiber atrophy previously reported in various animal models (152, 159, 160, 184, 208), organ donors (24, 197) and a recently published series of living mechanically ventilated patients (27, 136). Our results suggest that decreases in diaphragm thickness are common during mechanical ventilation and are associated with impaired diaphragmatic function, consistent with previously documented in vitro contractile dysfunction (136).

This study provides strong evidence that mechanical ventilation injures the diaphragm in the clinical setting through its effects on inspiratory effort. Diaphragm thickness did not change following extubation or in non-ventilated ICU patients (controls). Moreover, in both primary and secondary analyses there was a strong dose-response relationship between the level of diaphragm contractile activity and changes in diaphragm thickness over time. The level of contractile activity associated with stable diaphragm thickness corresponded to normal levels of inspiratory effort during resting breathing in healthy subjects (thickening fraction of 25-40%) (69, 204). Average ventilator driving pressure over the first 72 hours was also correlated with the change in diaphragm thickness during the first week of MV. Titrating ventilatory support to maintain some adequate level of diaphragm activity (“muscle-protective” mechanical ventilation) may therefore prevent changes in diaphragm configuration. Because diaphragm contractile activity varies considerably within modes of ventilation and levels of ventilator driving pressure, such titration strategies would require direct monitoring of diaphragm activity.

A minority of patients exhibited an increase in diaphragm thickness over time. While increased diaphragm thickness following exercise training has been associated with increased strength (34, 101), function measurements obtained in three of these patients revealed marked weakness, suggesting that this increase in thickness may reflect structural injury rather than hypertrophy. Inferences related to this finding are significantly limited by the small size of this group. An early rapid increase in diaphragm thickness can occur following traumatic diaphragm injury (209, 210). Given that increases in thickness over time were associated with both higher thickening fraction and higher SOFA scores in our study, it is possible that an injurious increase in thickness may result from excess inspiratory loads during ventilation or from systemic
inflammation. High inspiratory loads can cause myofibrillar and sarcolemmal injury (174, 175, 185, 186, 211-213). Systemic inflammation impairs diaphragm function apart from causing atrophy (214) and can sensitize the diaphragm to load-related injury (142). Further work is required to establish the structural basis of increases in diaphragm thickness in this setting.

In our study, thickening fraction was not significantly associated with sedation level, pH or PaCO₂ after adjusting for ventilator settings and SOFA score. This likely reflects the complex interplay between these variables in the clinical setting: low pH and high PaCO₂ might be expected to increase thickening fraction in the absence of sedation while in the presence of sedation, they may reflect a blunted respiratory drive associated with reduced diaphragm thickening. Alternatively, high thickening fraction might be expected to increase pH and lower PaCO₂. The significance of the observed association between SOFA and thickening fraction is unclear; it is possible that residual confounding factors associated with greater severity of illness reduce inspiratory effort.

Our findings are subject to a number of limitations. First, while the reproducibility of end-expiratory right hemidiaphragm thickness measurement is adequate (repeatability coefficient of 0.2 mm), the reproducibility of the thickening fraction measurement is only moderately acceptable (repeatability coefficient of 16%) (76, 204). It is also unknown whether inspiratory thickening fraction measured at a single point in time is representative of diaphragmatic activity over the whole course of the day. To minimize systematic bias, we obtained measurements at a similar time period on a daily basis (between 8 am and 12 pm). Importantly, the ‘noise’ resulting from measurement imprecision would be expected to obscure any observed associations and bias effects toward the null hypothesis. Consequently, the influence of inspiratory effort level on the rate and direction of changes in diaphragm thickness may in fact be underestimated in this study.

Second, we ascertained diaphragm function by measuring diaphragm thickening fraction during a maximal inspiratory effort. Although diaphragm thickening fraction does not actually “measure” pressure generation by the diaphragm, this measurement provides a valid method of estimating diaphragmatic contractile force in ventilated patients (76, 103, 204). Maximum inspiratory efforts can be challenging to obtain in ventilated patients (38) and our technique has not yet been validated against the current gold standard, magnetic twitch transdiaphragmatic pressure (41, 47). In some subjects, maximal thickening fraction was measured under quasi-is-
lung volume conditions with the airway occluded and this may reduce the degree of thickening observed at a given level of inspiratory effort (70), although maximal thickening fraction measured under these conditions is generally above 50% in healthy subjects (71, 72). Further studies are required to confirm that changes in diaphragm thickness are associated with true contractile weakness.

Nevertheless, previous studies suggest that maximal diaphragm thickening fraction is strongly associated with maximal inspiratory pressure in outpatients (33, 71) and ventilated patients (73) and was recently shown to predict extubation success when measured during a spontaneous breathing trial in two separate studies (73, 74). Maximal thickening fraction measurements therefore likely reflect true diaphragm function. We took great care to obtain the most reliable measurements (204) but the number of patients is limited and future studies are required to confirm that changes in diaphragm thickness are associated with contractile weakness.

Third, we did not obtain serial diaphragm tissue specimens for histological analysis to correlate with the changes in diaphragm thickness observed on ultrasound. The structural changes accounting for the observed variation in diaphragm thickness over time are therefore uncertain. Aside from changes in myofibrillar structure, tissue edema or tonic diaphragm activity might account for variation in diaphragm thickness. However, these phenomena would be expected to increase muscle thickness; they are unlikely to account for early rapid decreases in diaphragm thickness. Decreases in diaphragm thickness on ultrasound were recently found to be correlated with reduced myocyte cross-sectional area in a porcine model of VIDD (183).

Fourth, the interpretation of the primary regression model is subject to a number of limitations. We cannot exclude the possibility that atrophy causes weakness and consequently reduces contractile activity (reverse causation of association). However, contractile activity levels were lowest at baseline before significant changes in diaphragm thickness occurred and contractile activity increased over time (while diaphragm thickness decreased), arguing against such reverse causation. Furthermore, inspiratory effort was documented to be normal or even above normal in ventilated patients with diaphragm dysfunction (127).

The observed association between inspiratory effort and changes in thickness over time may have arisen from residual confounding unaccounted for in our model. For example, a considerable subset of patients enrolled in the study received large doses of corticosteroids
following transplantation. However, significant changes in diaphragm thickness were observed across all admission categories.

Fifth, we cannot draw inferences about the impact of changes in diaphragm thickness on clinical outcomes. While diaphragm dysfunction is associated with prolonged mechanical ventilation (57, 68), it remains unclear whether preventing diaphragm atrophy would accelerate liberation from mechanical ventilation. Our findings do not provide definitive evidence of causality; it therefore remains unknown whether optimizing inspiratory effort level would protect against deleterious changes in diaphragm structure and function during mechanical ventilation.

In summary, we describe important changes in diaphragm muscle thickness that may be caused by excessive or inadequate ventilatory support that may impair muscle function (VIDD). These changes in diaphragm configuration might be prevented by muscle-protective ventilation strategies titrated to optimize patient inspiratory effort.
Table 5-1. Model parameters for linear mixed model of end-expiratory diaphragm thickness

<table>
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<th>Model Variable</th>
<th>Missing data</th>
<th>Beta coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaphragm thickness (natural logarithm of measured value)</td>
<td>0/527</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Intercept</td>
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<td>0.73</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diaphragm thickening fraction (%)</td>
<td>7/527</td>
<td>-0.46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Day of mechanical ventilation (days)</td>
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<td>-0.033</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>0.002</td>
<td>0.11</td>
</tr>
<tr>
<td>Sex (=male)</td>
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<td>0.074</td>
<td>0.11</td>
</tr>
<tr>
<td>Sepsis (=yes)</td>
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<td>0.008</td>
<td>0.69</td>
</tr>
<tr>
<td>SOFA score*</td>
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<td>-0.003</td>
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<tr>
<td>SAPS.II</td>
<td>0/527</td>
<td>0.0009</td>
<td>0.51</td>
</tr>
<tr>
<td>Diaphragm thickening fraction (%) * day of mechanical ventilation</td>
<td>n/a</td>
<td>0.038</td>
<td>0.002</td>
</tr>
<tr>
<td>Sepsis (=yes) * day of mechanical ventilation</td>
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<td>0.001</td>
<td>0.85</td>
</tr>
<tr>
<td>SOFA * day of mechanical ventilation</td>
<td>n/a</td>
<td>0.002</td>
<td>&lt;0.001</td>
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### Table 5-2. Clinical Characteristics of Study Cohort and Patient Subgroups According to Change in Diaphragm Thickness Over Time

<table>
<thead>
<tr>
<th>Age (years) [mean (SD)]</th>
<th>Overall Study Population (n=107)</th>
<th></th>
<th>Change in diaphragm thickness during the first week of mechanical ventilation</th>
<th></th>
<th></th>
<th></th>
<th>&gt; 10% Decrease (n=47)</th>
<th>Within 10% of Baseline (n=47)</th>
<th>&gt; 10% Increase (n=13)</th>
<th>p-value</th>
</tr>
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<tr>
<td>Overall Study Population (n=107)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>39 (36%)</td>
<td></td>
<td>21 (45%)</td>
<td>15 (32%)</td>
<td>3 (23%)</td>
<td>0.25</td>
<td></td>
<td></td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>25.9 (21.5–28.7)</td>
<td></td>
<td>26.0 (21.5 – 28.6)</td>
<td>26.0 (22.1 – 28.9)</td>
<td>24.2 (20.7 – 26.8)</td>
<td>0.81</td>
<td></td>
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<td></td>
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<tr>
<td>SAPS II</td>
<td>42 (33 – 56)</td>
<td></td>
<td>44 (33 – 55)</td>
<td></td>
<td>40 (32 – 56)</td>
<td>49 (35 – 61)</td>
<td>0.54</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Change in diaphragm thickness during the first week of mechanical ventilation</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&gt; 10% Decrease (n=47)</td>
<td>60.7 (15.1)</td>
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<td>56.7 (16.6)</td>
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<tr>
<td>Within 10% of Baseline (n=47)</td>
<td></td>
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<tr>
<td>&gt; 10% Increase (n=13)</td>
<td></td>
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<tr>
<td>p-value</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) [mean (SD)]</td>
<td>59.6 (15.6)</td>
<td></td>
<td>60.7 (15.1)</td>
<td>56.7 (16.6)</td>
<td>66.8 (11.7)</td>
<td></td>
<td></td>
<td></td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>39 (36%)</td>
<td></td>
<td>21 (45%)</td>
<td>15 (32%)</td>
<td>3 (23%)</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.9 (21.5–28.7)</td>
<td></td>
<td>26.0 (21.5 – 28.6)</td>
<td>26.0 (22.1 – 28.9)</td>
<td>24.2 (20.7 – 26.8)</td>
<td>0.81</td>
<td></td>
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<tr>
<td>SAPS II</td>
<td>42 (33 – 56)</td>
<td></td>
<td>44 (33 – 55)</td>
<td></td>
<td>40 (32 – 56)</td>
<td>49 (35 – 61)</td>
<td>0.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Failing Organs at Baseline – n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>31 (30%)</td>
<td></td>
<td>13 (28%)</td>
<td>17 (38%)</td>
<td>1 (8%)</td>
<td>0.20</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2</td>
<td>40 (38%)</td>
<td></td>
<td>18 (38%)</td>
<td>13 (29%)</td>
<td>9 (69%)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>18 (17%)</td>
<td></td>
<td>8 (17%)</td>
<td>9 (20%)</td>
<td>1 (8%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>16 (15%)</td>
<td></td>
<td>8 (17%)</td>
<td>6 (13%)</td>
<td>2 (15%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Primary reason for ventilation – n (%)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Respiratory dysfunction</td>
<td>15 (14%)</td>
<td></td>
<td>7 (15%)</td>
<td>7 (15%)</td>
<td>1 (8%)</td>
<td>0.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular dysfunction</td>
<td>10 (9%)</td>
<td></td>
<td>5 (11%)</td>
<td>3 (6%)</td>
<td>2 (15%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepsis</td>
<td>30 (28%)</td>
<td></td>
<td>15 (32%)</td>
<td>12 (26%)</td>
<td>3 (23%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transplant</td>
<td>26 (24%)</td>
<td></td>
<td>13 (28%)</td>
<td>12 (26%)</td>
<td>1 (8%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other organ dysfunction</td>
<td>17 (16%)</td>
<td></td>
<td>5 (11%)</td>
<td>7 (15%)</td>
<td>5 (38%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-operative</td>
<td>9 (8%)</td>
<td></td>
<td>2 (4%)</td>
<td>6 (13%)</td>
<td>1 (8%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline mode of mechanical ventilation – n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controlled mode (ACVC, PCV)</td>
<td>76 (71%)</td>
<td></td>
<td>37 (79%)</td>
<td>32 (68%)</td>
<td>7 (54%)</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial assist mode (PSV)</td>
<td>31 (29%)</td>
<td></td>
<td>10 (21%)</td>
<td>15 (32%)</td>
<td>6 (46%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ventilator settings (average over first 3 days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tidal volume (ml/kg PBW)</td>
<td>6.4 (5.4 – 8.0)</td>
<td></td>
<td>6.1 (5.4 – 8.0)</td>
<td>6.6 (5.5 – 7.9)</td>
<td>6.2 (5.7 – 7.0)</td>
<td>0.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applied driving pressure (cm H₂O) [mean (SD)]</td>
<td>11.0 (6.3)</td>
<td></td>
<td>12.2 (6.3)</td>
<td>10.4 (5.4)</td>
<td>8.5 (8.6)</td>
<td>0.04b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>21 (18 – 24)</td>
<td></td>
<td>21 (18 – 24)</td>
<td>20 (19 – 24)</td>
<td>23 (18 – 28)</td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive end-expiratory pressure (cm H₂O)</td>
<td>8 (5 – 10)</td>
<td></td>
<td>8 (5 – 10)</td>
<td>7 (5 – 9)</td>
<td>9 (6 – 10)</td>
<td>0.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIO₂</td>
<td>0.45 (0.40 – 0.50)</td>
<td></td>
<td>0.45 (0.40 – 0.50)</td>
<td>0.45 (0.38 – 0.50)</td>
<td>0.45 (0.40 – 0.60)</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial blood gases (average over first 3 days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.38 (7.34 – 7.42)</td>
<td></td>
<td>7.38 (7.34 – 7.43)</td>
<td>7.39 (7.33 – 7.42)</td>
<td>7.36 (7.31 – 7.41)</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA CO₂ (mm Hg)</td>
<td>40 (34 – 47)</td>
<td></td>
<td>42 (35 – 48)</td>
<td>41 (35 – 47)</td>
<td>36 (32 – 46)</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA O₂ (mm Hg)</td>
<td>97 (80 – 113)</td>
<td></td>
<td>98 (79 – 111)</td>
<td>99 (83 – 117)</td>
<td>82 (78 – 97)</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical management during first week of mechanical ventilation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuromuscular blockade administered by infusion at any time – n (%)</td>
<td>23 (21%)</td>
<td></td>
<td>10 (21%)</td>
<td>10 (21%)</td>
<td>3 (23%)</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids administered at any time – n (%)</td>
<td>45 (42%)</td>
<td></td>
<td>23 (49%)</td>
<td>17 (36%)</td>
<td>5 (38%)</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days in controlled mode of ventilation</td>
<td>1 (0 – 2)</td>
<td></td>
<td>1 (0 – 2.5)</td>
<td>1 (0 – 2)</td>
<td>1 (0 – 3)</td>
<td>0.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days under heavy sedation (SAS 1-2)</td>
<td>1 (0 – 2)</td>
<td></td>
<td>1 (0 – 3)</td>
<td>1 (0 – 2)</td>
<td>1 (1 – 2)</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All data presented as either n (%) for categorical variables or median (IQR) for continuous variables, except where indicated.

*b after removal of a single highly influential outlier (p=0.12 before removal)
Table 5-3. Clinical characteristics of control subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age</th>
<th>Sex</th>
<th>Admitting Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>Male</td>
<td>Intracranial hemorrhage</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>Male</td>
<td>Intracranial hemorrhage</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>Female</td>
<td>Sepsis</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>Male</td>
<td>Upper gastrointestinal hemorrhage</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>Female</td>
<td>Sepsis</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>Male</td>
<td>Retroperitoneal hemorrhage</td>
</tr>
<tr>
<td>7</td>
<td>56</td>
<td>Male</td>
<td>Post-operative monitoring</td>
</tr>
<tr>
<td>8</td>
<td>59</td>
<td>Male</td>
<td>Decompensated cirrhosis</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>Male</td>
<td>Sepsis</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>Female</td>
<td>Sepsis</td>
</tr>
</tbody>
</table>
Table 5-4. Diaphragm function measurements in mechanically ventilated patients

<table>
<thead>
<tr>
<th>Day of Study at Assessment</th>
<th>Ventilation Status at Assessment</th>
<th>Diaphragm thickness at assessment (% of baseline)</th>
<th>Diaphragm Thickness Category at assessment</th>
<th>Inspiratory maneuver obtaining maximal thickening fraction measurement*</th>
<th>Maximal Inspiratory Thickening Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>PSV</td>
<td>61%</td>
<td>&gt;10% loss</td>
<td>Coached sniff maneuver</td>
<td>40%</td>
</tr>
<tr>
<td>4</td>
<td>Exubated**</td>
<td>67%</td>
<td>&gt;10% loss</td>
<td>Coached deep inspiration</td>
<td>21%</td>
</tr>
<tr>
<td>6</td>
<td>PSV</td>
<td>74%</td>
<td>&gt;10% loss</td>
<td>ETT occlusion maneuver</td>
<td>2%</td>
</tr>
<tr>
<td>6</td>
<td>Exubated</td>
<td>75%</td>
<td>&gt;10% loss</td>
<td>Coached deep inspiration</td>
<td>10%</td>
</tr>
<tr>
<td>8</td>
<td>PSV</td>
<td>85%</td>
<td>&gt;10% loss</td>
<td>ETT occlusion maneuver</td>
<td>68%</td>
</tr>
<tr>
<td>4</td>
<td>PSV</td>
<td>87%</td>
<td>&gt;10% loss</td>
<td>Coached deep inspiration</td>
<td>17%</td>
</tr>
<tr>
<td>10</td>
<td>PSV</td>
<td>88%</td>
<td>&gt;10% loss</td>
<td>ETT occlusion maneuver</td>
<td>18%</td>
</tr>
<tr>
<td>5</td>
<td>Exubated</td>
<td>92%</td>
<td>Near baseline</td>
<td>Coached deep inspiration</td>
<td>38%</td>
</tr>
<tr>
<td>4</td>
<td>Exubated</td>
<td>93%</td>
<td>Near baseline</td>
<td>Coached deep inspiration</td>
<td>24%</td>
</tr>
<tr>
<td>9</td>
<td>Exubated</td>
<td>95%</td>
<td>Near baseline</td>
<td>Coached sniff maneuver</td>
<td>36%</td>
</tr>
<tr>
<td>9</td>
<td>Exubated</td>
<td>96%</td>
<td>Near baseline</td>
<td>Coached deep inspiration</td>
<td>15%</td>
</tr>
<tr>
<td>7</td>
<td>Exubated</td>
<td>97%</td>
<td>Near baseline</td>
<td>Coached sniff maneuver</td>
<td>27%</td>
</tr>
<tr>
<td>9</td>
<td>Exubated</td>
<td>100%</td>
<td>Near baseline</td>
<td>Coached deep inspiration</td>
<td>79%</td>
</tr>
<tr>
<td>6</td>
<td>Exubated</td>
<td>100%</td>
<td>Near baseline</td>
<td>Coached deep inspiration</td>
<td>86%</td>
</tr>
<tr>
<td>6</td>
<td>Exubated</td>
<td>100%</td>
<td>Near baseline</td>
<td>Coached deep inspiration</td>
<td>37%</td>
</tr>
<tr>
<td>9</td>
<td>PSV</td>
<td>100%</td>
<td>Near baseline</td>
<td>ETT occlusion maneuver</td>
<td>38%</td>
</tr>
<tr>
<td>8</td>
<td>PSV</td>
<td>109%</td>
<td>Near baseline</td>
<td>ETT occlusion maneuver</td>
<td>24%</td>
</tr>
<tr>
<td>7</td>
<td>Exubated</td>
<td>113%</td>
<td>&gt;10% gain</td>
<td>Coached deep inspiration</td>
<td>21%</td>
</tr>
<tr>
<td>8</td>
<td>PSV</td>
<td>117%</td>
<td>&gt;10% gain</td>
<td>ETT occlusion maneuver</td>
<td>0%</td>
</tr>
<tr>
<td>7</td>
<td>PSV</td>
<td>140%</td>
<td>&gt;10% gain</td>
<td>Coached deep inspiration</td>
<td>10%</td>
</tr>
</tbody>
</table>

*If patient inspiratory effort was not initially convincing to the study examiner (EG), different maneuvers were employed (deep inspiration, inspiratory sniff, endotracheal tube occlusion) and the maximal thickening fraction value from the maneuver achieving the greatest visible patient inspiratory effort was recorded. There was no significant difference in the maximal inspiratory thickening fraction obtained between different maneuvers (p=0.4) or between ventilation status at assessment (p=0.10).

**Extubated patients were studied on the day of extubation.
Table 5-5. Model parameters for linear mixed model of diaphragm thickening fraction during mechanical ventilation

<table>
<thead>
<tr>
<th>Model Variable</th>
<th>Missing data</th>
<th>Beta coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaphragm thickening fraction (%) – dependent variable</td>
<td>7/527</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Intercept</td>
<td>n/a</td>
<td>22.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>pH</td>
<td>10/527</td>
<td>0.02</td>
<td>0.23</td>
</tr>
<tr>
<td>P&lt;sub&gt;CO&lt;/sub&gt;₂</td>
<td>10/527</td>
<td>-0.07</td>
<td>0.30</td>
</tr>
<tr>
<td>SAS*</td>
<td>16/527</td>
<td>0.43</td>
<td>0.45</td>
</tr>
<tr>
<td>SOFA*</td>
<td>28/527</td>
<td>-0.52</td>
<td>0.01</td>
</tr>
<tr>
<td>Mode (Controlled)</td>
<td>0/527</td>
<td>-4.16</td>
<td>0.02</td>
</tr>
<tr>
<td>Tidal volume (ml)</td>
<td>4/527</td>
<td>0.003</td>
<td>0.48</td>
</tr>
<tr>
<td>Total respiratory frequency (breaths/min)</td>
<td>4/527</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Ventilator driving pressure (cm H₂O)</td>
<td>7/527</td>
<td>-0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>PEEP (cm H₂O)</td>
<td>0/527</td>
<td>0.26</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*missing data imputed from patient median value
Figure 5-1. CONSORT diagram outlining study screening and enrolment.

498 patients screened for eligibility

255 eligible for enrollment

138 enrolled (128 MV patients, 10 controls)

117 underwent study evaluation (107 MV patients, 10 controls)

Function measurements obtained in 20 MV patients and 4 controls

- 164 – Extubation expected within 24 hours
- 9 – Readmission or previously intubated
- 18 – Lack of commitment to life support
- 12 – Physical limitation
- 3 – Airborne isolation
- 1 – Diaphragm resection
- 1 – Diaphragm hernia
- 1 – Death anticipated to be imminent
- 34 – Expected to leave unit in 24 hours

- 12 – MD refused study participation
- 76 – SDM refused consent
- 16 – Consent not obtained within 72 hour window
- 1 – Transferred to different institution
- 1 – Died before consent could be obtained
- 1 – Enrolled in other study
- 4 – Missed
- 5 – Study full (no capacity for more US)
- 1 – Study paused

- 2 withdrew consent
- 4 without baseline US measurements
- 15 with only baseline US measurements

- 77 – MV patients enrolled before protocol modified to include function measurements
- 1 – measurement missed
- 4 – discharged over weekend
- 1 – died before function measurement could be obtained
- 4 – unable to obtain inspiratory efforts due to sedation or delirium
- 6 controls – measurement not obtained
Figure 5-2. Variation in diaphragm thickness over time the first week of mechanical ventilation.

Subjects were categorized according to the magnitude and direction of change in diaphragm thickness during the first week of MV: 47 (44%) decreased by more than 10%, 47 (44%) remained unchanged, and 13 (12%) increased by more than 10%. Mean and standard deviation are plotted for each group for each study day. Numbers shown next to each data point indicate the number of patients remaining on the ventilator in each group on each study day. Trend line and confidence intervals (shaded area) were fitted by Loess smoothing.
Figure 5-3. Variation in diaphragm thickness in individual subjects over the first 14 days of mechanical ventilation.

Patients are grouped according to the category of overall change in thickness over the first week of ventilation.
Figure 5–4. Changes in diaphragm thickness according to mode of mechanical ventilation.

Early changes in diaphragm thickness over time were similar between patients ventilated in controlled modes of ventilation for at least 2 of the first 3 days of mechanical ventilation (blue) and patients ventilated in partially assisted modes of ventilation (predominantly pressure support ventilation) for at least 2 of the first 3 days on mechanical ventilation (red)—though some later separation is observed between the curves and heterogeneity between patients is marked. Error bars indicate standard deviations. The trend lines were generated using LOESS smoothed regression.
Figure 5-5. Diaphragm thickness variation over time in non-ventilated control patients and in ventilated patients following extubation.

Diaphragm thickness was stable over time in non-ventilated patients admitted to the intensive care unit (Panel A, n=10) and following extubation in patients who had been ventilated (Panel B, n=29). In panel B, day 0 represents the day of extubation. Subjects are represented by individual lines.
Figure 5-6. Diaphragm thickness before and after extubation.

Diaphragm thickness measurements were obtained following extubation in 29 subjects (numbered above each plot). Measurements prior to (or on the day of) extubation are shown in black, measurements after extubation are shown in red.
Figure 5-7. Diaphragm function is impaired by changes in diaphragm thickness over time during mechanical ventilation.

The diaphragm thickening fraction during a maximal inspiratory effort, was significantly lower in patients with a significant decrease in thickness (n=6, p=0.01), or a significant decrease in thickness (n=3, p=0.01), both compared to control subjects (n=4). Maximal thickening fraction in patients with near baseline thickness (n=10) was not significantly different from controls (p=0.14). The dotted grey line displays a previously reported threshold value for severe diaphragm dysfunction (69). Function measurements were obtained on the day of extubation (if earlier than 1 week) or after 1 week of ventilation.
Figure 5-8. Relationship between changes in diaphragm thickness and the rate of severe diaphragm dysfunction.

Diaphragm dysfunction was defined as maximal diaphragm thickening fraction < 20% during a maximal inspiratory effort as previously described (69). The rates of diaphragm dysfunction were higher in patients with significant increases or decreases in diaphragm thickness compared to patients with no significant change (p=0.06).
Figure 5-9. Diaphragm contractile activity (inspiratory effort) is associated with the rate and direction of change in diaphragm thickness during mechanical ventilation.

At low contractile activity levels, diaphragm thickness declines over time (lower arrow) while at high contractile activity levels diaphragm thickness increases over time (upper arrow) (p=0.002 for effect modification). Diaphragm thickness was stable over time at levels of contractile activity that are typically observed in healthy subjects during resting tidal breathing (blue band on y-axis) (102, 204). Variation in thickness is adjusted for the effects of age, sex, baseline SAPS II score, daily SOFA score, and diagnosis of sepsis.
Figure 5-10. Organ dysfunction modifies the relationship between diaphragm inactivity and changes in diaphragm thickness over time.

The relationship between diaphragm contractile activity and the rate of change in diaphragm thickness predicted by multivariable linear mixed effects modeling is shown for the 25<sup>th</sup> and 75<sup>th</sup> percentiles of SOFA score.
Figure 5-11. Diaphragm contractile activity over the first three days of ventilation and changes in diaphragm thickness over the 1st week of MV.

Changes in diaphragm thickness during the first week of mechanical ventilation are correlated with the average daily diaphragm thickening fraction over the first 72 hours of mechanical ventilation (p=0.003, \( R^2=0.07 \)).
Figure 5-12. Relation between set driving pressure and change in diaphragm thickness over time.

Ventilator driving pressures applied during the early course of mechanical ventilation are associated with the overall change in diaphragm thickness during the first week of mechanical ventilation (MV). Mean driving pressure was significantly different across categories of change in thickness after removal of a single highly influential outlier (n=106, p=0.04, p=0.12 before outlier removal).
Figure 5-13. Variation in diaphragm contractile activity over during the first week of mechanical ventilation.

Diaphragm contractile activity (quantified by the diaphragm thickening fraction) varied significantly over the course of ventilation. Error bars indicate the standard deviation in thickening fraction across all patients for each day of mechanical ventilation.
Figure 5-14. Effect of ventilator settings on diaphragm contractile activity.

Diaphragm contractile activity (quantified by the thickening fraction) is modulated by both the ventilator driving pressure set by clinicians (left, p<0.0001, R^2=0.12) and the mode of mechanical ventilation (right, p<0.0001 for differences across level of exposure to controlled mechanical ventilation). Note that diaphragm contractile activity overlaps significantly across all levels of set driving pressure and exposure to controlled ventilation.
Chapter 6

Monitoring Diaphragm Activity and Neuromuscular Coupling During Mechanical Ventilation: Feasibility and Preliminary Findings

1 Introduction

Respiratory muscle dysfunction is an important complication of critical illness (144, 145). In patients receiving invasive mechanical ventilation for acute respiratory failure, diaphragm dysfunction has been linked to increased mortality (56) and difficulty weaning from mechanical ventilation (195, 196). A range of factors associated with critical illness impair diaphragm function, including sepsis, hypercapnia, and pharmacologic agents (143, 144). Importantly, mechanical ventilation itself may injure the diaphragm (ventilator-induced diaphragm dysfunction—VIDD) (215, 216). Changes in diaphragm thickness and function occur rapidly following initiation of ventilation, affecting up to 50% of ventilated patients (150, 152, 153, 160, 202).

Three possible mechanisms for VIDD have been identified. In animal models, suppressing diaphragm contractile activity induces the rapid onset of muscle atrophy mediated by oxidative stress leading to alterations in protein synthesis and proteolysis signaling pathways (25, 161, 215, 217). A recent clinical study found that diaphragm atrophy occurs more rapidly when diaphragm contractile activity is reduced (202). Alternately, excess inspiratory loads can injure the diaphragm by disrupting sarcomeric architecture and inducing muscle inflammation (175, 186). Thirdly, eccentric (lengthening) muscle contractions—which may result from certain types of patient-ventilator dyssynchrony such as ineffective efforts or reverse triggering—are thought to be particularly injurious (177, 180).

Given these concerns, ventilation strategies designed to prevent diaphragm injury—muscle-protective ventilation—could accelerate liberation from mechanical ventilation. However, the depth and duration of diaphragm inactivity during mechanical ventilation and the frequency of eccentric diaphragm contractions remain poorly described. Further, the impact of these
mechanisms on diaphragm structure and function in the clinical setting requires further delineation. We undertook to address these uncertainties by monitoring diaphragm activity and patient-ventilator synchrony longitudinally over the first week of ventilation. Here we report the feasibility of our proposed study design and present the preliminary findings of our study in the first 10 patients enrolled. Some of our findings were previously reported in the form of an abstract (218).

2 Methods

This study was conducted in two medical-surgical intensive care units at University Health Network, Toronto, Canada. Written informed consent was obtained from substitute decision makers or deferred until recovery and obtained from the patient directly if no substitute decision maker was available. The study was approved by the Institutional Review Board of the University Health Network and was performed in accordance with the ethical standards laid down in the 2008 Declaration of Helsinki.

2.1 Experimental Subjects

We enrolled adult patients requiring invasive mechanical ventilation for moderate or severe acute respiratory distress syndrome (188), septic shock (189), or severe acute brain injury (defined as traumatic brain injury or intracranial hemorrhage causing coma with Glasgow Coma Scale score below 9 prior to intubation).

Patients were excluded from the study if they were receiving invasive mechanical ventilation for more than 24 hours, if they were deemed unlikely to remain on the ventilator for 7 days (based on a predictive algorithm derived from a pre-existing dataset, see Appendix 2 for details), if they had a high spinal cord injury or neuromuscular condition interfering with diaphragm function, if they had esophageal varices or recent upper gastrointestinal tract surgery (such that esophageal catheter placement was unsafe), or if there was any history of obstructive lung disease.

2.2 Experimental Measurements

Upon enrollment, a nasogastric catheter fitted with balloons to measure esophageal and gastric pressure and 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, $\Delta$Edi) by the Servo-I mechanical ventilator (Maquet, Solna, Sweden) according to previously published methods (59). Airway pressure (Paw), flow and Edi 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).

Esophageal and gastric balloons were coupled to pressure transducers (MPX2050DP, Freescale Semiconductor, Inc., Tempe, AZ) and pressures (Pes and Pga, respectively) were acquired and recorded in real time at a sampling frequency of 62.5 Hz by the same personal computer and software system. Transdiaphragmatic pressure (Pdi) was obtained by real-time digital subtraction of Pes from Pga. Study catheter positioning was confirmed by the electrode signals (59) and esophageal pressure validity was assessed by the occlusion maneuver (190). Positioning was reconfirmed on a daily basis for the duration of the study.

Diaphragm neuromuscular coupling (NMC, computed as the ratio of $\int$Pdi to $(\int$Edi)$^{2/3}$, integrated from onset to end of inspiration as described in Chapter 3) (47, 61) was measured from spontaneous patient inspiratory efforts made during brief airway occlusions applied for a single breath at 20-30 random intervals over a period of 10 minutes. Patients were advised that they might feel some difficulty breathing, but were not coached to increase inspiratory efforts.

Diaphragm thickness was measured by ultrasound as previously described (76, 194, 199). Briefly, a 13 MHz linear array transducer (HFL-38xe, FUJIFILM Sonosite Inc., Bothell WA) was placed in the ninth or tenth intercostal space near the midaxillary line and angled perpendicular to the chest wall (30). In this location, the diaphragm is identified as a three-layered structure just superficial to the liver, consisting of a relatively nonechogenic muscular layer bounded by the echogenic membranes of the diaphragmatic pleura and peritoneum (101). Diaphragmatic thickness was measured at end-expiration (Tdi,ee) and peak inspiration (Tdi,pi – i.e. peak thickness value during inspiration) as the distance between the diaphragmatic pleura and the peritoneum using M-mode (69, 72). Measurements of Tdi,ee and Tdi,pi were always made on two breaths visualized in a single M-mode image. Diaphragm thickening during inspiration ($\Delta$Tdi) was taken as the difference between Tdi,pi and Tdi,ee. Diaphragm thickening fraction (TFdi) was defined as the percentage change in diaphragm thickness during inspiration (computed from the quotient of $\Delta$Tdi and Tdi,ee).

2.3 Experimental Protocol
Following initiation of the study, Paw, flow and Edi were recorded for 5 minutes of every hour. NMC, Tdi and TFdi were measured once daily. Measurements were continued until patients were extubated, died, or completed 7 days of study monitoring.

2.4 Signal Analysis

Hourly signal recordings were analyzed using automated analysis software (Orion, Neurovent Inc., Toronto, Canada). This software uses a previously validated algorithm (219) to identify the onset, peak and end of inspiratory effort using the Edi 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, ΔEdi and ΔPaw were averaged across all mechanical or spontaneous breaths for each 5-minute recording. The timing of the onset of neural inspiration (onset of rise in Edi), offset of neural inspiration (decline to 70% of peak Edi), and onset and offset of mechanical insufflation (defined by ventilator cycling status) measured for each breath was used to classify whether breaths were synchronous or dyssynchronous and to classify the type of dyssynchrony. The dyssynchrony classification system (Table 6-1) was developed on an a priori basis using previously published definitions and clinical and physiological judgment. Randomly sampled recordings were inspected to identify classification errors (at least 10 recordings for each type of dyssynchrony) and definitions were modified as needed to reduce any observed errors in days synchrony detection. Histograms of neural inspiratory time, Edi and ∫Edi (integrated with respect to time per breath) were examined to identify potential thresholds to differentiate Edi artefact from genuine neural inspiratory events. A conservative threshold was selected to avoid including artefactual neural events in the dataset, at the risk of excluding some true neural events.

2.5 Feasibility Assessment

We assessed feasibility of the study design based on the rate of enrollment (subjects enrolled/month), the sensitivity and specificity of the study eligibility criteria to select patients who would remain alive and on the ventilator for at least 7 days, the proportion of eligible patients who were enrolled in the study (and the barriers to enrollment), and the proportion of enrolled patients who completed the planned 7-day monitoring protocol. We also ascertained the feasibility of planned longitudinal, hourly automated signal acquisition based on the frequency of
missing measurements and by documenting whether the monitoring protocol interfered with routine respiratory therapy or nursing care.

2.6 Statistical Analysis

Descriptive statistics were expressed as means and standard deviations where appropriate. Because the primary objective of this preliminary study was to establish the feasibility of the study protocol, we planned to continue the study until at least 10 patients were enrolled and at least 5 enrolled patients completed the 7-day monitoring protocol.

3 Results

3.1 Feasibility: Enrolment and Protocol Completion

Screening and enrolment are summarized in Figure 6-1. Of 136 patients screened over a period of 7 months, 102 met various exclusion criteria. Among the 56 patients excluded because they were deemed unlikely to remain alive and on the ventilator for 7 days, 24 (43%) remained on the ventilator for 7 days or longer. Of the 34 eligible patients, 10 were enrolled (average enrolment rate of 1.4 patients/month); 24 eligible patients were not enrolled in the study for various reasons, most often because they presented on the weekend or because eligibility was missed during screening. Among truly eligible subjects, 18 (53%) remained alive and on the ventilator for at least 7 days (5 of 10 enrolled subjects, 13 of 24 eligible non-enrolled subjects). The overall sensitivity and specificity of the predictive algorithm employed to identify patients who would remain alive and on the ventilator for at least 7 days was 44% and 68%, respectively.

Patient and enrolment characteristics for enrolled subjects are summarized in Table 6-2. Patients were enrolled a median of 11.9 hours (IQR 10.0-18.5 hours) following intubation. The duration of longitudinal recording ranged between 3 and 164 hours, for a total of 1068 study hours. Two hundred and forty five hourly recordings were not obtained (23% missing), and the proportion of missing recordings ranged from 0% to 65% across subjects (median 32% missing). The most common reason for missing recordings was temporary ventilator disconnection (for tracheal suctioning or patient transport) with failure to restart monitoring after resuming ventilation. Four subjects completed the full 7-day monitoring protocol. In one subject, the esophageal catheter was removed during an endotracheal tube change on study day 6 and the patient’s family declined to have the catheter reinserted. Three subjects died before completing 7 days of
monitoring and 2 subjects recovered and were electively extubated before completing 7 days of monitoring.

### 3.2 Feasibility: Signal Acquisition and Analysis

In 823 hourly recordings obtained from 10 subjects, a total of 129,359 mechanical ventilator breaths were recorded and 110,753 neural inspiratory events were detected using the automated signal analysis algorithm. Inspection of the distributions of Edi and neural inspiratory time for these neural inspiratory events suggested that many were not consistent with true neural inspiratory efforts (many values fell below previously observed minimum levels for these values (219, 220)). Visual review of associated recordings suggested that many of these non-physiological electrical events were possibly due to signal interference from esophageal muscular activity or electrocardiographic artefact. Inspection of the \( \int Edi \) histogram revealed a bimodal distribution (Figure 6-2) with local minimum of approximately 1.5 \( \mu \text{V}*\text{seconds} \). Given that this value is approximately consistent with a minimum level of inspiratory effort (64, 82), this value was used as a cut-off to filter out artefactual neural events, leaving 82,242 neural events for further analysis. Visual inspection of 10 randomly sampled recordings with frequent events with \( \int Edi \) below 1.5 \( \mu \text{V}*\text{seconds} \) confirmed that such events were not consistent with genuine inspiratory efforts.

### 3.3 Preliminary Findings: Diaphragm Contractile Activity

Diaphragm contractile activity (quantified by \( \Delta Edi \)) varied significantly within and between patients over the first week of mechanical ventilation (Figure 6-3). Neural inspiration was entirely absent during 0-51% of recordings (Table 6-3); diaphragm activity was minimal (\( \Delta Edi < 5 \, \mu \text{V} \)) during 6%-86% of recordings. \( \Delta Edi \) did not exhibit significant diurnal variation (daytime median 7.9 \( \mu \text{V} \) [IQR 2.2-12.9 \( \mu \text{V} \)], nocturnal median 7.0 \( \mu \text{V} \) [IQR 2.8-12.1 \( \mu \text{V} \)], \( p=0.74 \)).

Diaphragm thickening fraction measured once daily was weakly correlated with the average value of diaphragm electrical activity over 24 hours (Figure 6-4, \( R^2=0.09 \), \( p=0.04 \)).

### 3.4 Preliminary Findings: Patient-Ventilator Synchrony

Each form of patient-ventilator dyssynchrony was observed at varying time points during the monitoring period (Table 6-3). Reverse triggering was observed in 9 patients at varying rates
(range 1% to 55% of recordings). Double triggering was observed in 7 patients at varying frequencies (range 1% to 33% of recordings). Eccentric contractions were observed in 7 patients at relatively low frequencies (range 1% to 13% of recordings). The rate of eccentric contractions was associated with the frequency of reverse triggering (Figure 6-5, $R^2=0.72$, $p<0.0001$); 52% of reverse triggered breaths met criteria for eccentric contractions. Uncoupled neural events were observed in 5 patients at relatively low frequencies (range 1%-12%).

### 3.5 Preliminary Findings: Neuromuscular coupling and Diaphragm Thickness

NMC measurements were not obtained on 24 of 56 study days due to the absence of patient inspiratory effort. Mean daily NMC ranged between -0.1 to 3.6 cm H$_2$O-µV$^{-2/3}$ (Figure 6-6) and the average value of NMC varied significantly between patients (mean 1.4 cm H$_2$O/µV, between-subjects SD 0.6 cm H$_2$O/µV, between-days SD 0.7 cm H$_2$O/µV). Using the method described by Bellani and colleagues (64) to compute ΔPdi from hourly values of NMC interpolated from daily NMC measurements, we estimated the range of ΔPdi corresponding to given levels of ΔEdi (Figure 6-7). These computations suggest that ΔEdi of 5 µV corresponds to ΔPdi of 5 cm H$_2$O or less; the estimated ΔPdi at ΔEdi of 15 µV varied considerably from about 5-15 cm H$_2$O.

Diaphragm thickness measurements were obtained on 37 study days. Diaphragm thickness evolved considerably over time for most patients (Figure 6-8); the rate and direction of change in diaphragm thickness was associated with the average Edi for each 24-hour period between measurements (Figure 6-9, $p=0.0004$). On days when diaphragm thickness differed by more than 10% from baseline (either increases or decreases), median NMC was lower compared to days when diaphragm thickness was close to baseline, but differences were not statistically significant (Figure 6-10, n=24 measurements, $p=0.24$). NMC was inversely correlated with the average hourly rate of eccentric contractions (Figure 6-11, $p=0.05$).

### 4 Discussion

In this study, we found that longitudinal monitoring of diaphragm activity and neuromuscular coupling during invasive mechanical ventilation for acute respiratory failure is feasible. Furthermore, our preliminary findings support the hypothesis that diaphragm inactivity and
eccentric contractions are important determinants of diaphragm injury during mechanical ventilation in the clinical setting, corroborating recently published observations on changes in diaphragm thickness and function during mechanical ventilation (202) as outlined in Chapter 5.

The most important obstacle to study completion was enrolment of patients who were expected to remain alive and on the ventilator for the duration of the 7-day monitoring protocol. Because two opposing outcomes compete to determine the duration of ventilation—death vs. recovery—it is challenging to accurately predict duration of ventilation within 24 hours of initiating invasive ventilation. A significant minority of patients excluded from the study because they were deemed unlikely to complete the 7-day protocol remained alive and ventilator-dependent for at least 7 days, while only 50% of patients eligible for the study reached the same end-point. The use of less restrictive eligibility criteria would enhance enrolment considerably, at the cost of including more patients who do not remain ventilator-dependent for the target time period.

The majority of patients eligible for the study could not be enrolled because they presented on the weekend (when study staff are not actively screening). This barrier might be addressed by engaging non-study staff (medical and allied health team) in screening efforts and by extending the window for enrolment beyond 24 hours.

A unique strength of this study was the automated collection of detailed physiological recordings 24 hours per day; this approach allows for accurate descriptions in the variation of diaphragm electrical activity over time. It also presents important methodological challenges. First, signal acquisition was sometimes interrupted if patients were briefly disconnected from the Servo-i ventilator for suctioning or transport. Failure to resume monitoring resulted in the loss of several hours of recordings until study staff came by to check the acquisition. Over the course of the study, this seemed to occur less frequently as the clinical team became more familiar with the monitoring equipment. Second, Edi signal artefact resulting from possible displacement and malpositioning of the catheter presents an important challenge to accurately ascertaining the true rates of the various types of dyssynchrony. We employed a threshold cut-off of $\int_{\text{Edi}} (1.5 \mu V \cdot \text{seconds/breath})$ in order to differentiate between artefactual and actual neural inspiratory activity, a cut-off derived from the bimodal distribution of $\int_{\text{Edi}}$. Visual inspection of randomly selected tracings supported the validity of this cut-off, but it is possible that some Edi events below this threshold represented true neural inspiratory activity. Other authors reported that a
cut-off of 0.15 µV*seconds/breath was sufficient to remove signal artefact (219) but in the studies employed to derive this conclusion patients were continuously monitored by research staff and catheter position was carefully continuously checked and confirmed. The same authors reported that ‘subventilatory’ Edi activity is common; it is unclear whether such events constitute true ineffective efforts. Further work is required to clarify the physiological significance of Edi events in the range of 0.15-1.5 µV*seconds/breath.

Diaphragm activity and dyssynchrony rates varied widely over the course of ventilation and between individual patients. Some patients experienced prolonged periods of diaphragm inactivity or minimal activity. Importantly, we found a significant association between the rate of change in diaphragm thickness over time and the level of diaphragm activity, quantified by Edi. This provides important corroboration for a recently published study that ascertained diaphragm contractile activity using ultrasound to measure thickening fraction (202). Reverse triggering was the most common form of dyssynchrony, observed in up to 55% of recordings. Eccentric contractions were also very common. Importantly, just over half of eccentric contractions resulted from reversed triggering and the rate of eccentric contractions was strongly correlated with the rate of reverse triggering. Moreover, eccentric contractions were associated with lower neuromuscular coupling, supporting the hypothesis that eccentric contractions are injurious to muscle (although the validity of neuromuscular coupling as a measure of diaphragm function has not yet been firmly established, as outlined in Chapter 3). This observation raises the possibility that reverse triggering may contribute to diaphragm injury during mechanical ventilation by exposing the diaphragm to injurious loading conditions. If this were the case, neuromuscular blockade might be effective in preventing both lung injury (221) and diaphragm injury associated with mechanical ventilation, despite the risk of inactivity-induced atrophy (222). Further work is required to confirm this association and to confirm the hypothesized effects of eccentric contractions on diaphragm function during mechanical ventilation.

In this study, we employed measurements of neuromuscular coupling (NMC) to ascertain diaphragm function. Because NMC measurements require volitional inspiratory efforts against an occluded airway, NMC could not be ascertained on days when patient inspiratory effort was totally absent due to sedation or neuromuscular blockade. NMC varied significantly over time and it was difficult to identify a clear overall trend in the change in diaphragm function over time. Because NMC reflects the global efficiency of diaphragm contraction, it is sensitive to any
factor that impairs diaphragm performance whether changes in myofibrillar contractile function, myofibrillar cross-sectional area (i.e. atrophy/hypertrophy), or changes in end-expiratory lung volume. Muscle function may be affected by different factors at different time points during ventilation (i.e., low frequency fatigue/sepsis at an early stage; VIDD/medication effects at a later stage). Consequently, it may be difficult to draw inferences about the impact of diaphragm inactivity on diaphragm function based on trends in NMC over time. It is important to emphasize that the validity of NMC as a measurement of diaphragm function has not yet been fully established (see Chapter 3). Of note, median NMC varied with changes in diaphragm thickness (whether increased or decreased) in a pattern similar to that observed in a recently published cohort (202) (see Figure 5-7), although the association was not statistically significant. This coheres with (but cannot confirm) the hypothesis that changes in muscle thickness during ventilation impair diaphragm function and may therefore impede liberation from mechanical ventilation.

This study is subject to a number of limitations. It was designed to assess the feasibility of the proposed monitoring protocol; any inferences about the frequency of diaphragm inactivity or overactivity and the rate of dyssynchrony therefore require further confirmation in a larger cohort of patients. Definitive inferences about the impact of diaphragm inactivity on diaphragm function cannot be drawn, despite intriguing preliminary observations.

Second, while Edi was recorded hourly, Pdi was only measured once daily. Because the esophageal and gastric balloons deflate fairly quickly over a period of 1-2 hours, Pdi could not reliably be obtained without study personnel in attendance to confirm proper balloon inflation. Moreover, because the relationship between ∆Edi and ∆Pdi is variable depending on the inspiratory flow (21), the end-expiratory lung volume (21), and muscle function (20), the actual ∆Pdi remains somewhat uncertain. We employed 5 µV as a cut-off for minimal Edi activity based on previous observations of the range of Edi in spontaneously breathing ventilated patients. The validity of this cut-off was supported by our observation that ∆Pdi estimated by the Bellani method was typically very low at or below an ∆Edi level of 5 µV. The estimated ∆Pdi at ∆Edi of 15 µV varied widely, depending on the measured NMC value.

Third, due to the massive number of physiological recordings, individual inspection of every breath was not possible. Consequently, detailed observations about intrinsic PEEP, abdominal
muscle activity, and diaphragmatic expiratory braking activity could not be ascertained. Furthermore, signal artefact on the Edi tracing is an important concern because of ECG artefact breakthrough or interference from gastroesophageal sphincter activity. We chose a conservative threshold for ∫Edi based on its bimodal distribution. Further work is required to develop and validate reliable algorithms to discriminate between signal artefact and genuine neural inspiratory activity, if possible.

Fourth, the validity of neuromuscular coupling as a measure of diaphragm function remains uncertain (see Chapter 3). Observed associations between muscle thickness change or eccentric contraction rate and neuromuscular coupling must be interpreted cautiously.

In summary, we found that it is feasible to enroll patients in a longitudinal physiological monitoring study involving automated hourly recordings of diaphragm activity and ventilation. Moreover, we conclude that the acquired signals appear to provide meaningful insights about the rates of diaphragm inactivity and dyssynchrony. Our preliminary findings corroborate recent reports of the impact of diaphragm inactivity on muscle thickness and suggest that eccentric contractions may be injurious to diaphragm function.
Table 6-1. Dyssynchrony classification system

<table>
<thead>
<tr>
<th>Type of Dyssynchrony</th>
<th>Definition</th>
<th>Comments/Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double triggering</td>
<td>• Two pneumatic events occurring within a single neural event</td>
<td>• Standard definition employed in published literature (108)</td>
</tr>
</tbody>
</table>
| Reverse triggering           | • Neural Edi onset occurs at least 200 msec after onset of mechanical insufflation  
• Neural phase angle is greater than 30 degrees  
• Ventilator driving pressure (peak pressure – PEEP) is greater than 8 cm H₂O  
• Neural Edi exceeds artefact threshold (1.5 µV * seconds)  | • Phase angle criterion required to exclude some breaths with delayed EMG onset (occurring when patient is on CPAP)  
• Driving pressure criterion required to exclude some breaths with delayed EMG onset (occurring when patient is on CPAP)  
• Artefact threshold employed to increase probability that reverse triggered neural event is associated with a mechanical contraction |
| Uncoupled neural events*     | • Neural Edi offset occurs before onset of mechanical insufflation         | • Standard definition employed in published literature  
• Artefact threshold employed to increase probability that reverse triggered neural event is associated with a mechanical contraction |
| Eccentric contractions       | • Neural inspiration begins within 500 milliseconds of offset of mechanical insufflation  
• Neural inspiration begins at least 500 milliseconds before next mechanical insufflation commences  
• Neural expiration begins at least 300 milliseconds after offset of mechanical insufflation  
• Exhaled tidal volume during mechanical expiration exceeds 100 cc  
• Neural Edi exceeds artefact threshold (1.5 µV * seconds)  | • Neural inspiratory timing criteria designed to ensure that neural inspiration peaks near to peak expiratory flow  
• Neural expiratory timing criterion designed to ensure that the diaphragm is exposed to lengthening conditions for a sufficient period of time to cause injury (arbitrary cut-off based on personal judgment derived from inspecting tracings)  
• Exhaled tidal volume criterion employed to increase probability that diaphragm is lengthening while it contracts  
• Artefact threshold employed to increase probability that reverse triggered neural event is associated with a mechanical contraction |

*Commonly referred to as ineffective (wasted) efforts; renamed as uncoupled neural events given some uncertainty about the true degree of effort and the possibility that such events may represent signal artefact
### Table 6-2. Clinical Characteristics and Study Protocol Outcomes of Enrolled Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Duration of ventilation before enrolment (hours)</th>
<th>Duration of monitoring in study (hours)</th>
<th>Missing recordings (hours, % of monitoring duration)</th>
<th>NMC Measurements (n)</th>
<th>Protocol Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>Male</td>
<td>Intracranial hemorrhage (non-SAH)</td>
<td>12.0</td>
<td>165</td>
<td>69 (42%)</td>
<td>6</td>
<td>Completed protocol</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>Male</td>
<td>Subarachnoid hemorrhage</td>
<td>11.7</td>
<td>157</td>
<td>21 (13%)</td>
<td>4</td>
<td>Completed protocol</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>Female</td>
<td>Pneumonia</td>
<td>6.9</td>
<td>142</td>
<td>44 (31%)</td>
<td>5</td>
<td>Discontinued on day 6 due to self-extubation</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>Male</td>
<td>Pneumonia</td>
<td>11.2</td>
<td>3</td>
<td>0 (0%)</td>
<td>1</td>
<td>Extubated on study day 1</td>
</tr>
<tr>
<td>5</td>
<td>78</td>
<td>Female</td>
<td>Pneumonia</td>
<td>9.6</td>
<td>63</td>
<td>21 (34%)</td>
<td>2</td>
<td>Extubated on study day 4</td>
</tr>
<tr>
<td>6</td>
<td>53</td>
<td>Female</td>
<td>Intracranial hemorrhage (non-SAH)</td>
<td>5.0</td>
<td>103</td>
<td>28 (27%)</td>
<td>1</td>
<td>Died on study day 5</td>
</tr>
<tr>
<td>7</td>
<td>52</td>
<td>Female</td>
<td>Sepsis (non-pulmonary)</td>
<td>21.4</td>
<td>159</td>
<td>13 (8%)</td>
<td>5</td>
<td>Completed protocol</td>
</tr>
<tr>
<td>8</td>
<td>68</td>
<td>Male</td>
<td>Pneumonia</td>
<td>20.5</td>
<td>95</td>
<td>5 (5%)</td>
<td>5</td>
<td>Died on study day 5</td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>Female</td>
<td>Pneumonia</td>
<td>12.6</td>
<td>163</td>
<td>33 (20%)</td>
<td>6</td>
<td>Completed protocol</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>Female</td>
<td>Non-pulmonary sepsis</td>
<td>23.4</td>
<td>18</td>
<td>11 (65%)</td>
<td>0</td>
<td>Died on study day 2</td>
</tr>
</tbody>
</table>
Table 6-3. Frequency of Diaphragm Activity and Dyssynchrony in Enrolled Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Duration of Monitoring (hours)</th>
<th>Absent diaphragm activity</th>
<th>Minimal diaphragm activity</th>
<th>Reverse triggering</th>
<th>Double triggering</th>
<th>Uncoupled electrical activity</th>
<th>Eccentric contractions</th>
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<td>4.4% (2.3%-13.6%)</td>
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<td>0.4% (0%-6.7%)</td>
<td>12.3% (1.5%-15.9%)</td>
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*Recordings counted towards prevalence of each phenomenon were those recordings where diaphragm activity was either absent (no neural events) or minimal (Edi below 5 µV) or where at least 5 events in each recording met study criteria for the various types of dyssynchrony
Figure 6-1. Feasibility of screening and enrolment.
Figure 6-2. Histogram showing the distribution of $\int E_{\text{di}}$ per breath (integrated with respect to time from onset to end-inspiration) for all neural inspiratory events recorded in the study. The local minimum occurs at approximately 1.5 $\mu V$*seconds/breath (indicated by the dashed blue vertical line).
Figure 6-3. Variation in diaphragm electrical activity during invasive mechanical ventilation.

Local non-parametric (LOESS) smoothing was employed to evaluate trends over time. Grey shading indicates 95% confidence intervals.
Figure 6-4. Relationship between single daily measurements of diaphragm thickening fraction and mean Edi over 24 hours.

Diaphragm thickening fraction is correlated with the average level of diaphragm electrical activity over 24 hours ($R^2=0.09$, $p=0.04$). Grey shaded region indicates 95% confidence intervals.
Figure 6-5. Reverse triggering is associated with an increased risk of eccentric diaphragmatic contractions.

The frequency of eccentric contractions was strongly correlated with the rate of reverse triggering in a given recording ($R^2=0.72$, $p<0.0001$).
Figure 6-6. Variation in neuromuscular coupling of the diaphragm over time during mechanical ventilation.
Figure 6-7. The estimated range of Pdi levels associated with Edi measurements.

Pdi was estimated from Edi and NMC measurements according to the method described by Bellani and colleagues (64) to estimate the level of inspiratory effort corresponding to a given level of diaphragm electrical activity. At an Edi of 5 µV (cut-off for minimal diaphragmatic activity), Pdi was estimated to be in the range of 2-5 cm H₂O. At an Edi of 15 µV, estimated Pdi ranged from 5-15 cm H₂O. Grey shaded region indicates 95% confidence intervals.
Figure 6-8. Variation in end-expiratory diaphragm thickness over time during mechanical ventilation.
Figure 6-9. Impact of inspiratory effort on changes in diaphragm thickness over time.

The rate and direction of change in diaphragm thickness over time varies with the level of diaphragm contractile activity (ascertained by daily average Edi).
Figure 6-10. Relationship between changes in diaphragm thickness and diaphragm function.

Neuromuscular coupling varies with the magnitude of change in diaphragm thickness from baseline (n=24 measurements, p=0.24 for differences between groups).
Figure 6-11. Eccentric diaphragm contractions are associated with impaired diaphragm function.

Diaphragm function (measured by neuromechanical efficiency) is inversely correlated with the frequency of eccentric contractions ($p=0.05$, $R^2=0.10$). Grey shaded area indicates 95% confidence intervals.
Chapter 7
Synthesis and Future Directions

1 Discussion of Key Findings

The central hypothesis of this body of work is that diaphragm inactivity and/or injurious diaphragm contractile activity cause diaphragm injury and dysfunction during mechanical ventilation. To that end, we hypothesized that changes in diaphragm thickness and contractile activity could be ascertained by bedside ultrasound and that changes in diaphragm function could be detected by monitoring neuromuscular coupling. Here we will review and discuss the key methodological findings of this work (Chapter 3, 4, and 6), and then reconsider the main hypothesis in light of the results reported in Chapters 5 and 6.

As discussed at some length in Chapter 1, the difficulty of feasibly and reliably ascertaining diaphragm structure and function in mechanically ventilated patients has proven to be a major barrier to establishing the impact of mechanical ventilation on the diaphragm in ventilated patients and its significance for major clinical outcomes. In an effort to overcome these barriers, we examined two novel measurement techniques for bedside evaluation of diaphragm activity, structure and function.

1.1 Measurement Technique: Diaphragm Ultrasound

Ultrasound has become a standard clinical tool for bedside assessment and clinical decision-making in the intensive care unit. Ultrasound imaging of diaphragm thickness in the zone of apposition was first described over 20 years ago (31), initially as a research technique to characterize diaphragm contractile behavior in vivo and later as a clinical technique for monitoring diaphragm thickness and function in respiratory patients, primarily in the outpatient setting (69). Two very recent studies (published after the studies outlined in this work were initiated) suggest that ultrasound measurements of diaphragm thickening in patients subjected to a spontaneous breathing trial predict the outcome of extubation with reasonable sensitivity and specificity (73, 74). Our findings support the utility of this technique: we found that ultrasound
measurements of the thickness of the right hemidiaphragm were highly feasible and sufficiently precise to detect relatively small changes in diaphragm thickness within a patient over time (±10%). Furthermore, we found that quantifying the increase in diaphragm thickness during inspiration provides a feasible and valid means of quantifying patient inspiratory effort under mechanical ventilation. These findings constitute an important methodological advance – as discussed in Chapter 1, standard techniques for estimating inspiratory effort during mechanical ventilation are considerably more complex. Furthermore, muscle atrophy is a key morphological feature of ventilator-induced diaphragm dysfunction; ultrasound is therefore well-suited to detecting the development of disuse atrophy during mechanical ventilation.

Diaphragm ultrasound nevertheless exhibits some important limitations. The reproducibility coefficient of diaphragm thickening fraction (16-17%) is close to the normal value under resting tidal conditions. Thickening fraction is therefore not as sensitive to variation in diaphragm contractile activity as might be desired and this ‘noise’ makes it more difficult to differentiate between low levels of diaphragm contractile activity and complete quiescence. Measurements of left hemidiaphragm thickness proved technically challenging and are not feasible for research or clinical practice. This technique cannot therefore be employed to assess for isolated left hemidiaphragm paralysis (as may result from intrathoracic surgical procedures). The utility of diaphragm ultrasound as a continuous monitoring tool is uncertain; measurements require the availability of the clinical ultrasound device and can take up to 5 minutes to obtain, depending on patient positioning and co-operation. Our preliminary observations (reported in Chapter 6) suggest that a single daily measurement of diaphragm thickening fraction is correlated with average diaphragm electrical activity over 24 hours; whether repeated measurements could be used as a tool for titrating ventilatory support over minutes to hours or to automatically detect patient-ventilator dyssynchrony remains to be established.

1.2 Measurement Technique: Neuromuscular Coupling

The central challenge to assessing diaphragm muscle function in critically ill patients is obtaining maximal volitional inspiratory efforts (47). Many critically ill patients are sedated or delirious and acute brain injury (impairing consciousness and co-operation) is a common reason for mechanical ventilation; as a consequence, obtaining patient co-operation for respiratory muscle testing can be difficult in this setting. Magnetic twitch stimulation of the phrenic nerve
overcomes this difficulty but is not always well-tolerated and it can be challenging to achieve conditions necessary for valid measurements (54). Because the diaphragm generates pressure during its contraction in proportion to the magnitude of diaphragm electrical activity, measuring the ratio of Pdi to Edi (neuromuscular coupling) provides a means of assessing the mechanical performance of the muscle (20). Because neuromuscular coupling incorporates the electrical activity of the diaphragm, it is theoretically independent of volitional effort, assuming that transdiaphragmatic pressure and diaphragm electrical activity are linearly related. In Chapter 3, we set out to examine this assumption and to ascertain the normal variation of this measurement over time.

Our observations confirmed those of previous investigations: the relation between Pdi and Edi is consistently curvilinear, rather than linear. As a consequence, measured neuromuscular coupling decreases considerably as inspiratory effort increases. As discussed in Chapter 3, the curvilinearity appears to result from the combined effects of the flow-velocity relationship of muscle and changes in thoracoabdominal configuration during inspiration. Measurements obtained during airway occlusion (quasi-iso-lung volume conditions) in one of the healthy volunteers were considerably more linear, but the Pdi-Edi relation was curvilinear when measured during airway occlusions in mechanically ventilated patients, suggesting that diaphragm is shortening and changing shape to some extent during inspiratory efforts even in the absence of changes in lung volume. Neuromuscular coupling measurements were also more variable over time than twitch Pdi measurements. Based on these findings, the utility of neuromuscular coupling measurements to monitor diaphragm function during mechanical ventilation appears limited: neuromuscular coupling is confounded by the level of inspiratory effort during the measurement and the measurement is not sufficiently precise to detect small but potentially clinically relevant changes in diaphragm function.

Given the relatively consistent exponential relationship between Pdi and Edi described non-linear regression modelling, it is possible that a routine mathematical transformation could render the Pdi-Edi relation linear; however, it is unclear whether this computational approach can be generalized outside of the study population and further validation is required. An alternate approach to computing neuromuscular coupling by taking measurements of Pdi at a standard value of Edi also merits further investigation. Strategies to reduce the dependence of
neuromuscular coupling on inspiratory effort may also reduce day-to-day variability in this measurement.

1.3 Feasibility of Clinical Investigation of Diaphragm Function During Mechanical Ventilation

At the outset of this program, we recognized that mechanical ventilation likely only causes clinically important diaphragm injury in patients who remain alive and ventilator-dependent for at least 5-7 days. In light of this consideration, we employed two different approaches to identifying potentially eligible subjects for clinical studies of ventilator-induced diaphragm dysfunction. In the ‘inclusive’ approach, we enrolled any patient with acute respiratory failure within 36 hours of intubation (as per the study outlined in Chapter 5). The relative logistical ease of obtaining daily measurements of diaphragm thickness permitted this broadly inclusive enrolment strategy, which proved highly successful: in a period of approximately 48 weeks, we enrolled over 100 subjects. Approximately 50% of those patients remained alive and on the ventilator for at least 7 days. Moreover, diaphragm injury occurred at an earlier stage than expected (predominantly over the first 72 hours). Therefore, we were able to describe significant changes in diaphragm thickness over time in this population.

The second enrolment strategy (as described in Chapter 6) was more ‘specific.’ We specifically focused on patients believed to be at high risk of remaining alive and ventilator-dependent for at least 7 days using predictive models developed to render this prediction within 24 hours of intubation with optimal specificity. A ‘specific’ enrolment approach was employed in this study because of concerns about resource costs (in terms of time and equipment expenses) associated with the more invasive and rigorous nature of the proposed measurement protocol. However, the sensitivity and specificity of the overall inclusion and exclusion criteria were suboptimal and many eligible patients were missed because of the selective criteria. Indeed, it’s noteworthy that this approach enrolled a very similar proportion of patients remaining alive and on the ventilator for at least 7 days to the ‘sensitive’ approach described above. Therefore, the highly selective approach appears only to slow patient accrual.

Taken together, these findings suggest that a more broadly inclusive approach is more likely to be successful in terms of maximizing the enrolment rate without significantly increasing the
number of patients who are liberated from mechanical ventilation before developing (potentially) clinically important diaphragm injury.

The rate of missing hourly physiological recordings presents an important challenge to valid inferences about the time course and variation in diaphragm activity and patient-ventilator dyssynchrony over time. The principle reason for missing recordings was disconnection of ventilator from the computer monitoring system when the ventilator was placed on standby for endotracheal suctioning or patient transport. Educating the clinical team about the importance of resuming the monitoring system following such events may help to reduce the degree of missing data.

1.4 Impact of Mechanical Ventilation on the Diaphragm in the Clinical Setting

Is mechanical ventilation responsible for clinically significant diaphragm injury in the clinical setting? This is the central question we set out to examine in this body of work. As outlined in Chapter 1, experimental work in a number of animal models (rat, rabbit, mouse, pig) clearly demonstrated that mechanical ventilation can cause diaphragm injury (152, 153, 155, 223). Histopathological changes in diaphragm tissue obtained from mechanically ventilated patients similar to those obtained in animal experiments provided circumstantial evidence that ventilation-induced diaphragm dysfunction occurs in the clinical setting (7, 24, 26). However, the prevalence and magnitude of such injury was uncertain, and it remained unclear whether diaphragm disuse (or other putative mechanisms of ventilator-induced diaphragm injury) were specifically responsible for diaphragm dysfunction in the clinical setting, where many other factors predispose to muscle weakness (119).

Our findings provide important new evidence of the impact of mechanical ventilation on the human diaphragm in the clinical setting. Diaphragm thickness was observed to change rapidly over the first 72 hours following initiation of ventilatory support, whereas diaphragm thickness varied minimally over time in non-ventilated critically ill patients. Close to 50% of patients exhibited significant (>10%) reductions in muscle thickness, some losing as much as 30-40% of muscle thickness in this short period of time. Unexpectedly, a small subset of patients exhibited an increase in diaphragm thickness following initiation of ventilatory support. Maximal thickening fraction was lower in those patients with either increased or decreased diaphragm
thickness compared to patients with unchanged diaphragm thickness, suggesting that these patients had a relative impairment in muscle function, consistent with the hypothesis that these changes in muscle thickness documented by ultrasound signify muscle injury.

The precise nature of the injury giving rise to the observed changes in thickness remains uncertain. We did not obtain tissue specimens for histological examination, an important limitation of this work. Decreases in muscle thickness on ultrasound were correlated with myofibrillar atrophy in a recently reported porcine model of ventilator-induced diaphragm dysfunction (183). Furthermore, the significant relationship between changes in diaphragm thickness and the level of diaphragm contractile activity corroborates our suggestion that decreases in thickness are related to muscle disuse.

We conclude that diaphragm injury due to mechanical ventilation is frequent in the clinical setting.

1.5 Insights on the Mechanisms of Ventilator-Induced Diaphragm Dysfunction

1.5.1 Disuse Atrophy

Diaphragm thickness decreased most rapidly on days when patient inspiratory effort was lowest. This finding was clearly documented in the study outlined in Chapter 5, where the inspiratory thickening fraction of the diaphragm was employed as the measure of inspiratory effort. This was further corroborated by similar preliminary results presented in Chapter 6, where inspiratory effort was ascertained by diaphragm electrical activity. This consistent observation strongly suggests that diaphragm inactivity causes atrophy of the diaphragm during mechanical ventilation. Ventilatory strategies tailored to maintain some level of inspiratory effort may prevent diaphragm injury and accelerate liberation from mechanical ventilation.

1.5.2 Excess Pressure Loading

The cellular and histological basis for observed increases in diaphragm thickness is less certain but a number of findings provide clues as to the physiological significance of this phenomenon. First, increases in diaphragm thickness were associated with lower maximal thickening fraction, consistent with muscle injury rather than muscle hypertrophy. Second, increases in muscle
thickness were associated with high levels of inspiratory effort well above the normal range. This association raises the possibility that insufficient ventilatory support may contribute to load-induced muscle injury, similar to that described in previous animal and human studies. Load-induced injury results in muscular inflammation, potentially leading to edema and an increase in thickness (171, 185). As discussed in Chapter 5, traumatic diaphragmatic injuries are characterized by localized muscle thickening (224). Third, increases in muscle thickness were also associated with higher SOFA scores, suggesting that systemic inflammation, aggressive fluid resuscitation or other factors associated with multi-organ failure could contribute to an increase in diaphragm thickness. Additionally, these factors may interact, as systemic inflammation has been shown to render the diaphragm more sensitive to load-related injury (142). Further attention to the potentially deleterious effects of excess loading of the diaphragm and other respiratory muscles in acute respiratory failure patients is warranted, particularly in those with sepsis or multi-organ failure. Indeed, if inspiratory loads are frequently excessive during acute respiratory failure prior to intubation, one might hypothesize that earlier initiation of ventilatory support could prevent diaphragm injury and facilitate faster recovery. Alternately, suppression of inspiratory muscle effort – even neuromuscular blockade – may actually protect the muscle from load-induced injury in the context of sepsis and multi-organ failure.

1.5.3 Eccentric contractions

Eccentric (lengthening) contractions are known to cause acute injury to striated muscle, including the diaphragm (180). Relatively little attention has been paid to the possibility that the diaphragm may contract eccentrically under certain conditions during mechanical ventilation. As outlined in Chapter 6, we found that eccentric contractions of the diaphragm occurred frequently in two patients and were present in at least 10% of recordings in four others, suggesting that eccentric loading conditions are reasonably common during mechanical ventilation. Eccentric contractions were associated with reverse triggering, as the inspiratory effort resulting from mechanical insufflation of the chest was often sufficiently delayed to occur during the onset of inspiration. Neuromuscular coupling tended to be lower on days when eccentric contractions were more frequent, consistent with the hypothesis that such contractions are injurious to muscle function, though it is important to note that neuromuscular coupling requires further standardization before it can serve as a valid measure of diaphragm function. Thus our
preliminary findings suggest that eccentric diaphragm contractions warrant further study as a possible mediator of ventilator-induced diaphragm dysfunction.

2 Future Directions

Here I briefly outline persistent research questions and future lines of investigation.

2.1 Monitoring diaphragm structure

First, what is the precise cellular and histological basis for the observed decreases and increases in diaphragm thickness documented in these studies? While there are plausible explanations for these changes based on previous studies as discussed in Chapter 5, we did not obtain tissue samples for radiological-pathological correlation. Characterizing the histological and ultrastructural changes accounting for acute changes in diaphragm thickness would provide important mechanistic insights into the clinical importance of (and possible strategies for preventing) these changes in diaphragm thickness.

Second, can ultrasound be employed to identify changes in the diaphragm muscle aside from changes in dimension? Some recent studies have reported measurements of diaphragm echogenicity during mechanical ventilation. In quadriceps muscle, increases in echogenicity reflect myofibre necrosis (225). Describing the changes in tissue echogenicity during mechanical ventilation may further illuminate the mechanisms responsible for the development of diaphragm dysfunction and the factors associated with diaphragmatic inflammation and/or edema.

Third, can diaphragm function be assessed accurately by measuring the maximal thickening fraction? We showed differences in maximal thickening fraction in patients whose diaphragm thickness changed significantly during the first week of ventilation. As discussed in chapter 5, several studies have shown that maximal thickening fraction or spontaneous thickening fraction during a spontaneous breathing trial predicts MIP and weaning success (71, 73, 74). One recent study reported a correlation between maximal thickening fraction and twitch airway pressure (226). The literature therefore seems to support the validity of this approach to assessing diaphragm function but formal diagnostic validation studies are required. The validity of maximal thickening fraction measurements obtained under iso-lung volume vs. isotonic conditions merits exploration, as an airway occlusion (the Marini maneuver) may sometimes be required to stimulate maximal inspiratory effort.
Fourth, what is the clinical significance of changes in diaphragm thickness during the first days of mechanical ventilation? Our preliminary findings suggest that both increases and decreases in diaphragm thickness have a deleterious effect on muscle function. However, the impact of such changes on patient-centered clinical outcomes such as survival, duration of ventilation, risk of tracheostomy, and discharge location remain to be determined. Building on the observations reported in Chapter 5, we plan to describe the impact of changes in diaphragm thickness on clinical outcomes in a large multicenter cohort of mechanically ventilated patients.

2.2 Measuring and applying neuromuscular coupling

As mentioned above, further work is required to determine whether transforming Edi to compute NMC provides valid and consistent estimates of NMC. Because the transformation approach employed in Chapter 3 was developed empirically rather than from first principles, the distribution of parameters (slope and exponent) describing the Pdi-Edi curvilinear relation in the general population of mechanically ventilated patients remains uncertain. This computational approach requires validation in an independent cohort of patients. Enhancing the validity of NMC measurements by increasing the linearity of the Pdi-Edi relation would permit more accurate estimates of patient inspiratory effort from measuring NMC and Edi during NAVA. Such estimates might provide a sound approach for titrating NAVA in the clinical setting, and further investigation of this approach is warranted.

We examined the reproducibility of NMC in healthy volunteers. Although variability in NMC over time was much lower in healthy volunteers compared to mechanically ventilated patients, it was nevertheless considerably higher than the variability of Pdi,tw over time. Some of this variability may be related to lack of standardization of various determinants of the Pdi-Edi relation, such as effort, inspiratory flow, and diaphragmatic motion. Thus, additional work is required to determine whether measuring NMC during inspiration against an occluded airway and computing NMC at a pre-specified level of Edi (i.e. 5 μV) would further reduce NMC variability over time. Because we measured NMC in healthy volunteers using an apparatus other than the Servo-I ventilator, the mean values of NMC were quite different than those obtained in mechanically ventilated patients. The distribution and variability of NMC acquired by the Servo-I in healthy volunteers would provide useful insights into interpreting measurements of NMC in mechanically ventilated patients.
2.3 Intervening to prevent diaphragm injury

The results of the large clinical ultrasound study (Chapter 5) provide strong support for the hypothesis that diaphragm may be injured by disuse and/or excess loading during mechanical ventilation. Preliminary observations using near-continuous measurements of Edi and ventilator flow and pressure independently support these findings and suggest that eccentric contractions may also be an important mediator of injury—although further results are awaited. These findings have important implications for the prevention of diaphragm injury during mechanical ventilation. First, these results suggest that titrating ventilatory support to achieve some optimal level of patient inspiratory effort may mitigate VIDD. Such patient-specific titration would require a clinically feasible means of monitoring inspiratory effort. Several options are available for such monitoring; in particular, our findings suggest that ultrasound may provide such an approach. Devices for near-continuous monitoring of diaphragm thickening have been reported but require testing and validation in the adult ICU setting.

Second, it is uncertain whether it is always feasible to achieve near-normal levels of inspiratory effort during mechanical ventilation, particularly during the first 72-96 hours following intubation. Patients sometimes require heavy sedation or neuromuscular blockade to optimize hemodynamics and ventilation during severe hemodynamic shock or severe hypoxemic respiratory failure. Acutely ill patients often have high levels of respiratory drive due to high minute volume, high physiological dead space fraction, or from central effects of systemic inflammation; suppressing this drive to achieve lung protective ventilation often requires complete ablation of respiratory drive as most sedative agents primarily reduce respiratory rate rather than inspiratory effort (227). These concerns are theoretical and anecdotal; barriers to achieving muscle-protective ventilation in the clinical setting need to be systematically characterized. Furthermore, it is unclear whether inspiratory effort always responds to changes in ventilator support as expected; the brainstem medullary respiratory control centres receive input from cortical and subcortical centres, peripheral pulmonary and chest wall receptors and peripheral and central chemoreceptors. Titrating ventilatory support in combination with extracorporeal CO₂ clearance might permit ‘fine-tuning’ of inspiratory effort levels (228) but further work is required to characterize how ventilatory pattern responds to these interventions and whether they exert synergistic or additive effects.
Our preliminary observations suggest that eccentric contractions may be most frequent in the context of frequent reverse triggering and that, as expected, eccentric contractions are associated with impaired diaphragm function. While concerns have already been raised about the deleterious impact of reverse triggering dyssynchrony on VILI, our findings suggest that reverse triggering may also contribute to VIDD. Further efforts to detect and treat reverse triggering may therefore prevent diaphragm injury. This finding supports the importance of continuous monitoring of effort and synchrony. Indeed, given the potential for severe muscular injury related to repeated eccentric contractions, the use of neuromuscular blockade may actually protect the diaphragm from injury, but this hypothesis requires further exploration and confirmation. Caution is warranted about interpreting these findings given their preliminary nature.

Importantly, our proposed approach to ascertaining the burden of diaphragm inactivity, overactivity, and eccentric contractions proved feasible. We therefore plan to continue enrolment in this study with a number of design modifications. First, we have extended the enrolment window to 36 hours in an effort to enhance enrolment success. Second, in view of the uncertainty about the interpretation of neuromuscular coupling measurements, we have added additional measurements of respiratory muscle function at the completion of the monitoring protocol, including maximal thickening fraction and maximal transmit-brag attic pressure. Third, we plan to enroll 50 patients in order to permit more generalizable inferences about the frequency and pathophysiological impact of diaphragm inactivity, overloading, and eccentric contractions.

### 2.4 Treating established diaphragm injury

While preventing diaphragm injury is a critical priority, treating established diaphragm injury and dysfunction might help to improve outcomes for patients who are difficult to liberate from mechanical ventilation. Our findings may inform treatment strategies for patients with diaphragm dysfunction. First, ultrasound may provide a feasible means of diagnosing diaphragm dysfunction in difficult-to-wean patients, thereby identifying patients who could benefit from respiratory muscle rehabilitation. Future investigation is required to determine whether ultrasound-guided clinical evaluation of patients who are difficult to wean enhances the specificity of clinical interventions and accelerates liberation from ventilation. Second, the effect of inspiratory flow and diaphragm motion on the relationship between Pdi and Edi highlight the importance of the interaction between the diaphragm and other inspiratory muscle and expiratory
muscle groups. The impact of critical illness on the structure and function of these other muscles has not been well-described. Future work is required to develop ultrasound-based techniques to monitor these muscles and describe changes in structure and function during mechanical ventilation. Such data might assess efforts to prevent injury and weakness in the intercostal and abdominal muscles, muscles that are crucial to optimizing diaphragm function as well as cough and secretion clearance. Finally, comprehensive respiratory muscle rehabilitation strategies that aim to strengthen the diaphragm and abdominal muscles in difficult-to-wean patients need to be developed and systematically evaluated in clinical studies. Changes in diaphragm thickness and maximal thickening fraction may provide an important surrogate measure to track the effectiveness of respiratory muscle training protocols.

3 Summary

The studies outlined in this thesis have yielded a number of interesting and important physiological and clinical insights. Our findings open up many future avenues of investigation with the potential to significantly improve understanding of the physiological mechanisms at play in the outcomes of acute respiratory failure, and to improve the care and outcomes for mechanically ventilated patients around the world.
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Appendix 1: Evolution of Diaphragm Thickness During Mechanical Ventilation

1 Study Methods

1.1 Study Population and Setting

The study was conducted at three tertiary academic intensive care units in Toronto, Canada. The Research Ethics Boards at the University Health Network and St. Michael’s Hospital approved the study protocol. Potentially eligible patients were screened daily from Monday to Thursday in the ICU. Patients were eligible for enrolment if they had received invasive mechanical ventilation for acute respiratory failure for fewer than 72 hours, and after the first 53 subjects, enrolment was restricted to patients receiving mechanical ventilation for fewer than 36 hours. Patients were excluded if they were expected to be liberated from mechanical ventilation within 24 hours of screening or if they had received invasive mechanical ventilation for greater than 48 hours in the previous six months. We also enrolled a control group consisting of non-ventilated patients admitted to the ICU for any reason. Patients were enrolled within 36 hours of ICU admission. Patients were excluded as controls if they had received mechanical ventilation for more than 48 hours in the previous six months. Written informed consent was obtained prior to enrollment.

1.2 Diaphragm Thickness Measurements

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 midaxillary lines in the zone of apposition (32). Ultrasound probe placement location was marked to enhance day-to-day measurement consistency. Two separate consecutive measurements of end-expiratory diaphragm thickness were obtained at each measurement session (204). 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. Three trained observers performed the measurements.
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.

1.3 Diaphragm Function Measurements

At a later stage in the study, diaphragm function was measured in participants who were awake and breathing spontaneously after 1 week of mechanical ventilation or on the day of extubation (whichever came first). Diaphragm function was ascertained by measuring maximal inspiratory diaphragm thickening fraction during coached maximal inspiratory efforts (69, 74) and maximal inspiratory sniff maneuvers (43, 46) while in CPAP mode. For sniff maneuvers, intubated patients were coached to make a short, sharp sniff maneuver similar to the maneuver used for sniff nasal inspiratory pressure measurement (41). Intubated subjects were coached repeatedly until they could perform this intuitive inspiratory maneuver while generating flow through the endotracheal tube, rather than through the airway and nasopharynx as usual. For all maneuver types, evidence of maximal volitional effort was obtained by clinical examination of accessory muscle activation, facial expression and chest wall movement. Both maneuvers were repeated several times as needed until a consistent maximal value was obtained.

In participants who were unable to follow instructions, the endotracheal tube was transiently occluded to stimulate maximal inspiratory efforts as previously described by Marini et al. (38). The highest value obtained for thickening fraction during repeated inspiratory efforts was taken as the measurement of muscle function.

1.4 Risk Factors and Outcomes

Demographic data, comorbidities, admission diagnosis, and severity of illness (Severe Acute Physiology Score [SAPS] II) were collected at baseline. Ventilator settings, arterial blood gas tensions, and Severity of Organ Failure Assessment (SOFA) scores were collected on a daily basis for the duration of the study. For each study day, patients were classified as having severe sepsis if they satisfied 2/4 criteria for the Systemic Inflammatory Response Syndrome (SIRS)
and were receiving antibiotic medication. Data were collected on a daily basis for the first fourteen days of mechanical ventilation.

Ventilator driving pressure was ascertained from the set driving pressure (in pressure-targeted mode) or from the difference between peak and end-expiratory pressures (in flow-targeted modes).

1.5 Statistical Analysis

Data are expressed as mean (SD), median (interquartile range), and absolute and relative frequencies, as indicated. ANOVA or Kruskal-Wallis tests were used to compare continuous variables and chi-square tests were used for categorical variables.

Missing data for SOFA score (5% of observations) were imputed from the patient median value. Model variables and rates of missingness for each variable are listed in Table E1.

To assess the impact of pre-specified exposure variables on the change in diaphragm thickness over time (i.e. atrophy) we constructed a linear mixed model (primary analysis). The dependent variable was daily end-expiratory diaphragm thickness (expressed as the natural logarithm of the measurement to account for covariance with baseline values). Model exposure variables included pre-specified confounders and the interactions between pre-specified exposure variables and day of mechanical ventilation. The coefficients for these interaction terms represent the magnitude by which the exposures modified the variation in diaphragm thickness over time. A random effects term for was included to account for correlation within subjects.

The primary model results were corroborated by examining whether the magnitude of changes in diaphragm thickness over the first week of mechanical ventilation were related to the average diaphragm thickening fraction during the first three days of mechanical ventilation, or the proportion of the first three days spent in a controlled mode of ventilation. For these secondary models, the change in diaphragm thickness during the first week of ventilation was defined as the percentage change in diaphragm thickness from baseline to the final measurement made on or before day 8 of ventilation (i.e. if a patient was extubated on MV day 4, the percentage change in diaphragm thickness was quantified from baseline to day 4).
A second linear mixed effects model was constructed to evaluate the relationship between diaphragm contractile activity and pre-specified factors (Table E3). Details regarding model structure and the rationale for model covariate selection are provided below.

We examined whether model effects were stable across study epochs (2013 versus 2014). We examined model diagnostics to confirm satisfaction of statistical assumptions. Measures of influence and collinearity were examined and the effect of removing highly influential data points on model effects was assessed.

We planned to enroll 120 mechanically ventilated patients and 10 control patients. These sample size estimates were based on the following considerations: first, the linear regression sample size requirement was computed assuming an $R^2=0.10$ (estimated from the correlation between natural logarithm of diaphragm thickness and time in the preliminary dataset), beta=0.1, and alpha=0.05; this gives an expected sample size of 100. Second, given that significant changes in diaphragm thickness were expected to require at least 4-5 days to manifest and given the difficulty of predicting duration of mechanical ventilation and expected early completion of the study in some subjects, we decided that it was prudent to plan to enroll more patients than required. Third, we planned to implement a model with 10 predictors (7 covariates, 3 interactions) and we conservatively wanted to include at least 10 subjects per predictor. We planned to enroll 10 control patients based on clinical judgment about the number of subjects required to demonstrate stable thickness over time.

All statistical analyses were conducted using R software, version 3.0.2 (www.r-project.org).

2 Linear Mixed Model of Diaphragm Thickness

Dependent Variable

End-expiratory diaphragm thickness (natural logarithm of measured value – employed to account for covariance with baseline values of diaphragm thickness within patients)

Fixed Effects

Day of mechanical ventilation

Diaphragm thickening fraction (%)
Age (years)
Sex
Sepsis
SOFA score
SAPS II score (baseline value)

**Interactions**

Diaphragm thickening fraction * day of mechanical ventilation
Sepsis * day of mechanical ventilation
SOFA * day of mechanical ventilation

**Random Effects**

Subject

### 2.1 Pre-specified Rationale for Model Variable Selection

Day of mechanical ventilation – this variable was employed to assess the effect of time on diaphragm thickness (i.e. to establish the magnitude and rate of atrophy)

Diaphragm thickening fraction – this variable was employed as a surrogate for inspiratory diaphragm activation during mechanical ventilation, a measure of muscle activity

Age – this variable was deemed *a priori* to be a potentially important determinant of diaphragm thickness and atrophy

Sex – this variable was deemed *a priori* to be a potentially important determinant of diaphragm thickness and atrophy
Sepsis – based on previous observations regarding the effect of sepsis on muscle function and injury during critical illness (28, 75, 229), this variable was deemed *a priori* to be a potentially important determinant of diaphragm thickness and atrophy.

Daily SOFA score - based on previous observations regarding the effect of multiple organ failure on muscle function and injury during critical illness (207), this variable was deemed *a priori* to be a potentially important determinant of diaphragm thickness and atrophy.

SAPS II score – since severity of illness is associated with greater diaphragm inactivity, potentially more significant muscle injury (56) and a longer duration of ventilation, this variable was deemed *a priori* to be a potentially important determinant of diaphragm thickness and atrophy.

Diaphragm thickening fraction * day of mechanical ventilation – since multiple previous laboratory models suggest that disuse is an important mechanism of diaphragmatic atrophy (152, 153, 184), we planned to examine whether diaphragm thickening modified the effect of time (i.e. day of mechanical ventilation) on diaphragm thickness.

Sepsis * day of mechanical ventilation – since multiple previous studies suggest that sepsis mediates diaphragmatic and peripheral muscle injury (28, 56, 145), we planned to examine whether sepsis modified the effect of time (i.e. day of mechanical ventilation) on diaphragm thickness.

SOFA score * diaphragm thickening fraction – since a previous seminal study linked multiple organ failure to peripheral muscle atrophy during critical illness (207), we planned to examine whether variation in the daily SOFA score modified the effect of time (i.e. day of mechanical ventilation) on diaphragm thickness.

### 3 Mathematical Coupling: Sensitivity Analysis

The primary regression model in this study included the term for end-expiratory diaphragm thickness on both sides of the equation:

1. \( TF_{di} = (T_{di,ei} - T_{di,ee})/T_{di,ee} \)

2. \( T_{di,ee} = Age + Sex + SAPSII + SOFA + Sepsis * MV.Day + TF_{di} * MV.day \)
where TFdi = diaphragm thickening fraction during inspiration, Tdi,ei = end-inspiratory diaphragm thickness, Tdi,ee = end-expiratory diaphragm thickness

Consequently, it is possible that any relationship between the dependent variable and Tdi,ee may result from mathematical coupling (230). Direct coupling is only a concern insofar as Tdi,ei and Tdi,ee are independent of one another (231). However, given that they are physiologically coupled (since percentage change in diaphragm thickness correlates with inspiratory effort, patients with thicker diaphragms will exhibit greater end-inspiratory thickness for a given level of inspiratory effort) we expect a high degree of correlation between Tdi,ee and Tdi,ei. This greatly mitigates any concern about mathematical coupling. However, because Tdi,ei and Tdi,ee are correlated, random error in the Tdi,ee term could mathematically couple the relationship between TFdi and Tdi,ee (231).

To assess the probability that mathematical coupling due to random measurement error accounts for the interaction between TFdi and time demonstrated in the present study, we simulated the model results assuming that Tdi,ee and TFdi are randomly distributed independent variables (with no real world association).

(3) Tdi,ee = TFdi * time; random effect = subject
(4) (Tdi,ee + Error) = (TFdi + TFdi.Error) * time; random effect = subject

where:

Tdi,ee is a random normally distributed variable with mean 2.0 mm, SD 0.8 mm

It was then transformed as a percent of baseline value for each subject

TFdi is a random normally distributed variable with mean 0.2, SD 0.1

time is a consecutively ordered ordinal variable ranging from 1 to 5 days

Error is a random normally distributed variable with mean 0.0 mm, SD 0.1 mm

(based on the Bland-Altman limit of agreement of 0.2 mm, see Goligher et al. (204))
TFdi.Error is the alteration in computed TFdi resulting from the addition of the Error term to Tdi,ee

Subject is the term included to describe the correlation between diaphragm thickness values within each study subject

The simulation produced the following two datasets:

We then constructed linear mixed models as described in equations 3 and 4 for the randomly derived datasets with and without the random error term. Our goal was to compute by simulation the proportion of statistically significant findings occurring from purely random data (where no true association exists) due to mathematical coupling.

The impact of the addition of the error term on the coefficients and statistical significance of the model relationships was evaluated by simulation involving 1000 repetitions. During each cycle, model coefficients were computed from a randomly generated dataset of 7 observations for 50 subjects for the models described in equations (3) and (4).

**Table.** Frequency of random statistical associations in simulated linear mixed models

<table>
<thead>
<tr>
<th>Simulated dataset</th>
<th>Regression Model Term</th>
<th>Proportion of p-values below 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>No error term – Equation (3)</td>
<td>TFdi</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>TFdi*time</td>
<td>12%</td>
</tr>
<tr>
<td>Error term included – Equation (4)</td>
<td>TFdi</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>TFdi*time</td>
<td>8%</td>
</tr>
</tbody>
</table>
If anything, the inclusion of random measurement error appears to mitigate against chance statistical associations due to mathematical coupling. Because the simulated frequency of significant p-values was close to the significance threshold of 5%, we conclude that mathematical coupling is unlikely to produce false positive statistical significance in this model design.

4 Variability of Diaphragm Thickness over Time in Different Study Groups

Table E1. Variability of diaphragm thickness in study groups

<table>
<thead>
<tr>
<th>Variability characteristic</th>
<th>Control subjects (n=10)</th>
<th>Post-extubation (n=29)</th>
<th>During Ventilation (n=107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of variation (within subjects)</td>
<td>9.2%</td>
<td>10.6%</td>
<td>13.6%</td>
</tr>
<tr>
<td>Beta coefficient (for effect of time on thickness, average across subjects)</td>
<td>0.015 (p=0.44)</td>
<td>0.029 (p=0.16)</td>
<td>-0.028 (p&lt;0.0001)</td>
</tr>
<tr>
<td>Standard deviation of beta coefficient (for effect of time on thickness) between subjects</td>
<td>0.004</td>
<td>0.082</td>
<td>0.049</td>
</tr>
</tbody>
</table>

5 Linear Mixed Model of Diaphragm Thickening Fraction

Outcome

Diaphragm thickening fraction (%)

Fixed Effects

SAS score

Arterial pH

Arterial pCO₂

Ventilator mode category (control vs. spontaneous)
Set driving pressure (cm H\textsubscript{2}O) [in case of volume-cycled ventilation, set driving pressure was taken as mathematical difference between peak driving pressure and PEEP]

Total respiratory rate (/min)

Tidal volume (cc/kg predicted body weight)

Positive end-expiratory pressure (PEEP, cm H\textsubscript{2}O)

**Random Effects**

**Subject**

**Pre-specified Rationale for Model Variable Selection**

1. Sedation Agitation Scale score (SAS) – level of arousal is a known physiological determinant of ventilatory drive
2. Arterial pH – acid-base status is a known physiological determinant of ventilatory drive
3. Arterial P\textsubscript{a}CO\textsubscript{2} – carbon dioxide tension is a known physiological determinant of ventilatory drive
4. Ventilator mode category – ventilatory drive is commonly perceived to be higher, in general, in patients receiving a partial assist mode of mechanical ventilation (i.e. pressures support) compared to those receiving assist-control mechanical ventilation (regardless of whether the mode is pressure targeted or volume limited)
5. Set driving pressure – increasing set driving pressure is associated with attenuated inspiratory muscle effort (232)
6. Total respiratory rate – respiratory frequency is associated with respiratory motor output and patients with higher respiratory rate are generally thought to have greater ventilatory drive during mechanical ventilation
7. Tidal volume – increasing tidal volume during pressure targeted ventilation can suggest an increase in ventilatory motor output
8. Positive end-expiratory pressure – higher values of PEEP result in greater end-expiratory intrathoracic volume and diaphragm lengthening. This may impact measurements of diaphragm thickening during mechanical ventilation.
Appendix 2: Predicting Risk for Prolonged Ventilation

1 Development of Algorithm to Predict Probability of Remaining Alive and on the Ventilator for at least 7 days

In order to maximize the number of patients enrolled in our longitudinal monitoring study who remained alive and ventilator-dependent for at least 7 days (such that they would complete the monitoring protocol), we undertook to develop a clinical prediction tool to help predict duration of ventilation at baseline.

We derived the predictive algorithm from the dataset described in the third International Study of Mechanical Ventilation (203). This prospective cohort study enrolled 8,151 subjects and followed them up for up to 28 days of mechanical ventilation until liberation from ventilation or death. Clinical and physiological characteristics collected in this study included demographics, baseline severity of illness, organ dysfunction, and admitting diagnosis. Mechanical ventilation characteristics and organ function variables were ascertained on a daily basis. Outcomes collected included death, duration of ventilation, extubation failure and tracheostomy.

Because the duration of ventilation is determined by two competing forces, recovery and death, and because severity of illness characteristics are related in opposite directions to these two forces, we judged that attempting to model the probability of remaining alive and ventilator-dependent for at least 7 days as a linear function of disease severity and patient characteristics would be unsuccessful. Accordingly, we chose a non-parametric modeling technique, Classification and Regression Tree (CART) analysis, to develop the predictive model (233).

We first examined the average duration of ventilation associated with various types of admitting diagnoses. Three categories of admitting diagnosis were associated with the highest probability of remaining alive and on the ventilator for at least 7 days: acute lung injury (acute respiratory distress syndrome and/or pneumonia), severe sepsis, and acute severe brain injury (Glasgow Coma Scale less than 9 following intracranial hemorrhage, ischemic stroke, and traumatic brain injury). Because the prognostic significance of various variables was likely to depend on the
admitting diagnosis, we elected to build separate classification and regression tree models in these three populations.

Model performance was assessed by computing the sensitivity and specificity of the model to predict outcome in the derivation cohort and by evaluating the percentage of patients who would be deemed eligible. To enable more rapid and feasible model-based predictions, SAPS II was excluded from the predictive models (as this variable requires knowledge of a number of physiological variables); model predictive performance was not significantly affected. We aimed to compute models with a positive predictive value of 70%, such that approximately 70% of patients enrolled in the study would be likely to complete the study protocol.

The final models are displayed in Figure E1. Model performance is reported in Table E2. All models achieved positive predictive values of close to 70% in the derivation cohort; sensitivity and specificity varied considerably between the models. Most patients intubated for acute lung injury diagnosis had a high predicted probability of remaining alive and ventilator-dependent for 7 days; a minority of patients with acute brain injury or severe sepsis were eligible for study inclusion based on their predicted probability of completing the study protocol.

These models were implemented for use in bedside prediction of duration of ventilation during assessment for study eligibility via a web-based computational module, available at www.paradigmstudy.ca.
Table E2. Recursive partitioning model performance

<table>
<thead>
<tr>
<th>Population</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Percent eligible</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALI</td>
<td>81</td>
<td>51</td>
<td>67</td>
<td>30</td>
<td>67</td>
</tr>
<tr>
<td>ABI</td>
<td>41</td>
<td>87</td>
<td>69</td>
<td>34</td>
<td>25</td>
</tr>
<tr>
<td>Sepsis</td>
<td>54</td>
<td>84</td>
<td>74</td>
<td>31</td>
<td>33</td>
</tr>
</tbody>
</table>
Figure E1. Classification and regression tree (CART) prognostic models to predict the probability of remaining alive and on the ventilator for at least 7 days in patients with acute lung injury (a), severe acute brain injury (b), and severe sepsis (c). Each node identifies the variable and cut-off employed for prediction. The boxes indicate the likely outcome (on or off vent), the probability of being either off or on the ventilator at day 7 (fractions), and the percentage of patients who fall within the relevant cut-off values to reach that probability.