The Effects of Positive End-Expiratory Pressure on the Position and Function of the Human Diaphragm

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Preface

Beste lezer,

Voor u ligt mijn afstudeerthesis van de master Technical Medicine, richting Medical Sensing & Stimulation, aan de Universiteit Twente. De afgelopen tien maanden heb ik stage gelopen op de afdeling Intensive Care Volwassenen in het VU Medisch Centrum te Amsterdam waar ik met veel plezier aan dit afstudeeronderzoek heb gewerkt. Vele mensen hebben bijgedragen aan de totstandkoming van deze thesis, die ik bij deze graag zou willen bedanken.

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Ik hoop dat u deze thesis met veel plezier zult doorlezen; ik heb er de afgelopen tien maanden in ieder geval met veel plezier aan gewerkt.

Vriendelijke groet,
Judith Elshof
List of Abbreviations

ARDS acute respiratory distress syndrome
ATP adenosine triphosphate
BMI body mass index
COR-Mid coronal mid plane
CT computer tomography
CV coefficient of variation
EAdi electrical activity of the diaphragm
ECG electrocardiography
EMG electromyography
FRC functional residual capacity
ICU intensive care unit
MRI magnetic resonance imaging
NIV non-invasive ventilation
NME neuromechanical efficiency
PEEP positive end-expiratory pressure
Pes esophageal pressure
Pga gastric pressure
SAG-L sagittal left plane
SAG-R sagittal right plane
SBT spontaneous breathing trial
SD standard deviation
# Contents

## Preface

## List of Abbreviations

## 1 Introduction

## 2 Study Objectives

- 2.1 Primary objectives
- 2.2 Secondary objectives

## 3 Outline

## 4 Clinical Background

- 4.1 Respiration
- 4.2 Mechanical ventilation
- 4.3 Diaphragm
- 4.4 Muscle physiology
- 4.5 Diaphragm weakness
  - 4.5.1 Disuse atrophy
  - 4.5.2 Excessive loading

## 5 Technical Background

- 5.1 Methods to assess the position of the diaphragm
  - 5.1.1 Ultrasonography
  - 5.1.2 Computer tomography
  - 5.1.3 Magnetic resonance imaging
  - 5.1.4 X-ray
  - 5.1.5 Fluoroscopy
- 5.2 Methods to assess the function of the diaphragm
  - 5.2.1 Ultrasonography
  - 5.2.2 Maximal (sniff) maneuvers
  - 5.2.3 Transdiaphragmatic pressure
  - 5.2.4 Diaphragm electromyography
  - 5.2.5 Neuromechanical efficiency
  - 5.2.6 Stimulation of the phrenic nerves

## 6 The Acute Effects of PEEP on the Diaphragm in Healthy Subjects

- 6.1 Introduction
- 6.2 Methods
Critically ill patients admitted to the intensive care unit (ICU) rapidly develop diaphragm weakness. It is associated with adverse outcomes, including prolonged duration of mechanical ventilation and higher mortality. To prevent or treat diaphragm weakness, understanding of the underlying mechanisms is essential. It has recently been suggested that positive end-expiratory pressure (PEEP) may contribute to diaphragm weakness. In mechanically ventilated patients, PEEP is used to prevent airway collapse and to maximize alveolar recruitment by preventing cyclic derecruitment during expiration. Especially in patients with acute respiratory distress syndrome, high levels of PEEP are needed to maintain oxygenation.

As PEEP increases the end-expiratory lung volume, it is thought that the diaphragm moves caudally and shortens in the zone of apposition. Furthermore, it is hypothesized that this diaphragm shortening leads to a decrease in force generating capacity, since the diaphragm no longer operates on its optimum length in the force-length relation. Muscles are capable of adapting in response to a change in force generating capacity. Therefore, the diaphragm shortening in response to mechanical ventilation with PEEP could be clinically relevant as the diaphragm is able to adapt structurally.

Lindqvist et al. are the first ones to study the effects of mechanical ventilation with PEEP on the diaphragm in both critically ill patients and rats. In both study populations, mechanical ventilation with PEEP led to a caudal displacement of the diaphragm. Furthermore, their study in rats showed that this displacement resulted in a reduced diaphragm muscle fiber length due to a reduction in the length of the individual sarcomeres, i.e. the smallest contractile units of a muscle fiber. After 18 hours, mechanical ventilation with PEEP led to adaptation of the diaphragm by absorbing sarcomeres in series; a mechanism referred to as longitudinal atrophy. Due to longitudinal atrophy, the sarcomeres are restored to their original, physiological length thereby optimizing the force-length relationship. For a schematic overview of these processes, see Figure 1.1A-C.

It is hypothesized that similar adaptation may occur in mechanically ventilated patients, especially if patients are ventilated for multiple days. This adaptation might lead to problems during a spontaneous breathing trial (SBT) since PEEP is abruptly removed. Consequently, the end-expiratory lung volume is reduced, which could lead to an overstretch of the newly-adapted diaphragm fibers. Stretched muscle fibers are not working at their optimal length on the force-length relation, thereby possibly contributing to diaphragm weakness, as depicted in Figure 1.1D.

The goal of this study is to evaluate the acute and long term effects of PEEP on the diaphragm position and function in humans. Accordingly, it is the first step towards understanding the role of PEEP on the diaphragm in mechanically ventilated ICU patients, hopefully leading to better understanding of diaphragm weakness in the ICU.
Figure 1.1: Schematic overview of the muscle fibers during mechanical ventilation with PEEP. A) Sarcomere and muscle length during normal breathing, depicted at end-expiration. B) The acute effects of PEEP on sarcomere and muscle: reduced fiber and sarcomere length. C) Long term effect of mechanical ventilation with PEEP: absorption of sarcomeres leading to a restored sarcomere length. D) During a spontaneous breathing trial, PEEP is removed, which leads to the stretch of diaphragm fibers beyond optimal sarcomere length.
2 | Study Objectives

2.1 Primary objectives

The primary goal of this proof-of-concept study is to evaluate the acute effects of PEEP on the position, length and contractility of the diaphragm in vivo. The hypothesis that mechanical ventilation with PEEP leads to a caudal displacement of the diaphragm is tested in both healthy subjects and in ICU patients. The hypothesized consequence of a decrease in length and force generating capacity is tested in healthy subjects by applying different levels of PEEP.

2.2 Secondary objectives

Throughout the study in healthy subjects, the effect of PEEP on tidal volume, respiratory rate, I:E ratio and minute volume is evaluated. Furthermore, a secondary objective is to evaluate the accuracy of ultrasound for measurements in diaphragm motion in comparison with magnetic resonance imaging.
This thesis focuses on the effects of PEEP on the diaphragm structure and function in vivo. In chapter 4, clinical background information is given to explain the basic physiology and anatomy to be able to understand the problem of diaphragm weakness in ICU patients. Methods to assess both the position and the function of the diaphragm are discussed in chapter 5. In chapter 6, the acute effect of PEEP on the diaphragm in healthy subjects are evaluated. The rationale and development of the clinical trial will be included in this chapter. Furthermore, preliminary data is shown and some trends are described. In chapter 7, the effect of PEEP on the position of the diaphragm in mechanically ventilated ICU patients is evaluated. This chapter is part of a recently published article in the American Journal of Respiratory and Critical Care Medicine (doi: 10.1164/rccm.201709-1917OC). The long-term effect of PEEP on the diaphragm in ICU patients is the subject of chapter 8. Due to the limited time of my internship, no patients are yet included in this study. Therefore, this chapter focuses on the rationale and study design to evaluate the effects of long term ventilation with PEEP. The last chapter, chapter 9, includes a general discussion of all what has been learned and future perspectives will be discussed.
4 | Clinical Background

4.1 Respiration

The primary goal of the respiratory system is to exchange oxygen and carbon dioxide between the atmosphere and blood. The respiratory system can accommodate to changing metabolic demands of the body, which are reflected by changes in PaO$_2$, PaCO$_2$ and pH. In this way, homeostasis can be achieved.

Lung volume is determined by the interaction between the thoracic wall and the lungs. The lungs have the tendency to collapse, while the thoracic wall has the tendency to pull outwards (elastic recoil). If these opposing forces are equal, normal lung volume at end-expiration (functional residual capacity: FRC) is maintained. The intrapleural space is the space between the parietal and visceral pleura and serves as the interaction between the thoracic wall and lungs. The pressure in this space, the intrapleural pressure, is less than barometric pressure since both the lungs and the thoracic wall pull away from the space.

The elastic recoil of the chest wall can be controlled using the muscles of respiration. The muscles of inspiration increase the elastic recoil of the thoracic wall by expanding the chest, thus making the intrapleural pressure more negative. The lungs passively expand due to the enhanced vacuum and thoracic volume increases. As a result, the alveolar pressure is decreased which leads to the inflow of air along the pressure gradient. Inspiration ends when the atmospheric and intra-alveolar pressures are equalized. The primary muscles of inspiration are the diaphragm and external intercostal muscles. The following accessory muscles can help during a forced inspiration: scalenes, sternocleidomastoids, neck and back muscles and the upper-respiratory-tract muscles. A quiet expiration is normally passive, which is ensured by the elastic recoil of the lung. During forced expiration, expiratory muscles come into play: abdominal muscles (internal and external oblique, recto-abdominal and transverse abdominal muscles), internal intercostal muscles and back muscles.

4.2 Mechanical ventilation

When respiration is insufficient, critically ill patients may be in need for mechanical ventilation, for example due to pneumonia, neuromuscular disorders or an acute exacerbation of chronic obstructive pulmonary disease. The earliest mechanical ventilators were negative pressure ventilators which simulated the decreasing pleural pressure during normal breathing. Nowadays, mechanical ventilation is done using positive pressure ventilation in which a positive pressure is delivered to the airway. Many different modes of mechanical ventilation exist, for example pressure-control, volume-control or pressure support ventilation. However, simply put, each mode is a modification of the manner in which positive pressure is given and the interplay between the mechanical ventilator and the patient’s own respiratory efforts.

In almost all ventilated patients, PEEP is given to prevent airway collapse and to maximize alveolar recruitment by preventing cyclic derecruitment during expiration. However, too high values of PEEP create alveolar overdistention, so setting PEEP correctly is crucial. Various methods exist to select the ideal level of PEEP, for example, methods based on the compliance of the respiratory system, oxygenation values or volume-pressure curves. In clinical practice, levels of PEEP mostly vary between 5 and 15.
cmH$_2$O. Taken the respiratory system’s compliance of a patient with acute respiratory distress syndrome (ARDS) and a healthy subject into account, a PEEP of 10 cmH$_2$O leads to a change in end-expiratory lung volume of approximately 380 ml and 1000 ml, respectively.\textsuperscript{11,12}

The process of discontinuing mechanical ventilation is called weaning. A common weaning strategy is to first set the mode of mechanical ventilation to a partially assisted mode. For example, it can be set to pressure support in which the patient triggers the ventilator and a certain level of pressure support is given at each inhalation. Secondly, the level of pressure support and PEEP is gradually decreased until low levels given (e.g. around 10 and 5 cmH$_2$O, respectively) and a spontaneous breathing trial (SBT) can be considered. During a SBT, the patient is disconnected from the mechanical ventilator and the endotracheal tube is attached to a humidification filter to which additional oxygen can be connected. It is possible perform a SBT with PEEP, however, in daily clinical practice, a SBT is mostly performed without PEEP to prevent respiratory insufficiency after extubation.\textsuperscript{10}

### 4.3 Diaphragm

The diaphragm is the main inspiratory muscle and forms a partition between the thoracic and abdominal cavity. During inspiration, the diaphragm contracts and the dome of the diaphragm moves in caudal direction and the dome flattens. This action increases the thoracic volume, which decreases the thoracic pressure after which air enters the lungs. The diaphragm is innervated by the phrenic nerves, originating from cervical nerves 3-5.\textsuperscript{9} The diaphragm consists of a peripheral muscular part which converges radially to the central tendon: a trefoil-shaped aponeurotic part. The muscular part of the diaphragm can be divided into three parts, although these parts are merged into one muscular sheet. The first part is the sternal part and is attached to the posterior parts of the xiphoid process. The second part is the costal part which forms the right and left domes of the diaphragm. The costal part of the diaphragm attaches to the internal surface of the inferior six costal ribs and their cartilage. The zone of apposition is the area of the diaphragm where it attaches to the rib cage. The lumbar parts form the so-called right and left crura which ascend to the central tendon. This part is attached to the three superior lumbar vertebrae and two ligaments.\textsuperscript{13} An inferior view of the diaphragm can be seen in Figure 4.1. The diaphragm consists of both slow-twitch and fast-twitch fibers. These fiber types are approximately equally present in the costal diaphragm in healthy subjects.\textsuperscript{14}

![Figure 4.1: Inferior view of diaphragm](image)
4.4 Muscle physiology

Since this study focuses on the diaphragm, some basic physiology about muscles will follow. Each muscle fiber contains myofibrils, which consists of repeating units, called sarcomeres. In turn, these sarcomeres consist of smaller filaments called myofilaments, which come into two types: the thin and thick filaments. Thin filaments primarily consist of actin, while the thick filaments primarily consist of myosin, see Figure 4.2. Thin and thick filaments and its connections to the so-called Z-disk are responsible for the striped appearance of skeletal and cardiac muscle.

Simplified, the myosin molecule consists of two heads which can form cross-bridges with the thin filaments. Each head has a binding site for both actin and adenosine triphosphate (ATP). These binding sites are crucial for muscle contraction: muscle contraction is a cycle of myosin heads binding to actin, distortion of this cross-bridge, and myosin head detaching from actin filaments. The source of energy for this cycle is the hydrolysis of ATP.

The overlap between thin and thick filaments determines the force-length relationship as shown in Figure 4.3. The optimal length of a muscle is near the normal muscle length. As muscle length increases, the Z disk and thus the actin filaments are pulled away from each other. This can eventually lead to a state in which the actin filaments are pulled beyond the end of the myosin heads. In this state, no cross-bridge formation can occur and no force can be developed. If the length of the muscle is decreased from its resting state, actin filaments can slide over each other and eventually the myosin filaments hit the Z disks. In this case, the overlap of actin and myosin filaments is not optimal and force decreases.

Several studies have been performed to determine the in vivo force-length relationship of the diaphragm, as the relationship depicted in Figure 4.3 is determined in vitro. In these studies, the pressure generated by the diaphragm decreased if lung volume increased, i.e. the length of the diaphragm decreased. Surprisingly, the optimum length of the diaphragm is not at the resting position of the diaphragm (functional residual capacity), but at residual volume or even lower. So, in contrast to the theoretical in vitro relationship, no descending limb is present in the in vivo force-length relationship of the diaphragm. The in vivo force-length relationship of the diaphragm created by Braun et al. can be seen in Figure 4.4, indicating the decrease in force-generating capacity due to an decrease in diaphragm length.
Figure 4.3: Theoretical in vitro length-force relationship of a sarcomere\textsuperscript{21}

This force-length relationship determines the ability for muscle to adapt to a change in length. For example, hamstrings can adapt to eccentric contractions (i.e. active lengthening) by changing the optimum length\textsuperscript{20}.

This adaptability is not specific to skeletal muscle; also the diaphragm is able to adapt. Farkas et al\textsuperscript{8} found that the length-tension curve of the diaphragm in emphysematous hamsters was displaced to the left due to hyperinflation. This reduction in diaphragmatic length was mainly due to a loss of sarcomeres.

Prezant et al\textsuperscript{7} investigated the adaptability of the human diaphragm in patients on continuous ambulatory peritoneal dialysis. In these patients, the abdomen is virtually always filled with dialysate, which leads to a decrease in functional residual capacity due to the increase in intra-abdominal pressure. They stated that the human diaphragm is be capable of adapting to this constant decrease in FRC by shifting the force-length relationship to the right.

4.5 Diaphragm weakness

Diaphragm weakness is highly prevalent in ICU patients: it is present in approximately 80\% of the prolonged mechanically ventilated patients\textsuperscript{22-24}. Risk factors include sepsis, sedation and denutrition\textsuperscript{2}.

Furthermore, mechanical ventilation is a major contributor to diaphragm weakness in the ICU. Loss of force-generating capacity of the diaphragm related to the use of mechanical ventilation is often referred to as ventilator-induced diaphragm dysfunction\textsuperscript{25}. Jaber et al\textsuperscript{26} found that diaphragm weakness rapidly occurs in critically ill patients on mechanical ventilation. They used the gold standard for determining diaphragm weakness: the twitch transdiaphragmatic pressure after magnetic stimulation of the phrenic nerves, which will be explained in chapter 5.

Ventilator-induced diaphragm dysfunction is caused by two different mechanisms. On one hand, excessive ventilatory support can lead to diaphragm inactivity and consequently, disuse atrophy. On the other hand, insufficient ventilatory support can lead to diaphragm injury due to excessive loading\textsuperscript{2}.
4.5. DIAPHRAGM WEAKNESS

4.5.1 Disuse atrophy
Levine et al.\cite{levine1} were the first ones to find histological evidence of diaphragm atrophy in 14 brain-dead donors. They found that 18-69 hours of complete diaphragm inactivity due to mechanical ventilation led to diaphragm atrophy and increased diaphragmatic proteolysis. Interestingly, this rapid atrophy was not found in pectoralis muscle indicating that the diaphragm is more sensitive to disuse. Hooijman et al.\cite{hooijman} obtained diaphragm muscle fibers from 22 critically ill patients who received mechanical ventilation and determined the size and contractile strength of the individual muscle fibers. They found that these fibers display atrophy and severe contractile weakness. Furthermore, the ubiquitin-proteasome pathway, an important proteolysis pathway, was activated in the diaphragm fibers of critically ill patients. Picard et al.\cite{picard} and Levine et al.\cite{levine1} found that mitochondrial dysfunction and oxidative stress play an important role in diaphragm atrophy respectively. However, these studies were performed in animals and brain-dead patients. In critically ill patients, Van den Berg et al.\cite{vandenberg} found that diaphragm atrophy is present in the absence of mitochondrial dysfunction and oxidative stress. The study of Goligher et al.\cite{goligher} found that disuse atrophy, demonstrated by a reduced diaphragm thickness with ultrasound, is found in patient with low respiratory effort and high ventilatory assist. They suggest that diaphragm disuse atrophy can be attenuated by titrating ventilator support to maintain adequate levels of respiratory effort.

4.5.2 Excessive loading
Laghi et al.\cite{laghi} investigated the rate of recovery after a fatigue protocol in 12 healthy subjects. They found a marked decrease in diaphragmatic contractility after this protocol, even after 24 hours, indicating load-induced diaphragm injury. Furthermore, Orozco-Levi et al.\cite{orozcolo} obtained samples of the diaphragm in patients undergoing surgery after a threshold inspiratory loading protocol was performed. They found significantly more sarcomere disruption in the diaphragm biopsies after the loading task, again indicating load-induced diaphragm injury. Goligher et al.\cite{goligher} found that excessive loading, demonstrated by an increased diaphragm thickness with ultrasound, was found in patients with high respiratory effort and low ventilatory assist. They suggest that excessive loading, next to disuse atrophy, can be attenuated by maintaining adequate levels of respiratory effort.

Diaphragm weakness, regardless of its cause, is associated with increased ICU mortality, difficulties in weaning and prolonged duration of mechanical ventilation.\cite{davis}

\[ \text{Diaphragm weakness, regardless of its cause, is associated with increased ICU mortality, difficulties in weaning and prolonged duration of mechanical ventilation.} \]
5  Technical Background

Certain techniques will be needed to prove the hypothesis that PEEP results in a caudal shift of the diaphragm, leading to a shortening and a decrease in its force generating capacity. First, imaging techniques are needed to quantify the change in position and length after PEEP application. Second, techniques are needed to quantify the change in functionality after PEEP application due to this change in diaphragm position and length. Many methods are currently used in the clinical practice to determine diaphragm position and function of which the most used methods will be listed below.

5.1 Methods to assess the position of the diaphragm

5.1.1 Ultrasonography

Ultrasound is often used in clinical practice to assess the diaphragm. Two different windows can be used, of which the subcostal window could be used to monitor the change in position for instance due to PEEP application. With a low-frequency (2-5 MHz) probe placed subcostally, the diaphragm can be monitored as a hyperechoic line using the liver or spleen as an acoustic window. In M-mode, it is often used to monitor diaphragm excursions and assess diaphragm functionality during spontaneous breathing. The subcostal window can be interesting for this study since it can also be used to quantify the displacement of the diaphragm dome after a change in PEEP level by comparing the end-expiratory position of the diaphragm before and after a change in PEEP level, see Figure 5.1.

Ultrasound is non-invasive, easy to use, safe, has low costs and is applicable at the bedside. However, quantifying movement of the left hemidiaphragm is often difficult since the bowel and gas interposition often hide the diaphragm. Furthermore, a major disadvantage of ultrasound is its two-dimensional view of the diaphragm. It is unknown how the diaphragm acts in response to PEEP. Theoretically it would be possible that one part of the diaphragm displaces differently to PEEP than another which could be missed with ultrasound.

5.1.2 Computer tomography

Computer tomography (CT) has been used in several studies to monitor the position and length of the diaphragm. Especially the study of Pettiaux et al. is interesting, since CT was used for three-dimensional reconstruction of the diaphragm. During the study, 20 coronal and 30 sagittal images were reconstructed. Silhouettes of the diaphragm contour in both the zone of apposition and in the dome were digitized from which a three-dimensional reconstruction was made, see Figure 5.2. They stated that CT is a reliable method to obtain accurate measurements of muscle length, surface area and shape. However, a major disadvantage is the exposure to radiation. Next to this, CT is rather expensive and not applicable at the bedside. Especially for mechanical ventilated patients, performing a CT scan is quite cumbersome.
5.1. METHODS TO ASSESS THE POSITION OF THE DIAPHRAGM

Figure 5.1: Ultrasonographic view of the diaphragm in a critically ill patient in the region of the liver dome, with B-mode image on the left and M-mode image on the right. The M-mode image shows the acute effect of a 10 cmH$_2$O PEEP change on the position of the diaphragm, measured at end-expiration (indicated by dashed line); note that in this patient PEEP was decreased from 12 to 2 cmH$_2$O, resulting in a cranial diaphragm displacement.

Figure 5.2: Three-dimensional reconstruction of the diaphragm using CT at functional residual capacity.
CHAPTER 5. TECHNICAL BACKGROUND

5.1.3 Magnetic resonance imaging

As with CT, magnetic resonance imaging (MRI) has already been used to make a three-dimensional reconstruction of the diaphragm.\textsuperscript{37,38} However, in contrast to CT, MRI does not lead to radiation-exposure. Furthermore, MRI can be used to make dynamic three-dimensional reconstruction of the diaphragm.\textsuperscript{39} In this way, the shape, length and motion of the diaphragm can be monitored during the respiratory cycle. Craighero et al.\textsuperscript{39} also concluded that the spatial and temporal resolution was sufficient for studying diaphragm dynamics. However, like CT, MRI is quite expensive and not applicable at the bedside.

5.1.4 X-ray

Since X-rays are part of the clinical practice in the ICU, they could be used to monitor diaphragm position. Chest X-rays have especially been used to monitor the length of the diaphragm.\textsuperscript{17,40,41} Furthermore, X-ray is applicable at the bedside and easy to perform. Disadvantages are the exposure to radiation and the two-dimensional view. Furthermore, only the right hemidiaphragm can be assessed, since the heart shadow often conceals the left hemidiaphragm.\textsuperscript{17}

5.1.5 Fluoroscopy

In clinical practice, fluoroscopy is used to diagnose diaphragm paralysis.\textsuperscript{12,43} With fluoroscopy, dynamic images of the diaphragm can be obtained during the breathing cycle using X-ray. Furthermore, multiple fluoroscopy could be obtained to create a three-dimensional reconstruction. Major disadvantages are its exposure to radiation and its poor accessibility.

All imaging techniques that could be used to monitor the change in position of the diaphragm and its advantages and disadvantages are summed up in Table 5.1.

5.2 Methods to assess the function of the diaphragm

5.2.1 Ultrasonography

As discussed earlier, diaphragm ultrasound is often performed in two windows. In the zone of apposition, a measure of the function of the diaphragm can be assessed. Using a high-frequency probe (10 MHz) located between the 8th and 10th intercostal space in the mid-axillary or antero-axillary line, the diaphragm can be seen as a less echogenic structure between the pleura and the peritoneum.\textsuperscript{33} With this window, the thickness of the diaphragm can be assessed and the thickening fraction can be calculated as a change in thickness between inspiration and expiration.\textsuperscript{44,45} This thickening fraction can be used to estimate the work of breathing, since a significant correlation was found between the thickening fraction and the diaphragmatic pressure-time product, which is a tool for quantifying respiratory muscle effort. Despite the significant correlation, the variation between thickening fraction values at a specific value of the pressure-time product was high.\textsuperscript{46} Therefore, using the thickening fraction as a measure for work of breathing has its limitations, since the exact amount of work of breathing at a given thickening fraction is highly variable. Ultrasound has its clear advantages: it is easy to perform, accessible, cheap and applicable at the bedside.

5.2.2 Maximal (sniff) maneuvers

A method to measure global respiratory muscle strength is a maneuver that measures the maximal inspiratory pressure. It can be measured using a hand-held device while the subject inspires maximally. Since this maneuvers require voluntary actions, the outcome is extremely dependent on the subject’s
### Table 5.1: Overview of the techniques to assess diaphragm position

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameter</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasonography</td>
<td>Motion and change in position</td>
<td>➢ Applicable at the bedside</td>
<td>➢ Two dimensional view</td>
</tr>
<tr>
<td></td>
<td></td>
<td>➢ Easy to perform</td>
<td>➢ Difficult to view left hemidiaphragm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>➢ Accessible and cheap</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>Position, length and shape</td>
<td>➢ Three dimensional reconstruction</td>
<td>➢ Exposure to radiation</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>➢ Expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>➢ Not applicable at the bedside</td>
</tr>
<tr>
<td>MRI</td>
<td>Position, length, shape and motion</td>
<td>➢ Three dimensional reconstruction</td>
<td>➢ Expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>➢ Dynamic imaging</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>➢ No radiation exposure</td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>Position and length</td>
<td>➢ Applicable at the bedside</td>
<td>➢ Exposure to radiation</td>
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<td>➢ Easy to perform</td>
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<td>Fluoroscopy</td>
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<td>➢ Dynamic imaging</td>
<td>➢ Exposure to radiation</td>
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ability to understand comments and perform the maneuver. Therefore, when low values are found, it is not known whether it is a result of either decreased respiratory function or poor technique/effort. Furthermore, subjects can be asked to perform maximal sniffs, which is defined as a short sharp sniff as hard as possible. Miller et al. found that sniff maneuvers are more reproducible than maximal inspiratory maneuvers. However, just like the maximal inspiratory pressure, the sniff maneuver is a voluntary test which means that the outcome is again extremely dependent on the subject’s ability to understand comments and perform the maneuver rather than diaphragm function.

Different pressures can be assessed during these voluntarily maneuvers. The mouth pressure is easily available and therefore the easiest to use. However, change in mouth pressure during a maximal maneuver is more a parameter for global respiratory function rather than diaphragm function, because pressure generation of other inspiratory muscles is also incorporated. Therefore, a more specific method to measure diaphragm function is needed: the transdiaphragmatic pressure.

#### 5.2.3 Transdiaphragmatic pressure

The transdiaphragmatic pressure (Pdi) is the pressure developed across the diaphragm and is a specific measure of the diaphragm’s muscle strength. It is defined as the difference between abdominal and intrapleural pressure, which can be estimated by gastric (Pga) and esophageal pressure (Pes), respectively. Therefore, it is assumed that changes in Pes or Pga induced by mechanism other than diaphragm contraction, for example the use of intercostal muscles, are uniformly transmitted across the diaphragm from the thorax to the abdomen, so that an increase in Pdi is the result of a contraction of the diaphragm. Pes and Pga can be measured with invasive balloon catheters inserted via the nose or mouth. Insertion of the catheters may cause discomfort for subjects, but the discomfort of this small catheter can be minimized by adequate practice and good-quality equipment. Pdi values are highly reproducible in subjects, but
values between subjects vary over a wide range. Another limitation is that interpretation of Pes, Pga and Pdi measurements are rather complex and are therefore rarely used in the routine clinical care.

5.2.4 Diaphragm electromyography

Next to the pressure that the diaphragm can develop, the electrical activity of the diaphragm can be monitored. More precisely, it is a measure of the spatial and temporal summation of action potentials from the recruited motor units. Provided that the neuromuscular transmission and muscle fiber membrane excitability are intact, the electrical activity of the diaphragm should be a valid reflection of phrenic nerve activity and therefore, the neural respiratory drive of the patient. The electrical activity of the diaphragm can be monitored non-invasively using surface electrodes around the chest, often referred to as sEMGdi. Furthermore, the electrical activity of the diaphragm can be measured using an invasive esophageal catheter inserted via the nose or mouth, which is referred to as EAdi. In contrast to the sEMGdi, the EAdi measured by a catheter is less affected by obesity and cross-talk signals from other muscles. The esophageal catheter possesses metal electrodes which are placed at the level of the crural diaphragm. Therefore, the EAdi recorded by an esophageal catheter is mainly from the crural part of the diaphragm and is criticized for not being representative of the diaphragm as a whole. However, electrical activity in the crural and costal parts of the diaphragm are closely correlated. The EAdi is only useful if the signal is recorded accurately. Typical artifacts include esophageal peristalsis, the ECG, electrode motion and power line artefacts. Using the correct computer algorithms, these factors can be controlled for. High EAdi levels represent high neural drive, whereas low EAdi values could indicate low neural drive but can also be the consequence of catheter malposition, malfunction of the neuromuscular junction or phrenic nerve or the use of neuromuscular blocking agents or sedatives. Another limitation is the need for inserting an esophageal catheter which may uncomfortable in non-sedated patients. However, most ICU patients require a nasogastric catheter for feeding, which are available with EMG electrodes to measure EAdi simultaneously.

5.2.5 Neuromechanical efficiency

Neuromechanical efficiency (NME) is the ratio between Pdi and EAdi, i.e. the ability of the diaphragm to convert neural input (EAdi) into mechanical output (Pdi). Its advantage is that it is not influenced by the subject’s load of breathing such as airway compliance and resistance. NME can be used as a parameter for the diaphragm’s muscle strength, therefore a decrease in NME indicates diaphragm weakness whereas an increase indicates recovery.

Pdi, EAdi and NME can be monitored during tidal breathing or during voluntarily maneuvers as discussed earlier. Since voluntarily maneuvers have their clear disadvantages, a more specific method can be used to assess diaphragm’s strength: stimulation of the phrenic nerves.

5.2.6 Stimulation of the phrenic nerves

Stimulation of the phrenic nerves is a non-voluntary method for the evaluation of diaphragm strength. Stimulation can be induced electrically or magnetically, but magnetic stimulation is preferred since it is easier, faster to apply and more comfortable for the subject. An electric current flowing in the coils of the magnetic stimulator creates a magnetic field orthogonal to the flow of the current. This magnetic field is able to easily penetrate body tissue, for example skin. The magnetic field induces a secondary electric current into the structures and can depolarize the phrenic nerves if the duration and amplitude of the induced current is sufficient. This depolarization causes a twitch of the diaphragm, which can be expressed as the twitch mouth pressure or twitch transdiaphragmatic pressure.
### Table 5.2: Overview of the techniques to assess diaphragm function

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameter</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Values in healthy subjects</th>
</tr>
</thead>
</table>
| Ultrasound                    | Diaphragm thickness (Tdi) and thickening fraction (TF) | ➢ Easy to perform  
➢ Accessible and cheap  
➢ Non-invasive  
➢ TF: measure of work of breathing | ➢ Wide range of normal values for both Tdi and TF  
➢ TF: High variability as a measure of work of breathing | Tdi at end-expiration: 1.7-3.3 mmHg  
TF from residual volume to total lung capacity: 21-78%                                              |
| Maximal (sniff) maneuvers      | Global respiratory muscle function (MIP) or diaphragm strength (sniff Pdi / Pdi,max) | ➢ Easy to perform  
➢ Accessible and cheap  
➢ MIP: Non-invasive | ➢ Voluntarily maneuver  
➢ Requires full cooperation | MIP > 80 cmH2O  
Sniff Pdi > 80 cmH2O for females  
and > 100 cmH2O for males  
Pdi, max > 60 cmH2O                                           |
| Transdiaphragmatic pressures   | Diaphragm strength (Pdi)                       | ➢ Specific measure for diaphragm strength  
➢ Available in real-time | ➢ Invasive  
➢ Technique and interpretation is complex | No normal values available                                                                                                                         |
| Diaphragm EMG                 | Neural drive (EAdi)                            | ➢ Available in real-time | ➢ Invasive  
➢ Presence of artefacts  
➢ Low values do not exclude high drive | No normal values available                                                                                                                         |
| Neuromechanical Efficiency    | Ability of the diaphragm to convert drive to strength | ➢ Available in real-time | ➢ Invasive  
➢ Technique and interpretation is complex | NME: 1.4 cmH2O/μV                                                                                                                                 |
| Magnetic twitch pressures      | Diaphragm strength (Pno,tw / Pdi,tw)           | ➢ Non-voluntarily maneuver  
➢ Pdi,tw: Invasive | ➢ Technically difficult  
➢ Pdi,tw: Invasive | Dependent on stimulation technique, but these values are probably normal:  
Pno,tw < -11 cmH2O  
Pdi,tw > 15 cmH2O                                                                 |

Stimulation should be supramaximal to gain valid information about the maximal strength of the diaphragm. Therefore, bilateral anterolateral stimulation is preferred over other magnetic stimulation methods (e.g. cervical stimulation) since reliable supramaximal twitch transdiaphragmatic values are obtained and less co-activation of muscles occurs. The great advantage of magnetic stimulation is that it requires no voluntary actions of the subjects. However, subjects do not always tolerate repeated magnetic stimulations and the technique requires expertise since it is technically challenging.

For an overview of all discussed techniques for evaluating of diaphragm function, see Table 5.2.
6 The Acute Effects of PEEP on the Diaphragm in Healthy Subjects

6.1 Introduction

It has recently been found that PEEP may contribute to diaphragm weakness, which is an important problem in the ICU. The study of Lindqvist et al. showed that mechanical ventilation with PEEP resulted in a caudal displacement of the diaphragm in both critically ill patients and rats, since PEEP increases end-expiratory lung volume. Furthermore, their study in rats showed that this displacement resulted in a reduced fiber and sarcomere length on the short term. Since these shortened muscle fibers were not operating at their optimal position on their force-length relationship, diaphragm fibers of long-term ventilated rats (18 hours) adapted by absorbing serially-linked sarcomeres. In this manner, the optimal length on the force-length relationship was decreased (i.e. the force-length was shifted to the left) thereby restoring the sarcomere length to their original, physiological length.

The aim of our study is to evaluate the acute effects of PEEP on the position, length and function (i.e. force generating capacity) of the diaphragm in vivo. This is the first step to evaluate whether adaptation of muscle fibers could also occur in the human diaphragm since adaptation will only take place if the diaphragm is positioned at a suboptimal length on the force-length relationship. It is hypothesized that the immediate caudal shift of the diaphragm as a result of PEEP causes a reduction in diaphragm fiber length. Since short muscle fibers are not working at their optimal length of their force-length relationship, it is hypothesized that the diaphragm can generate less force at high PEEP levels. This physiological proof of concept study is the first step towards understanding the role of PEEP on the diaphragm in humans, which could lead to better understanding of diaphragm weakness in mechanically ventilated patients in the ICU. In this manner, new strategies to prevent or treat diaphragm weakness may be established.

6.2 Methods

6.2.1 Study design and population

This study was conducted in collaboration between the department of Intensive Care Adults and the department of Radiology and Nuclear Medicine of the Amsterdam UMC, location VUmc, Amsterdam, the Netherlands. The study population consisted of healthy subjects older than 18 years. In total, 15 healthy subjects will be included. Exclusion criteria were symptoms relating to respiratory or cardiovascular disease, history of pneumothorax, obesity (BMI > 30 kg/m²), contraindications for the placement of a nasogastric tube and contraindications for MRI. The study consisted of two parts: 1) physiological measurements during non-invasive mechanical ventilation with PEEP and 2) MRI measurements during non-invasive mechanical ventilation with PEEP. The different parts of the study were not necessarily performed on the same day.
6.2. METHODS

The protocol was approved by the local ethics review committee (approval number: 2017-590). Written informed consent was obtained from the subjects prior to the start of the study.

6.2.2 Part 1: Physiological study

6.2.2.1 Screening and study set-up

Screening of the subjects was performed which included a medical history, physical examination and a ECG. After screening, two CE-certified nasogastric catheters were inserted nasally to measure the electrical activity of the diaphragm (EAdi-catheter, Maquet Critical Care, Solna, Sweden), and the esophageal pressure (Pes) and gastric pressure (Pga) simultaneously (Nutrivent, Sidam, Mirandola, Italy). Both catheters were positioned according to clinical protocol. Transdiaphragmatic pressure (Pdi) was defined as Pga-Pes. EMG surface electrodes were placed on the left parasternalis muscle, rectus abdominis muscle and the external oblique muscle to monitor activity of the accessory inspiratory muscles and expiratory muscle activation, since earlier studies showed that healthy subjects may try to counteract PEEP by using their abdominal wall muscles. Furthermore, accessory respiratory muscles could be activated due to an increased work of breathing. Flow was continuously measured with a pneumotachograph (Adult Flow Sensor, Hamilton Medical, Bonaduz, Switzerland). All signals were acquired with a CE-certified measurement set-up (BIOPAC MP160, BIOPAC Systems Inc., Goleta, California, USA).

6.2.2.2 Mechanical ventilation with PEEP

PEEP was applied while patients were ventilated in pressure support mode (SERVO-i, Maquet Critical Care AB, Solna, Sweden) using a non-invasive ventilation (NIV) mask. During all measurements, the fraction of inspired oxygen was set to 21% (ambient air) and no additional inspiratory support was given. Before starting the experimental protocol, the PEEP level was set to 5 cmH$_2$O on the ventilator so that the subject could get used to the sensation of mechanical ventilation with PEEP.

6.2.2.3 Magnetic Stimulation

Magnetic bilateral anterolateral stimulation of the phrenic nerves was performed to measure twitch Pdi using two figure-of-eight coils and MagStim 200 stimulators (MagStim Co., Whitland, Dyfed, UK) according to clinical protocol. Before the experimental protocol, supramaximal stimulation was assessed by leveling off twitch Pdi-values during a stepwise increase of the stimulator intensity (70-80-90-100% of maximal power output). On each power setting, two stimulations were made. During the experimental protocol, power output was increased with a further 10% to compensate for slight changes in the quality of the stimulus. At least 30 seconds were taken between stimulations to avoid potentiation.

6.2.2.4 Neuromechanical Efficiency

The neuromechanical efficiency (NME) of the diaphragm was assessed during an end-expiratory hold given at the ventilator. In this manner, the NME was assessed during an isovolumetric contraction.

6.2.2.5 Ultrasound

Diaphragm ultrasound was performed in both in the right subcostal region and the zone of apposition to measure diaphragmatic displacement and diaphragm thickness respectively (Sonosite, Bothell, WA, USA). Probe positions were marked on the skin.
6.2.2.6 Experimental protocol

At the baseline PEEP of 2 cmH\(_2\)O, three different measurement were performed. First, three twitch Pdi values were measured. Second, ultrasound was performed to assess diaphragm thickness. Third, three NME values were measured. After performing baseline measurements, the PEEP level was changed. During each change in PEEP level, subcostal ultrasound was performed. The caudal-cranial displacement of the diaphragm was calculated from a M-mode image directly on the ultrasound machine. The investigated PEEP levels were 5, 10 and 15 cmH\(_2\)O since these levels are often used in clinical practice. For each PEEP level, one or two short moment of PEEP administration were given (lasting for ±25 seconds), after which a longer moment of PEEP administration was delivered (lasting for ±15 minutes). The short moments of PEEP administration were given so that more ultrasound images could be made to assess the change of the diaphragm’s position. At the longer moments of 5, 10 and 15 cmH\(_2\)O PEEP administration, the same measurements as the baseline measurements were performed: three twitch Pdi measurements, ultrasound measurements and three NME measurements. After finishing the measurements at 15 cmH\(_2\)O, a PEEP level of 5 cmH\(_2\)O was applied again to evaluate whether measurements in the first long moment of 5 cmH\(_2\)O PEEP were comparable to the later application of this same PEEP level. Periods of 2 cmH\(_2\)O were given between different PEEP levels. An overview of the PEEP levels is given in Figure 6.1. All measurements were performed in supine position.

This part of the study took approximately 3 hours to perform. Ultrasound movies in the zone of apposition and all signals acquired by the measurements set-up were saved for offline analysis.

6.2.3 Part 2: MRI study

6.2.3.1 Study preparations

Before the MRI measurements, the subject was randomized to receive either 5, 10 or 15 cmH\(_2\)O of PEEP. A flexible tube (7mm ID) filled with oil was fixated around the costal margin of each subject to identify the origin of the costal diaphragm fibers. The subject took place in the supine position in the 1.5T MRI scanner (MAGNETOM Avanto, Siemens AG) with the NIV mask positioned and connected to the same mechanical ventilator as used for the physiological part of the study. Initially, the PEEP was set to the baseline value of 2 cmH\(_2\)O. Steady state free precession MRI was used with the following operating conditions: slice thickness of 15 mm, echo time of 1.01 ms, flip angle of 50 degrees, temporal resolution of 99ms and a repetition time of 84.7 ms for the sagittal planes and 99.2 ms for the coronal planes.

6.2.3.2 Experimental protocol

Real-time MRI measurements were started at the PEEP level of 2 cmH\(_2\)O and after 30 seconds the PEEP level was increased to 5, 10 or 15 cmH\(_2\)O, depending on the allocated PEEP level. This PEEP level was applied for 60 seconds after which the PEEP level was reduced to 2 cmH\(_2\)O. MRI measurements continued
for ± 60 seconds at baseline PEEP level. After the MRI data were saved, real-time MRI measurements were started again. The same protocol was performed, but images were made in another plane. Measurements took place in the following planes:

- Sagittal right (SAG-R) – through the top of the right diaphragm dome
- Sagittal left (SAG-L) – through the top of the left diaphragm dome
- Coronal mid (COR-Mid) - positioned at the top of the diaphragm dome in the sagittal right plane

In total, this part of the study took 1 hour to perform.

6.2.4 Data analysis

6.2.4.1 Physiological parameters

Measurement variables of the physiological part were analyzed offline in Matlab R2016a (The Mathworks, Natick, MA). Twitch Pdi-values were calculated as the difference in Pdi from baseline to peak value. The NME of the diaphragm was calculated as the difference in transdiaphragmatic pressure from baseline to peak value divided by the peak value of the electrical activity of the diaphragm (ΔPdi/EAdi). Mean values of three measurements per PEEP level were calculated for both the twitch transdiaphragmatic pressure and the NME. Furthermore, some basic respiratory variables were calculated on a breath-by-breath basis during the longer period of PEEP ventilation (15 minutes). Median values of the respiratory breath-by-breath measurements were calculated. We defined the following variables:

- EAdi amplitude, as the peak of the EAdi signal
- Tidal volume, as the integral of the inspiratory flow over time
- I:E ratio, as the ratio between the inspiratory and the expiratory respiratory phase (based on flow)
- Respiratory rate, as the number of breaths (based on flow) in one minute
- Minute volume, as the product of the respiratory rate and the tidal volume

Ultrasound movies were analyzed with DICOM software (Sante DICOM Viewer, Santesoft, Athens, Greece). The thickening fraction was defined as the end-inspiratory thickness minus the end-expiratory thickness divided by end-expiratory thickness. Mean values of three breaths at each PEEP level were calculated.

6.2.4.2 MRI parameters

MRI images were analyzed using Agfa Xero Viewer 8.0.1 software (Agfa HealthCare, Mortsel, Belgium). For the length measurements, the diaphragm silhouette was divided in two zones: the dome (lung-apposed diaphragm) and the zone of apposition (rib cage-apposed diaphragm). The length of the diaphragm dome was measured by tracing the diaphragm contour. The length of the zone apposition was estimated with the use of a straight line. The lower limit of the zone of apposition was determined by the flexible tube filled with oil. Furthermore, the shape of the diaphragm was estimated by the K-dome parameter. K-dome is the diaphragm length divided by the diaphragm diameter where length is measured by tracing the diaphragm contour and diameter is measured as the shortest straight line between the begin and end of the diaphragm dome. For an overview of these parameters, see Figure 6.2. The caudal displacement of the diaphragm due to the application of PEEP was determined by calculating the difference between the position of the diaphragm dome at baseline PEEP and its position at the allocated PEEP level. The internal diameter of the thorax was determined at the level of the diaphragm dome at baseline PEEP. Each MRI parameter was measured for three breaths before and three breaths during PEEP application. All measurements were done at FRC, i.e. the resting position of the diaphragm. An overview of the primary study parameters for the study can be found in Table 6.1.
Figure 6.2: Representative magnetic resonance image in the mid coronal plane at functional residual capacity in subject 5. The length of the diaphragm dome (i.e. lung-apposed diaphragm) was measured by following the contour of the dome (green line). The length of the right and left zone of apposition (ZOA i.e. rib cage-apposed diaphragm) was estimated by straight lines (blue lines). The diameter of the diaphragm dome (i.e. shortest straight line) is depicted in red. The shape parameter K-dome was calculated by dividing the contour of the diaphragm dome by its diameter. Note the costal markers (white arrows) indicating the lower limits of the zone of apposition.

Table 6.1: Overview of the primary study parameters and their objective regarding the diaphragm at different PEEP levels

<table>
<thead>
<tr>
<th>Position</th>
<th>Length</th>
<th>Shape</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Twitch Pdi</td>
<td></td>
<td></td>
<td>✔️</td>
</tr>
<tr>
<td>NME</td>
<td></td>
<td></td>
<td>✔️</td>
</tr>
<tr>
<td>US - subcostal</td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US - zone of apposition</td>
<td></td>
<td></td>
<td>✔️</td>
</tr>
</tbody>
</table>

MRI = magnetic resonance imaging; Pdi = transdiaphragmatic pressure; NME = neuromechanical efficiency; US = ultrasound.
6.3 Results

Until now, six of the fifteen subjects were recruited. One subject was excluded due to the inability to position the nasogastric catheters. Baseline characteristics of the remaining subjects involved a male/female ratio of 2/3, mean age of 30.0±2.1 years, and a mean BMI of 23.7±1.1 kg/m². Due to technical difficulties, the electrical activity of the diaphragm of three subjects was not measured. MRI measurements were not performed yet in one of the five remaining subjects due to time constraints. Furthermore, one MRI scan was performed without the costal markers. Therefore, the length of the zone of apposition could not be measured in this subject. In the same subject, a few measurements in the coronal mid plane of the MRI scan were disregarded due to unclear diaphragm silhouettes. Furthermore, there were no serious adverse events during the experimental protocol.

6.3.1 Effect of PEEP on diaphragm position and length

Figure 6.2A shows the effect of a change in PEEP level on the position of the diaphragm measured by ultrasound. An increase in PEEP levels led to a caudal displacement of the diaphragm, varying from 0.5 cm when PEEP was increased from 2 to 5 cmH₂O, to 2.0 cm when PEEP was increased from 2 to 15 cmH₂O. Consequently, decreasing PEEP levels led to a cranial displacement of the diaphragm. Figure 6.2B shows the effect of PEEP on diaphragm thickness. A trend towards a thicker diaphragm at higher PEEP levels is seen. MRI measurements can be found in Table 6.2. It can be seen that PEEP led to a caudal displacement of the diaphragm, varying from 2 mm at a 2 to 5 cmH₂O change in PEEP level to 22 mm at a 2 to 15 cmH₂O change in PEEP level. Furthermore, an increase in PEEP level led to decrease in the length of the zone of apposition for all subject, varying from 2 to 50%. See Figure 6.3 for an example of a representative subject. The length and shape of the diaphragm dome did not change uniformly as a result of a change in PEEP level. The maximal increase in diaphragm dome length was 7% during a maximal PEEP increase to 15 cmH₂O. Furthermore, the thorax diameter increased with maximal 4% during a change in PEEP from 2 to 15 cmH₂O.
Figure 6.3: Representative magnetic resonance image in the left sagittal plane at functional residual capacity in subject 1 at two different PEEP levels. On the left, a PEEP level of 2 cmH\textsubscript{2}O is depicted, whereas on the right a PEEP level of 15 cmH\textsubscript{2}O is depicted. The diaphragm dome is marked in green and the zone of apposition in blue. Note the caudal displacement of the diaphragm and the concomitant reduction in length of the zone of apposition as a result of an increase in PEEP.

6.3.2 Effect of PEEP on diaphragm function

6.3.2.1 Twitch transdiaphragmatic pressure

The stimulator intensity was set to 100\% of maximal power output in most subjects; in one of the subjects a power output of 90\% was sufficient for supramaximal stimulation. Magnetic stimulation was well tolerated by all subjects. Figure 6.3 shows the effect of PEEP on the twitch pressures in one representative subject; twitch transdiaphragmatic pressure decreased if higher levels of PEEP were applied. Data of all subjects can be found in Figure 6.4. A trend towards lower transdiaphragmatic pressure during higher PEEP levels can be seen.

6.3.2.2 Additional functional measurements

An overview of the effects of PEEP on all functional measurements can be found in Table 6.3. The NME showed a trend of decreasing values when higher levels of PEEP were applied, but only two subjects were measured. The EAdi amplitude of the two subjects showed no analogous trend. The thickening fraction showed a trend towards higher values with increasing levels of PEEP with a thickening fraction of around 30\% at baseline PEEP to almost 80\% at the highest PEEP level. No increased activity of the additional respiratory muscles was seen with surface EMG except in subject 2 (data not shown). Individual data of the included subjects from the physiological part of the study is shown in appendix A.

6.3.3 Effect of PEEP on respiratory pattern

Table 6.4 shows the effects of mechanical ventilation with different levels of PEEP on the respiratory pattern. No clinical significant changes in the respiratory variables were found as a result of PEEP.
## 6.3. RESULTS

Table 6.2: Diaphragm position, length and shape measured with MRI at different PEEP levels. Both absolute values and change (%) in comparison with the baseline PEEP value of 2 cmH\textsubscript{2}O are depicted. Note that the length of the zone of apposition and a few parameters in the coronal images could not be measured in one of the subjects where a PEEP level of 5 cmH\textsubscript{2}O was applied.

<table>
<thead>
<tr>
<th>MRI parameter</th>
<th>Plane</th>
<th>Variable</th>
<th>PEEP 2 cmH\textsubscript{2}O</th>
<th>PEEP 5 cmH\textsubscript{2}O</th>
<th>PEEP 10 cmH\textsubscript{2}O</th>
<th>PEEP 15 cmH\textsubscript{2}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudal displacement</td>
<td>SAG-R</td>
<td>Change (mm)</td>
<td>-</td>
<td>1.9±1.2</td>
<td>14.3</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>SAG-L</td>
<td>Change (mm)</td>
<td>-</td>
<td>1.9±1.2</td>
<td>10.7</td>
<td>21.3</td>
</tr>
<tr>
<td></td>
<td>COR-Mid</td>
<td>Change (mm)</td>
<td>-</td>
<td>2.0±0.4</td>
<td>13.3</td>
<td>20.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left</td>
<td></td>
<td>2.5±1.1</td>
<td>8.3</td>
<td>20.3</td>
</tr>
<tr>
<td>Length zone of apposition</td>
<td>SAG-R</td>
<td>Absolute (mm) Anterior</td>
<td>63.2±13.9</td>
<td>47.3</td>
<td>66.3</td>
<td>39.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior</td>
<td>74.9±37.8</td>
<td>37.6</td>
<td>104.7</td>
<td>54.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%) Anterior</td>
<td></td>
<td>-15.8</td>
<td>-16.3</td>
<td>-26.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior</td>
<td>-</td>
<td>-5.5</td>
<td>-9.0</td>
<td>-22.4</td>
</tr>
<tr>
<td></td>
<td>SAG-L</td>
<td>Absolute (mm) Anterior</td>
<td>57.1±21.6</td>
<td>53.3</td>
<td>72.9</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior</td>
<td>63.3±32.4</td>
<td>30.5</td>
<td>90.6</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%) Anterior</td>
<td></td>
<td>-5.3</td>
<td>-7.8</td>
<td>-52.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior</td>
<td>-</td>
<td>-16.2</td>
<td>-8.8</td>
<td>-41.3</td>
</tr>
<tr>
<td></td>
<td>COR-Mid</td>
<td>Absolute (mm) Right</td>
<td>149.4±20.1</td>
<td>126.3</td>
<td>147.7</td>
<td>123.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left</td>
<td>116.9±21.7</td>
<td>97.4</td>
<td>132.0</td>
<td>84.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%) Right</td>
<td></td>
<td>-1.6</td>
<td>-12.2</td>
<td>-18.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left</td>
<td>-</td>
<td>-3.8</td>
<td>-9.2</td>
<td>-18.7</td>
</tr>
<tr>
<td>Length dome</td>
<td>SAG-R</td>
<td>Absolute (mm)</td>
<td>192.5±3.3</td>
<td>197.5±2.5</td>
<td>182</td>
<td>177.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-</td>
<td>1.6±0.6</td>
<td>-4.6</td>
<td>-6.6</td>
</tr>
<tr>
<td></td>
<td>SAG-L</td>
<td>Absolute (mm)</td>
<td>169.0±18.4</td>
<td>180.7±19.8</td>
<td>159</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-</td>
<td>1.2±2.0</td>
<td>-1.7</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>COR-Mid</td>
<td>Absolute (mm)</td>
<td>280.0±14.2</td>
<td>276.3</td>
<td>302.7</td>
<td>266.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-</td>
<td>-0.4</td>
<td>2.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>Shape diaphragm</td>
<td>SAG-R</td>
<td>Absolute (mm)</td>
<td>1.28±0.11</td>
<td>1.25±0.12</td>
<td>1.22</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-</td>
<td>1.6±1.6</td>
<td>-1.6</td>
<td>-4.2</td>
</tr>
<tr>
<td></td>
<td>SAG-L</td>
<td>Absolute (mm)</td>
<td>1.22±0.03</td>
<td>1.22±0.04</td>
<td>1.23</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-</td>
<td>0.4±1.2</td>
<td>-2.4</td>
<td>-0.8</td>
</tr>
<tr>
<td></td>
<td>COR-Mid</td>
<td>Absolute (mm)</td>
<td>1.13±0.01</td>
<td>1.14</td>
<td>1.15</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-</td>
<td>0.0</td>
<td>2.7</td>
<td>-1.8</td>
</tr>
<tr>
<td>Diameter thorax</td>
<td>SAG-R</td>
<td>Absolute (mm)</td>
<td>176.7±12.3</td>
<td>183.0±12.7</td>
<td>181.7</td>
<td>171.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-</td>
<td>0.8±1.5</td>
<td>1.9</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>SAG-L</td>
<td>Absolute (mm)</td>
<td>176.8±18.5</td>
<td>177.2±28.5</td>
<td>188.3</td>
<td>166.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-</td>
<td>-0.3±0.3</td>
<td>1.8</td>
<td>-0.2</td>
</tr>
<tr>
<td></td>
<td>COR-Mid</td>
<td>Absolute (mm)</td>
<td>275.9±19.0</td>
<td>278.5±19.1</td>
<td>296.0</td>
<td>259.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-</td>
<td>0.3±0.1</td>
<td>1.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

MRI = magnetic resonance imaging; PEEP = positive end-expiratory pressure; SAG-R = sagittal right plane; SAG-L, sagittal left plane; COR-Mid = coronal mid plane.
CHAPTER 6. ACUTE EFFECTS OF PEEP IN HEALTHY SUBJECTS

Figure 6.3: The effect of positive end-expiratory pressure (PEEP) on the esophageal (Pes), gastric (Pga) and transdiaphragmatic pressure (Pdi) after magnetic stimulation. Data is from one representative subject. An increase in PEEP level led to a decrease in twitch transdiaphragmatic pressure.

Figure 6.4: Effect of positive end-expiratory pressure (PEEP) on the twitch transdiaphragmatic pressure (Pdi) after magnetic stimulation of the phrenic nerves. A) Absolute values of twitch Pdi at different PEEP levels and (B) the change (in %) in comparison with the baseline PEEP level of 2 cmH₂O. Data are presented as mean±SD.
6.3. RESULTS

Table 6.3: Diaphragm functional measurements at different PEEP levels. Change is measured in comparison with the baseline PEEP level (2 cmH$_2$O). Note that the NME and EAdi amplitudes are measured in 2 subjects, in contrast to the twitch Pdi and thickening fractions where 5 subjects are measured. Data are presented as mean±SD.

<table>
<thead>
<tr>
<th></th>
<th>PEEP 2 cmH$_2$O</th>
<th>PEEP 5 cmH$_2$O</th>
<th>PEEP 10 cmH$_2$O</th>
<th>PEEP 15 cmH$_2$O</th>
<th>PEEP 5 cmH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Twitch Pdi (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (cmH$_2$O)</td>
<td>24.2±5.0</td>
<td>21.1±2.3</td>
<td>20.8±8.7</td>
<td>17.5±6.4</td>
<td>19.5±3.9</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-</td>
<td>-13.8±11.6</td>
<td>-14.8±24.1</td>
<td>-24.4±33.6</td>
<td>-17.9±18.4</td>
</tr>
<tr>
<td><strong>NME (n=2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (cmH$_2$O/μV)</td>
<td>0.71±0.18</td>
<td>0.55±0.27</td>
<td>0.52±0.16</td>
<td>0.48±0.02</td>
<td>0.58±0.01</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-</td>
<td>-36.6±34.6</td>
<td>-27.6±4.9</td>
<td>-30.8±14.3</td>
<td>-15.9±20.1</td>
</tr>
<tr>
<td><strong>EAdi amplitude (n=2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (μV)</td>
<td>24.8±13.2</td>
<td>17.9±9.0</td>
<td>21.3±0.3</td>
<td>27.1±0.1</td>
<td>21.3±6.2</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-</td>
<td>-27.4±2.3</td>
<td>-0.4±51.7</td>
<td>26.8±67.0</td>
<td>-7.9±24.0</td>
</tr>
<tr>
<td><strong>Thickening fraction (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute value (%)</td>
<td>29.6±13.4</td>
<td>42.6±19.7</td>
<td>33.8±23.5</td>
<td>79.3±46.4</td>
<td>43.2±27.8</td>
</tr>
</tbody>
</table>

PEEP = positive end-expiratory pressure; Pdi = transdiaphragmatic pressure; NME = neuromechanical efficiency; EAdi = electrical activity of the diaphragm.

Table 6.4: Respiratory variables for different PEEP levels. Both absolute values and change (%) in comparison with the baseline PEEP level (2 cmH$_2$O) are depicted. Data are presented as mean±SD.

<table>
<thead>
<tr>
<th></th>
<th>PEEP 2 cmH$_2$O</th>
<th>PEEP 5 cmH$_2$O</th>
<th>PEEP 10 cmH$_2$O</th>
<th>PEEP 15 cmH$_2$O</th>
<th>PEEP 5 cmH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiratory rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (breaths/minute)</td>
<td>12.8±3.4</td>
<td>12.1±2.9</td>
<td>12.3±3.0</td>
<td>13.2±4.1</td>
<td>13.5±3.6</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-</td>
<td>-5.8±5.6</td>
<td>-3.0±9.4</td>
<td>3.3±11.8</td>
<td>6.8±13.0</td>
</tr>
<tr>
<td><strong>Tidal volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (ml)</td>
<td>718±243</td>
<td>648±137</td>
<td>681±165</td>
<td>759±215</td>
<td>744±209</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-</td>
<td>-8.4±14.1</td>
<td>0.5±33.0</td>
<td>12.9±47.6</td>
<td>7.0±22.0</td>
</tr>
<tr>
<td><strong>Minute volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (L/s)</td>
<td>9.0±3.6</td>
<td>7.8±2.7</td>
<td>8.1±2.6</td>
<td>9.9±4.1</td>
<td>9.6±2.4</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-</td>
<td>-13.8±9.5</td>
<td>-4.7±26.4</td>
<td>15.2±42.2</td>
<td>12.5±19.6</td>
</tr>
<tr>
<td><strong>I:E ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.62±0.19</td>
<td>0.55±0.16</td>
<td>0.53±0.15</td>
<td>0.54±0.12</td>
<td>0.65±0.13</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-</td>
<td>-12.1±6.8</td>
<td>-12.5±15.1</td>
<td>-11.6±12.3</td>
<td>7.5±17.7</td>
</tr>
</tbody>
</table>

PEEP = positive end-expiratory pressure; I:E ratio = inspiratory time to expiratory time ratio.
6.4 Discussion

The present study is the first to evaluate the acute effects of PEEP on the position, length and function of the diaphragm in vivo. Preliminary data showed that PEEP resulted in a caudal displacement of the diaphragm in healthy subjects, measured with both ultrasound and MRI. Furthermore, PEEP led to a decrease in length of the diaphragm, mainly in the zone of apposition. In addition, a trend towards a reduction in diaphragm function was seen indicating that the diaphragm is operating at a non-optimal length on the force-length relationship as a result of PEEP. However, this proof-of-concept study is not yet completed; the sample size is limited to five subjects. Therefore, no statistical analysis were performed and no final conclusions can be drawn.

6.4.1 Effect of PEEP on diaphragm position and length

6.4.1.1 Diaphragm displacement measured with ultrasound

Ultrasound was used to evaluate the displacement of the diaphragm since it is accessible, non-invasive and easy to use. Figure 6.2 shows that mechanical ventilation with PEEP led to a caudal displacement of the diaphragm measured by ultrasound. The caudal displacement of the diaphragm as a result of an increase in PEEP was consistently larger than the cranial displacement as a result of a decrease in PEEP. This may be explained by the fact that the visualization of the diaphragm was limited during a reduction in PEEP since air of the intestines appeared in the field of view of the ultrasound image. It was hypothesized that the decrease in PEEP causes a similar but reversed displacement of the diaphragm than an increase in PEEP, which could be tested better with MRI than ultrasound measurements since ultrasound is limited in its 2-dimensional view. Furthermore, on average, it takes three breaths to create a new equilibrium after a change in PEEP level. Due to technical limitations of the ultrasound, diaphragm displacement can be calculated within a fixed time range of the M-mode. In subjects with a low respiratory rate, the displacement could be calculated after two breaths which might be an underestimation since a new equilibrium might not yet be reached.

A high intra-subject variation was seen in the displacement of the diaphragm with ultrasound (Figure A.1 of appendix A). This could be explained by the fact that it was difficult to place the M-mode of the ultrasound exactly perpendicular to the movement of the diaphragm. Therefore, these ultrasound images are of limited value.

6.4.1.2 Diaphragm displacement measured with MRI

MRI was used to monitor diaphragm motion at different PEEP levels because real-time images in multiple planes could be made in the absence of radiation exposure. MRI data as shown in Table 6.2 showed a caudal displacement of the diaphragm as a result of PEEP ranging from a few millimeters at low PEEP levels to more than two centimeters at high PEEP levels. This displacement was calculated as the difference between the position of the top of the diaphragm dome at different PEEP levels. In this manner, no differences in anterior or posterior sides are taken into account. Since the diaphragm displaces more posteriorly during tidal breathing than anteriorly, PEEP could also cause more displacement in the posterior region of the diaphragm. This could be evaluated by digitalizing the diaphragm contours at both PEEP levels and determining the difference between both positions at each requested region.

6.4.1.3 Comparison between ultrasound and MRI

Since ultrasound is often used in clinical practice, the accuracy of ultrasound for determining diaphragm displacement is of importance. Therefore, ultrasound data was compared to MRI data. The comparison between the displacement measured by both methods can be seen in Table B.1 of appendix B. It was
hypothesized that the displacement of the right dome in the coronal mid plane or in the sagittal right plane was most similar to the displacement measured by ultrasound since ultrasound was performed on the right side. In comparison with the MRI measurements in these planes, ultrasound seemed to overestimate the displacement for most subjects, but no conclusions can be drawn since only four subjects were measured by MRI.

6.4.1.4 Diaphragm length and shape

As a result of the caudal displacement, the length of the diaphragm in the zone of apposition was reduced, as shown in Table 6.2. Even during a minimal increase in PEEP from 2 to 5 cmH\textsubscript{2}O with a caudal displacement of only two millimeters, the length in the zone of apposition was reduced with maximal 16\%.

When PEEP is increased to 15 cmH\textsubscript{2}O, the length of the zone of apposition was decreased up to 50\%. The length of the diaphragm dome was barely decreased, which seemed logical since the diaphragm dome mainly consists of tendon, which is less compressible than the muscle in the zone of apposition. Therefore, the shape of the diaphragm dome barely changed as a result of PEEP. In theory, PEEP could lead to an increase in thoracic diameter thereby flattening the diaphragm dome without a reduction in diaphragm length. No relevant differences in thorax diameter were found, so this effect was less likely to occur. The trend towards increased end-expiratory thickness of the diaphragm at higher PEEP levels (Figure 6.2B) can be explained by the decrease in diaphragm length.

6.4.2 Effect of PEEP on diaphragm function

6.4.2.1 Twitch transdiaphragmatic pressure

Magnetic stimulation of the phrenic nerves was chosen as a primary functional measurements since it is a non-voluntarily test for diaphragm contractility and is thus effort independent. Furthermore, it the gold standard for determining diaphragm weakness and highly reproducible\textsuperscript{49} The transdiaphragmatic pressure was chosen since it is specific for the diaphragm in contrast to the esophageal pressure which is dependent on the accessory respiratory muscles\textsuperscript{42}

Figure 6.4 shows the effect PEEP on diaphragm function as measured by twitch P\textsubscript{di}. Despite the small sample size of 5 subjects, a trend towards less diaphragm contractility at higher PEEP levels was seen. This means that the diaphragm was likely to operate at a less optimal position on the length-force relationship, as supported by the decreased diaphragm length. As can be seen in Figure A.3 of appendix A, three of the five subjects exactly showed what was hypothesized: decreasing twitch pressures at higher levels of PEEP (see Figure 6.3 for a representative example). On the contrary, two of the subjects showed increasing levels of twitch pressures. In one of these subjects, subject number 2, PEEP was counteracted by the abdominal muscle activity (data not shown). This may explain the small caudal displacement of the diaphragm (see Figure A.1) and the fact that twitch pressures were not lower during the higher levels of PEEP. In the other subject that showed increasing twitch pressures with increasing levels of PEEP, extremely high variability in twitch pressures was seen which made it difficult to draw conclusions. The coefficient of variation (CV), defined as the standard deviation divided by the mean of all measurement at one PEEP level, was 21\% in this subject, in contrast to the mean CV of 13\% in the rest of the subjects. This high intra-subject variability may be explained by the occurrence of potentiation: an increase in contractility of a muscle as a results of previous contraction\textsuperscript{50}. However, all precautionary measures were taken to avoid potentiation: at least 30 seconds were taken between stimulations, the first stimulated was performed at least 20 minutes after the insertion of the nasogastric catheters, and magnetic stimulation was performed immediately after the increase to the PEEP level since an increased work of breathing could also cause potentiation. Furthermore, the position of the stimulation and the synchronization of the two stimuli were checked during the study protocol. Therefore, the high inter-subject variation cannot be explained. Even the CV of 13\% for the rest of the subjects is quite high in comparison with literature; Mills et al.\textsuperscript{60} found
a mean CV of 5.3%. However, this CV was found after performing ten measurements instead of three. To reduce the intra-subject variation, more stimulations could be performed. For example, five stimulations can be performed of which the three closest values are taken. Furthermore, it could be checked whether supramaximal stimulation is achieved by checking the levelling off of the twitch pressures during increasing levels of magnetic stimulation output, since supramaximal stimulation is crucial for reproducible values.

6.4.2.2 Neuromechanical efficiency

The NME was selected as a parameter for diaphragm function since it independent of the subject’s load of breathing (airway compliance and resistance).\textsuperscript{47} The EAdi signal, and thus the NME, of only two subjects was measured due to technical defects. The NME and especially the EAdi amplitudes showed high inter-subject variation (see Figure A.4 in appendix A) which makes interpretation of the absolute values more difficult. However, a trend towards lower NME values at higher PEEP levels was seen, which was particularly clear in the PEEP increase from 2 to 10 cmH\textsubscript{2}O and from 2 to 15 cmH\textsubscript{2}O. In one subject, decreasing EAdi amplitudes were found at higher PEEP levels while in the other subject increasing EAdi amplitudes were found (see Figure A.5 in appendix A). The latter seems more logical since it is thought that EAdi amplitudes would increase to compensate for the decreased efficiency and to restore Pdi values. More data is needed to draw conclusions of the effect of PEEP on the EAdi and NME.

NME data showed a large intra-subject variation (mean CV of 22\%). No studies are available on the repeatability of the NME based on the Pdi. However, a repeatability study with the NME based on the airway pressure was performed which is a good derivative for the NME based on the Pdi during an isovolumetric contraction.\textsuperscript{63} In this study, a great inter-subject variation (repeatability coefficient of 82\%) was found. However, filtered EAdi signals of the ventilator were used and it was not performed in healthy subjects.

6.4.2.3 Thickening fraction

Increased thickening fractions were seen during higher PEEP levels, especially during a PEEP of 15 cmH\textsubscript{2}O (Table 6.3). In other words, the effort of the diaphragm was increased, which may seem logical since it was hypothesized that the diaphragm is operating at a suboptimal length on the force-length relationship. However, it is difficult to translate the increase in thickening fraction to a measure of function, since no output (i.e. volume or pressure) was taken into account. It is more a measure of work of breathing rather than diaphragm function, although alternative measurements like pressure time product are superior in determining work of breathing.\textsuperscript{46}

High intra-subject variation was found in the preliminary data with a mean CV of 20\% (Figure A.6 in appendix A). This may partly be explained by limitations of ultrasound, such as its 2-dimensional view, limited resolution, and high operator-dependency.\textsuperscript{33} However, smaller intra-subject variations were seen during end-expiratory thickness measurements (Figure A.2 in appendix A), possibly indicating that the high variability cannot be explained by ultrasound itself and that the thickening fraction was genuinely variable in healthy subjects.

The PEEP level of 5 cmH\textsubscript{2}O was applied twice: at both the start and the end of the study protocol. In this manner, it could be tested whether potentiation occurred after application of the high PEEP level of 15 cmH\textsubscript{2}O. Furthermore, it could be tested whether the effects on the diaphragm function were reversible, which would support our hypothesis that a decreased length of the diaphragm as a result of PEEP caused an immediate effect on diaphragm function. Preliminary data suggested that the measurements at both moments of 5 cmH\textsubscript{2}O PEEP were comparable, indicating reversible effects of PEEP and no potentiation effect.
6.4. DISCUSSION

6.4.3 Effect of PEEP on respiratory pattern

The effect of PEEP on the respiratory pattern can be seen in Table 6.4. The respiratory rate did not alter substantially in these subjects. However, a trend towards lower I:E ratios was seen, which seems legitimate since PEEP hinders expiration. On average, tidal volume and minute volume increased with respectively 13% and 15%. However, both variables showed extremely high inter-subject variability so no conclusion can still be drawn. PEEP causes lung overdistention in healthy subjects and thereby increases physiological dead space ventilation. Therefore, minute volume could increase with increasing levels of PEEP to compensate for this increased dead space. This effect was seen in two studies evaluating the effect of PEEP on the respiratory pattern, where the increased minute volume was caused by an increase in tidal volume rather than respiratory rate. Both studies describe a reflex mechanism in which the increased expiratory load of PEEP induces activation of both expiratory and inspiratory muscles, thereby increasing tidal volume. Too limited data is present to compare the results of the present study with these previous studies.

6.4.4 Clinical implications

To the best of our knowledge, the present study is the first to evaluate the acute effects of PEEP on the diaphragm in vivo. It may seem logical that PEEP induces a downward shift of the diaphragm since end-expiratory lung volume is increased. However, no studies are yet performed to quantify this exact change in position and its consequently change in diaphragm length and function. If similar results of decreased diaphragm function during PEEP are found in mechanically ventilated ICU patients, the diaphragm could adapt structurally. By reducing the number of sarcomeres (i.e. longitudinal atrophy), the optimal length of the sarcomeres is restored and the function will restore. However, this adaptation ensures that the diaphragm length is decreased. The adaptation could have detrimental effects during a spontaneous breathing trial since PEEP is abruptly removed leading to an acute decrease in end-expiratory lung volume and an acute increase in diaphragm length. This could lead to a stretch in the adapted diaphragm muscle fibers resulting in a decrease in diaphragm function, thereby possibly contributing to diaphragm weakness. The effects of PEEP in healthy subjects cannot directly be translated to ICU patients. For example, PEEP may have a different effect in an ARDS patient with a decreased respiratory compliance. Furthermore, in clinical practice, abdominal muscle activity is occasionally increased in ICU patients which could diminish the effects of PEEP on the diaphragm. Therefore, more data in ICU patients is needed to evaluate the effects of PEEP on the diaphragm. In the meantime, low PEEP levels are preferred in clinical practice provided that oxygenation remains sufficient. In addition, a gradual decrease in PEEP before discontinuing mechanical ventilation may be beneficial. At last, it is known that disuse atrophy can be attenuated by maintaining small levels of respiratory effort. Similarly, longitudinal atrophy may be diminished by occasionally decreasing PEEP levels during the course of mechanical ventilation.

6.4.5 Study limitations

MRI images were acquired with limited spatial resolution since real-time images were made. The resolution was sufficient to follow the diaphragm contour, mostly as a result of the great difference in tissue properties of lung and liver. However, the zone of apposition was more difficult to follow and was therefore estimated with a straight line rather than following the exact contour, which may have led to a small miscalculation.

The lower limit of the zone of apposition was marked with an oil-filled flexible tube that was fixated around the costal margin of each subject. Especially on the anterior side, this seemed a reasonably valid indicator since the anterior part of the diaphragm originates from the rib-cage (i.e. costal part of the diaphragm). However, in the posterior region, the diaphragm does not originate from the ribs, which could lead to a miscalculation of the absolute length of the posterior zone of apposition. However, since
we were mostly interested in the change between two different PEEP levels, the perfect location for the marker seemed of limited importance.

Due to the limited time of my internship, MRI images were only analyzed in three planes. Additional images in coronal planes were made, for example in a plane positioned more posterior than the coronal mid plane. It was chosen to first evaluate the effect of PEEP in the these three planes since the muscle fibers contract in the same direction as these planes were viewed. Furthermore, additional analysis could be made, for example 3D reconstruction of the diaphragm, like the studies of Gauthier et al.\textsuperscript{37} and Paiva et al.\textsuperscript{38} Currently, we are conducting static full-coverage MRI images of the diaphragm at different PEEP levels with a higher spatial resolution. In this case, the 3D geometry of the diaphragm can be determined. Ideally, all images will be analyzed automatically of which a real-time 3D reconstruction is made.

MRI measurements did not take place at the same moment as the physiological measurements. Since there are no indications that a healthy subject does not respond similar to the same level of PEEP at different moments, this assumption was made. Furthermore, the MRI data is limited to one PEEP level per subject, since applying all PEEP values would be too time-consuming.

MRI and ultrasound images were not analyzed in a blind manner. When data is obtained from all subjects, the final analysis will be performed in a blind manner by two different observers.

A PEEP level of 2 cmH\textsubscript{2}O was used as a baseline value because it was the minimal level of PEEP the mechanical ventilator could generate. Since the end-expiratory lung volume is already increased with a PEEP of 2 cmH\textsubscript{2}O, the effect of PEEP may be underestimated. However, it is thought that this effect will be minimal since the end-expiratory lung volume is increased with less than 200 ml taken the compliance of a healthy respiratory system into account.\textsuperscript{11} Currently, we are performing MRI measurements to evaluate the effect of 2 cmH\textsubscript{2}O PEEP.

The change in end-expiratory lung volume as a result of PEEP could be analyzed by comparing the inspiratory and expiratory flow directly after a change in PEEP. Other parameters like swings in esophageal and transdiaphragmatic pressures, surface EMG of the expiratory muscles and the work of breathing at different PEEP levels may be also be of interest.

In conclusion, our preliminary data suggests that PEEP decreases diaphragm length in the zone of apposition by shifting the diaphragm dome to a more caudal position. A trend towards reduced diaphragm function was seen at higher PEEP levels. More data is needed for statistical analysis and to draw final conclusions. Furthermore, more research is needed in ICU patients since the decreased function as a result of PEEP may lead to structural adaptations in the long-term in the diaphragm of ICU patients, thereby possibly contributing to diaphragm weakness.
The Effects of PEEP on the Position of the Diaphragm in ICU Patients

7.1 Introduction

Diaphragm weakness is highly prevalent in critically ill patients admitted to the ICU and increases length-of-stay and mortality. Many contributors to diaphragm weakness are known such as inappropriate levels of inspiratory support, but the effects of PEEP have never been studied. Our hypothesis is that PEEP increases the end-expiratory lung volume and thereby shifts the diaphragm to a more caudal position which leads to shortening in the zone of apposition. Furthermore, it is hypothesized that this reduction in length affects the diaphragm’s capacity to produce force, since its force-length relationship is altered. The decrease in force-generating capacity could cause structural adaptations in the contractile units of the diaphragm, since it is known that muscles are capable to adapt to new lengths, thereby possibly contributing to diaphragm weakness. The first step of proving this hypothesis is to demonstrate that mechanical ventilation with PEEP shifts the diaphragm to a more caudal position. Therefore, the aim of this study is to prove the hypothesis that PEEP leads to a caudal shift of the diaphragm in mechanically ventilated patients.

7.2 Methods

Diaphragm ultrasound (sector array transducer, 5-1MHz, CX 50, Philips Inc., Bothell, WA, USA) was performed in mechanically ventilated ICU patients from the subcostal view at the right side using the liver as a window. The diaphragm was first imaged in the B-mode. Consequently, M-mode ultrasound images were acquired while PEEP was acutely reduced with either five or ten cmH\textsubscript{2}O within one breath cycle. The caudal-cranial displacement of the diaphragm was calculated from the M-mode images obtained during subsequent breaths (Figure 7.1). Analysis of diaphragm movement was performed on the ultrasound machine.

7.3 Results

Diaphragm ultrasound was performed in 15 critically ill patients admitted to the ICU with a clinical indication for PEEP reduction (Table 7.1). In all fifteen patients, acute PEEP reduction caused a cranial displacement of the diaphragm. Acute PEEP reduction of 5 cmH\textsubscript{2}O caused an average displacement of 0.40±0.10 cm (n=5) and a reduction of 10 cmH\textsubscript{2}O caused an average displacement of 0.89±0.17 cm (n=10), see Figure 7.2.
CHAPTER 7. ACUTE EFFECTS OF PEEP IN ICU PATIENTS

**Figure 7.1:** Ultrasonographic view of the diaphragm in a critically ill patient in the region of the liver dome, with B-mode image on the left and M-mode image on the right. The M-mode image shows the acute effect of a 10 cmH$_2$O PEEP change on the position of the diaphragm, measured at end-expiration (indicated by dashed line); note that in this patient PEEP was decreased from 12 to 2 cmH$_2$O, resulting in a cranial diaphragm displacement.

### 7.4 Discussion

The findings show that mechanical ventilation with PEEP causes a caudal movement of the diaphragm at end-expiration in critically ill patients. Previous studies showed that increased lung volumes led to diaphragm displacement, however, to our knowledge, this is the first study to quantify the change in diaphragm position due to PEEP alterations. It is the first step to prove our hypothesis that mechanical ventilation with PEEP may contribute to diaphragm weakness.

Using ultrasound for the purpose of measuring the diaphragmatic displacement has its limitations. In each patient, the ultrasound probe was placed at the same position so that approximately the same region of the diaphragm was imaged. However, we cannot rule out that the magnitude of displacement was different in other regions of the diaphragm, since ultrasound is a 2D technique. Furthermore, despite the fact that using the M-mode for diaphragm ultrasound is highly reproducible, the amount of displacement can be overestimated if the ultrasound beam is not perpendicularly directed at the diaphragmatic dome. In addition, only the right hemidiaphragm was assessed as it is easier to visualize than the left side. However, since ultrasound is fast, easy and applicable at the bedside, it was the most suitable method to use for this study.

This study shows that an acute reduction in PEEP led to a cranial displacement in all fifteen patients of this relatively heterogeneous study population. Therefore, the hypothesis that mechanical ventilation with PEEP leads to a caudal displacement of the diaphragm seems to be proven. More research should be conducted to assess the effects of this displacement on the length and force-generating capacity of the diaphragm and to assess its clinical consequences.
### Table 7.1: General characteristics of critically ill patients on whom ultrasound was performed

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Age (yr)</th>
<th>Sex (M/F)</th>
<th>BMI (kg/m²)</th>
<th>Relevant medical history</th>
<th>Reason of ICU admission</th>
<th>PEEP at baseline (cmH₂O)</th>
<th>Support pressure (cmH₂O)</th>
<th>Tidal volume (ml)</th>
<th>Delta PEEP (cmH₂O)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>63</td>
<td>F</td>
<td>19.1</td>
<td>COPD, pancreatitis</td>
<td>Pneumonia</td>
<td>5</td>
<td>18</td>
<td>371</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>F</td>
<td>29.4</td>
<td>Polymyalgia rheumatica</td>
<td>Septic arthritis</td>
<td>5</td>
<td>20</td>
<td>344</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>F</td>
<td>37.5</td>
<td>Tetraplegia</td>
<td>Sepsis</td>
<td>14</td>
<td>12</td>
<td>483</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>M</td>
<td>23.4</td>
<td>None</td>
<td>Cardiac arrest</td>
<td>10</td>
<td>14</td>
<td>478</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>M</td>
<td>30.6</td>
<td>Diabetes mellitus, CVA</td>
<td>Necrotizing fasciitis</td>
<td>10</td>
<td>4</td>
<td>595</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>M</td>
<td>25.2</td>
<td>COPD</td>
<td>Cardiac arrest</td>
<td>10</td>
<td>8</td>
<td>499</td>
<td>10</td>
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<tr>
<td>7</td>
<td>61</td>
<td>M</td>
<td>26.3</td>
<td>Diabetes mellitus</td>
<td>Septic arthritis</td>
<td>10</td>
<td>12</td>
<td>583</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>67</td>
<td>F</td>
<td>27.3</td>
<td>Myelofibrosis, Hypertension</td>
<td>ARDS</td>
<td>10</td>
<td>4</td>
<td>552</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>F</td>
<td>32.7</td>
<td>None</td>
<td>Multiple trauma</td>
<td>12</td>
<td>12</td>
<td>488</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>89</td>
<td>M</td>
<td>24.1</td>
<td>Benign prostatic hyperplasia; Paroxysmal atrial fibrillation</td>
<td>Neurotrauma (including multiple rib fractures)</td>
<td>10</td>
<td>6</td>
<td>435</td>
<td>10</td>
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<tr>
<td>11</td>
<td>70</td>
<td>F</td>
<td>21.6</td>
<td>Multiple myeloma</td>
<td>Pneumonia</td>
<td>12</td>
<td>23</td>
<td>332</td>
<td>10</td>
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<tr>
<td>12</td>
<td>77</td>
<td>M</td>
<td>27.9</td>
<td>CABG, right upper lobectomy due to adenocarcinoma</td>
<td>Cardiac arrest (in hospital)</td>
<td>10</td>
<td>10</td>
<td>385</td>
<td>10</td>
</tr>
<tr>
<td>13</td>
<td>56</td>
<td>M</td>
<td>21.6</td>
<td>None</td>
<td>Intracranial hemorrhage</td>
<td>10</td>
<td>14</td>
<td>404</td>
<td>10</td>
</tr>
<tr>
<td>14</td>
<td>48</td>
<td>M</td>
<td>24.7</td>
<td>None</td>
<td>Cardiac arrest</td>
<td>10</td>
<td>8</td>
<td>470</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
<td>F</td>
<td>29.4</td>
<td>Diabetes mellitus</td>
<td>Peritonitis</td>
<td>10</td>
<td>10</td>
<td>374</td>
<td>10</td>
</tr>
</tbody>
</table>

ARDS = Acute Respiratory Distress Syndrome; BMI = Body Mass Index; CABG = Coronary Artery Bypass Graft; COPD = Chronic Obstructive Pulmonary Disease; CVA = Cerebral Vascular accident; PEEP = Positive End Expiratory Pressure.
Figure 7.2: Cranial diaphragm displacement in critically ill patients caused by a 5 cmH$_2$O PEEP reduction (n=5) or by a 10 cmH$_2$O PEEP reduction (n=10); each data point is the average of three measurements per patient.
8 The Effects of PEEP on the Function of the Diaphragm in ICU Patients

8.1 Introduction

In our previous study, it was found that mechanical ventilation with PEEP leads to a caudal displacement of the diaphragm in ICU patients. This displacement could have an effect on the length of the diaphragm and therefore its force-generating capacity due to the length-force relationship of muscles. As explained earlier, it is hypothesized that the human diaphragm will adapt to this change in force-generating capacity by reducing the number of sarcomeres so that the force-length relationship of the individual sarcomere returns to normal. The aim of this study is to assess the functional effects of PEEP on the diaphragm in ICU patients. This study will be conducted in ICU patients who are mechanical ventilated for at least 3 days, since most decrease in diaphragm thickness and strength, associated with diaphragm weakness, occurs in the first three days of mechanical ventilation. Furthermore, it is thought that the diaphragm is adapted to PEEP ventilation after 3 days, corresponding to the state represented in Figure 8.1C. In rats, adaptation to PEEP occurred within 18 hours, but since adaptation might occur more rapidly in rats than in humans, it may be more safe to assume that adaptation in humans has occurred after 3 days of mechanical ventilation.

Study measurements will be performed before, during and right after an acute reduction in PEEP. It is hypothesized that after the reduction in PEEP, diaphragm fibers will be stretched since the end-expiratory lung volume is acutely reduced, corresponding to the state represented in Figure 8.1D. Therefore, the force-length relationship could be altered and the force generating capacity of the diaphragm can be reduced.

The study protocol described in the following paragraphs was send to the medical ethics committee of the VU University Medical Center as an amendment for another study. The goal of this study is to monitor expiratory muscle activity and function in critically ill ventilated patients. Since patients in this study population are already fully instrumented to perform the measurements for the PEEP study, it was decided that an amendment would be beneficial for both patients and the study team. The study protocol was approved by the medical ethics committee in January 2018. No patients are yet included in the study. Therefore, the study methods and some discussion points will only be addressed.

8.2 Methods

8.2.1 Study population

The study population will consist of patients (male/female, age > 18) who have been mechanically ventilated at the ICU for less than 48 hours and who are expected to receive mechanical ventilation for at least 72 hours. Signed consent from either the patient or the patient’s representative is needed. To be eligible for the measurements, patients have to be on an assisted mode of mechanical ventilation (e.g. pressure support or neurally adjusted ventilatory assist), since it is hypothesized that the diaphragm will only adapt
to mechanical ventilation with PEEP if the diaphragm is active. Exclusion criteria are contraindication for the placement of a nasogastric tube (e.g. upper airway/esophageal pathology or nasal bleeding disorders) and a medical history of neuromuscular disorders. In total, 20 patients will be included in this explorative physiological study.

### 8.2.2 Study protocol

Patients will be instrumented with dedicated nasogastric catheters to measure gastric pressure (Pga), esophageal pressure (Pes) and electrical activity of the diaphragm (EAdi). Using these measurements, the neuromechanical efficiency (NME) can be calculated as the difference of Pga and Pes (transdiaphragmatic pressure: Pdi) divided by EAdi. Furthermore, patients will be instrumented with surface EMG on the abdominal muscles to track expiratory muscle activity. This could be interesting since patients could activate their abdominal muscle during expiration to help the subsequent inspiration if the function of the diaphragm fall shorts after a PEEP reduction.

As mentioned before, measurements will be performed at least 72 hours after the initiation of mechanical ventilation. They will be carried out around the point that the level of PEEP will be reduced to zero according to clinical protocol, i.e. during a spontaneous breathing trial, during bronchial suctioning, or at the discretion of the attending physician. Before the reduction of the level of PEEP, recordings from the present nasogastric catheters and surface EMG will be started and three occlusion maneuvers are performed for the calculation of an isovolumetric NME. In addition, an ultrasound image of the thickening of the right hemidiaphragm and abdominal muscles will be made. The position of the ultrasound probe will be marked. When PEEP is being decreased, the change in position of the diaphragm will be visualized using the subcostal view of ultrasound to see whether PEEP ventilation has an effect of the position of the diaphragm in the specific patient. At the level of zero PEEP, another ultrasound image of the right
### 8.3. DISCUSSION

#### 8.3.1 Statistical Analysis

Changes in respiratory muscle function parameters before and after a reduction in PEEP level will be analyzed with paired t-tests or Wilcoxon signed-ranked test, based on whether the data is normally distributed.

#### 8.3.2 Discussion

To our knowledge, this study will be the first one to quantify diaphragm function of ICU patients before and after a reduction in PEEP level. Due to a delivery delay of the measurement equipment and the overflow of studies currently conducted at the department of the Intensive Care Adults, no patients are yet included in the study. Therefore, no results can be discussed, but some limitations of the study can already be considered.

The functional parameter that will be used in this study are the NME and thickness measurements using ultrasound. Other methods could be added to assess the functionality of the diaphragm, such as magnetic stimulation of the phrenic nerves since it is the gold standard to determine diaphragm strength. However, ultrasound and NME measurements are easy to use and applicable at the bedside, especially since patients are already instrumented with the nasogastric catheter for the expiratory muscle function study.

Furthermore, this study must be seen as an explorative physiological study so extensive research with more functional parameters should probably be performed based on the results in this study. Thickness measurements of the right hemidiaphragm are highly reproducible, thus likely to identify even small changes in the function of the diaphragm after PEEP reduction. The NME based on the Pdi and EAdi is a relatively new parameter and no repeatability studies are yet performed. Recently, a repeatability study with the NME based on Paw was performed, which is a good derivative for Pdi during the isovolumetric contraction in response to an occlusion maneuver. They found an unacceptable high variability between repeated measurements and therefore state that a single NME maneuver cannot be used. To reduce the

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**Figure 8.2:** Overview of the study protocol. EMG = electromyography; Pes = esophageal pressure; Pga = gastric pressure; US = ultrasound; NME = neuromechanical efficiency.
variability between measurements, multiple NME values will be measured before and after a reduction in PEEP. Furthermore, raw EAdi signals will be used in this study which are probably less variable than the filtered EAdi signal of the ventilator.

The hypothesis is that the functional parameters of the diaphragm will decrease after the reduction in PEEP, because the sarcomeres of the diaphragm muscle fibers are stretched after an acute reduction in the PEEP level. For this stretch to occur, the muscle fibers have to be adapted to the mechanical ventilation with PEEP by absorbing the sarcomeres in series. In rats, the adaptation occurred after 18 hours of mechanical ventilation with PEEP. However, it is unknown how fast this adaptation occurs in mechanically ventilated patients. If the number of series is not decreased, so the adaptation did not yet occur, no differences in diaphragm functionality before and after the withdrawal of PEEP would be measured. This phenomenon is minimized by measuring the patient at least 72 hours after initiation of mechanical ventilation; a turning point in diaphragm weakness based on ultrasound data.

Furthermore, it is unknown whether the amount of PEEP levels has an effect on the timing of longitudinal atrophy development. In rats, mechanical ventilation was applied with a PEEP of 2.5 cmH₂O, estimated to correspond to 5-6 cmH₂O PEEP in humans. In clinical practice, the amount of PEEP level frequently varies, especially in the first days of mechanical ventilation. It seems logical that longitudinal atrophy occurs faster with higher levels of PEEP, since the diaphragm fibers are shorter during higher levels of PEEP application, which causes a more reduced force due to the length-force relationship of a muscle. Since the minimal amount of PEEP does not often drop below 5 cmH₂O in clinical practice, the amount of PEEP is likely sufficient to cause longitudinal atrophy in ICU patients.
General Discussion

The studies performed in this thesis evaluated the in-vivo effects of PEEP on the diaphragm. In chapter 7, it was shown that a reduction of PEEP led to a cranial displacement of the diaphragm in ICU patients. Therefore, it was concluded that PEEP caused a caudal displacement of the diaphragm. In mechanically ventilated rats, this caudal displacement led to a reduced diaphragm and sarcomere length. In turn, this reduced length led to adaptation in the long-term by decreasing the number of sarcomeres thereby decreasing the optimal length of the diaphragm. To determine whether this effect also occurs in the human diaphragm, a proof of concept study was conducted to first prove the acute effects of PEEP in vivo. In chapter 6, it was shown that PEEP also led to a caudal displacement of the diaphragm in healthy subjects. MRI data suggested that this displacement led to a reduction in diaphragm length, mainly in the zone of apposition. Furthermore, a trend towards a decreased contractility during higher levels of PEEP was seen. This could indicate that the diaphragm may be on an unfavorable position on the force-length relationship, thereby possibly inducing diaphragm adaptation in the long-term.

More research is needed to prove this effect. First, the sample size of the study described in chapter 6 is too small to show significant results and to draw final conclusions and should be completed. Second, ICU patients may react differently to PEEP than healthy subjects for example due to a decreased compliance of the respiratory system. Therefore, more research is needed in ICU patients. For instance, the acute effects of PEEP on the position and length of the diaphragm in ICU patients could be established by performing MRI scans during a spontaneous breathing trial. To evaluate whether in-vivo adaptation occurs in mechanically ventilated patients seems more challenging. Ideally, the number of sarcomeres in diaphragm muscle fibers of long-term mechanically ventilated patients should be determined. However, this requires isolation of whole length (central tendon to rib cage) diaphragm muscle fibers. A more convenient and non-invasive way to determine whether adaptation likely has occurred is using functional diaphragm measurements. Such a method is described in chapter 8, where the study population consisted of ICU patients who have been on mechanical ventilation for at least 3 days. A decrease in functional measurements as a result of an acute reduction in PEEP indicates that adaptation has occurred. Together with the results of the acute effect, this study might possibly show that mechanical ventilation with PEEP may be involved in diaphragm weakness in ICU patients.

In the meantime, low PEEP levels are preferred in clinical practice, ideally guided by esophageal pressure measurements, provided that oxygenation remains sufficient. In addition, a gradual decrease in PEEP before discontinuing mechanical ventilation may be beneficial. Theoretically, PEEP levels during a weaning trial could be guided by functional measurements of the diaphragm. For example, if PEEP is decreased too rapidly, a decrease in neuromechanical efficiency or twitch transdiaphragmatic pressure might occur. At last, it is known that disuse atrophy can be attenuated by maintaining small levels of respiratory effort. Similarly, longitudinal atrophy may be diminished by occasionally decreasing PEEP levels during the course of mechanical ventilation.
References


REFERENCES


Appendices
Individual data of the subjects

The following figures show individual data of all included subjects during the physiological part of the study.

- Figure A.1 shows the effect of a change in PEEP level on the position of the diaphragm
- Figure A.2 shows the effect of PEEP on the diaphragm thickness
- Figure A.3 shows the effect of PEEP on twitch transdiaphragmatic pressures
- Figure A.4 shows the effect of PEEP on the neuromechanical efficiency of the diaphragm (n=2)
- Figure A.5 shows the effect of PEEP on the electrical activity of the diaphragm (n=2)
- Figure A.6 shows the effect of PEEP on the thickening fraction of the diaphragm
Figure A.1: Displacement of the diaphragm (in cm) of each subject (marked as S01-S05) as a result of a change in PEEP level (in cmH$_2$O) is shown. A displacement in the caudal direction is defined as a positive displacement, whereas a cranial displacement is defined as a negative displacement. Data are presented as mean plus standard deviation of three subcostal ultrasounds during changes in PEEP level. For example, a change in PEEP level from 2 to 5 cmH$_2$O in subject 1 led to an average caudal displacement of 0.7 cm. Note that not all measurements are performed in each subject.

Figure A.2: The end-expiratory thickness (cm) of each subject (marked as S01-S05) is shown at different PEEP levels. Data are presented as mean plus standard deviation of three breaths during at each PEEP level.
Figure A.3: The twitch transdiaphragmatic pressure (twitch Pdi in cmH$_2$O) of each subject (marked as S01-S05) is shown at different PEEP levels. Data are presented as mean plus standard deviation of the three magnetic stimulations created at each PEEP level.

Figure A.4: The neuromechanical efficiency (NME) of the diaphragm of two subjects is shown at different PEEP levels. Data is presented as mean plus standard deviation of three measurements.
Figure A.5: The amplitude of the electrical activity of the diaphragm of two subjects is shown at different PEEP levels. The amplitude at each level was calculated as the median value of breath-by-breath measurements during the long period of PEEP ventilation.

Figure A.6: Thickening fraction (in %) of each subject (marked as S01-S05) is shown at different PEEP levels. Data are presented as mean plus standard deviation of three breaths during at each PEEP level.
Comparison between ultrasound and MRI

Table B.1: Displacement of the diaphragm as a result of a change in PEEP level measured by ultrasound and MRI. Displacement data by the ultrasound is the mean of three measurements. The displacement in the MRI is measured in four different planes. The MRI data from the sagittal right plane is used for calculating the difference US and MRI data.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Change in PEEP (cmH₂O)</th>
<th>Displacement by US (cm)</th>
<th>Displacement by MRI (cm)</th>
<th>Δdisplacement by US and MRI (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2-15</td>
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<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
</tbody>
</table>

PEEP = positive end-expiratory pressure; US = ultrasound; SAG-R = sagittal right plane, SAG-L = sagittal left plane, COR-mid right = right dome in coronal mid plane; COR-mid left = left dome in coronal mid plane.