THE RELATIONSHIP BETWEEN DIAPHRAGM THICKNESS, DIAPHRAGM STRENGTH AND DIAPHRAGM ENDURANCE IN YOUNG, HEALTHY INDIVIDUALS

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Declaration

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Abstract

Introduction: In the intensive care unit population, approximately 40% of patients require mechanical ventilation and 20-25% of these patients will encounter difficulties in the discontinuation of mechanical ventilation. As mechanical ventilation affects the diaphragm, a better understanding of the structural and functional changes of the diaphragm is warranted.

Method: A scoping review was done to determine whether a relationship between diaphragm thickness, diaphragm strength and diaphragm endurance had been established. Seven databases were searched using a specific search strategy. Papers were identified based on pre-defined inclusion criteria. Data was extracted by the primary investigator (PI) into a self-developed excel spreadsheet. Criteria were developed for a more focused review to inform the planning of a primary study. The primary study investigated the relationship between diaphragm thickness, diaphragm strength and diaphragm endurance in young, healthy individuals. A sample of convenience was used; included healthy individuals (18-24); three activity-levels (sedentary; endurance- and strength related sporting activities); stratified for gender and BMI. Measurements included: Sonographic measurement of diaphragm thickness; mouth pressure manometer measurements for diaphragmatic strength; and fatigue resistance index as a measure of endurance. Participants were instructed to breathe through a pressure threshold device at 60% of PI_{max} until task failure. The fatigue resistance index was calculated as PI_{max} final/PI_{max} initial. Intra-rater reliability was established and testing procedures standardised a priori.

Results: 405 full texts were retrieved and assessed for inclusion into the review. Papers identified the evaluation of diaphragm function in a variety of populations. 23 papers were included in the focused review. Six papers were published on diaphragm thickness, six on diaphragm strength and eleven on diaphragm endurance. No papers identified the correlation between diaphragm thickness, diaphragm strength and diaphragm endurance. 55 subjects, males and females, were recruited for the primary study. Groups were similar at baseline with regards to gender, age and BMI. The mean age of the sample was 21.16 years (SD = 1.55), with a mean body mass index (BMI) of 25.43 kg/m2 (SD = 3.70). A moderate positive correlation was established between diaphragm thickness and diaphragm strength measurements ($r = 0.52$; $r^2 = 0.27$; $p < 0.01$). Diaphragm thickness was not correlated with diaphragm endurance ($r = -0.15$; $r^2 = 0.02$; $p = 0.29$). No relationship was found between the strength of the diaphragm and the endurance of the diaphragm ($r= -0.19$; $r^2 = 0.04$; $p= 0.16$).

Conclusion: Guidelines for the measurement of diaphragm function do exist, but they are not adhered to by the majority of studies. Study procedures are inconsistently reported and this may affect the reproducibility of techniques in future studies. We further conclude that a correlation exists between diaphragm thickness and diaphragm strength. The use of ultrasound to measure diaphragm thickness proved to be a reliable technology and gave a moderate indication of the strength of the diaphragm. This technology may help clinicians to detect and monitor dysfunction of the diaphragm in the early stages of admission to the acute setting.

Opsomming

Inleiding: Ongeveer 40% van pasiente wat in intensiewe sorgeenheid behandel word, benodig intubasie en meganiese ventilasie. Tot 25% van hierdie pasiënte sal probleme ondervind in die staking van meganiese ventilasie. Meganiese ventilasie beïnvloed die diafragma, daarom word n beter begrip van die strukturele en funksionele veranderinge van die diafragma benodig.

Metode: 'n Literatuur oorsig is gedoen om te bepaal of daar 'n verhouding bestaan tussen die dikte, krag en uithouvermoë van die diafragma. Sewe databasisse is deurgesoek aan die hand van spesifieke databasis gedefinieerde soektog strategie. Relevante artikels is geïdentifiseer aan die hand van pre-gedefinieerde insluiting kriteria. Data is onttrek en in ‘n self-ontwikkelde datablad opgesom deur die primêre ondersoeker (PI). Hierdie inligting is gebruik in die beplanning van ‘n primêre studie. Die doel van die primêre studie was om die verhouding tussen die diafragma dikte, krag en uithouvermoë in jong, gesonde individue te ondersoek. ‘n Gerieflikheids steekproef is gebruik; insluitend gesonde individue (18-24); drie aktiwiteits vlakke (passief; uithouvermoë- en krag verwante sportaktiwiteite) en breë spektrum vir geslag en ligaamsbou (BMI). Metings ingesluit: sonografiese meting van die diafragma se dikte; monddruk manometer metings vir diafragmatiese krag en ‘n moegheid/weerstand indeks as maatstaf van diafragmatiese uithouvermoë. Deelnemers is opdrag gegee om asem te haal deur toestel met druk maksimum gestel 60% van PI_{max}, tot mislukking. Die moegheid/weerstand indeks is bereken as PI_{max} finale / PI_{max} oorspronlik. Intra-meter betroubaarheid is bepaal en toets prosedures is gestandaardiseer voordat data ingesamel is.

Resultate: 405 vol teks artikels is uitgelig vir insluiting in die literatuur oorsig. Diafragmatiese funksie is ge-evalueer in 'n verskeidenheid bevolkings. Drie en twintig artikels is in die finale oorsig ingesluit. Ses artikels wat diafragma dikte evalueer, ses wat diafragmatiese krag evalueer en elf wat die diafragma se uithouvermoë evalueer is ingesluit in die oorsig. Geen van die artikels uitgelig het ‘n ooreenkoms tussen diafragma dikte, diafragma krag en diafragma uithouvermoë geïdentifiseer nie. 55 deelnemers is gewerf vir die primêre studie. Groepe was soortgelyk by basislyn met betrekking tot geslag, ouderdom en BMI. Die gemiddelde ouderdom van die toetsgroep was 21.16 jaar (SD=1.55), met 'n gemiddelde BMI van 25.43 kg/m2 (SD = 3.70). ‘n Middelmatige positiewe verhouding is waargeneem tussen diafragma dikte en krag ($r = 0.52$; $r^2 = 0.27$; $p < 0.01$). Geen verhouding is gevind tussen diafragma dikte en uithouvermoë nie ($r= -0.15$; $r^2 = 0.02$; $p = 0.29$). Daar is ook geen

verhouding waargeneem tussen diafragma krag en diafragma uithouvermoë nie. (r= 0.19; r^2 = 0.04; p = 0.16).

Gevolgtrekking: Daar bestaan wel riglyne vir die meting van die diafragma se funksie, maar in die meerderheid van studies word dit nie nagekom nie. Studie prosedures is nie konsekwent weergegee nie en dit kan die resultate van tegnieke beinvloed in toekomende studies. 'n Matige sterk verhouding is waargeneem tussen diafragmatiese dikte en krag. Die gebruik van ultraklank om die diafragma se dikte te meet is betroubare tegnologie en kan n redelike aanduiding gee oor die krag van die diafragma. Hierdie tegnologie kan praktisyne help om enige disfunksie van die diafragma te identifiseer en te monitor in die vroeë stadiums van toelating tot die akute omgewing.

Dedication

This one is dedicated to you, Gwenny!

You were my driving force through it all and my constant source of hope and determination. Wish you were here so I could share this with you, RIP

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Glossary of Terms

Balloon Catheter System is a widely used method for recording oesophageal pressure as a reflection of pleural pressure; and gastric pressure as a reflection of abdominal pressure. Air-containing latex balloons are sealed over catheters, which transmit pressures to the transducers. Pressures are measured by passing a pair of balloon catheters through the nose, following local anaesthesia of the nasal mucosa and pharynx (ATS/ERS Statement on Respiratory Muscle Testing, 2002)

Bilateral Anterior Magnetic Phrenic Stimulation is a form of phrenic nerve stimulation and reliably achieves supra-maximal nerve stimulation (ATS/ERS Statement on Respiratory Muscle Testing, 2002)

Cervical Magnetic Stimulation is a form of phrenic nerve stimulation and elicits a bilateral diaphragm contraction, co-activating muscles innervated by the brachial plexus. Achieving and confirming supra-maximal nerve stimulation is challenging (ATS/ERS Statement on Respiratory Muscle Testing, 2002)

Competitive is defined as regular participation in a sporting activity (Guenette et al, 2010)

Diaphragm is identified as a structure made of three distinct layers: A non-echogenic central muscular layer bordered by two echogenic membranes, the peritoneum and the diaphragmatic pleura (Matamis et al, 2013; Ueki et al, 1995)

Diaphragm Injury is associated with ultra-structural injury to the muscle fibres of the diaphragm (Jaber et al, 2011b)

Muscle Strength in skeletal muscles is described as the force developed under isometric conditions with a muscle at its optimal length (ATS/ERS Statement on Respiratory Muscle Testing, 2002)

Muscle Endurance is the ability to sustain a specific muscular task over time. It is a highly integrated and complex quality of a muscle and/or group of muscles that is related to resistance to fatigue (ATS/ERS Statement on Respiratory Muscle Testing, 2002)

Discontinuation is described as the progressive withdrawal from invasive ventilatory support (Savi et al, 2012)

Duchenne Muscular Dystrophy is a genetically inherited disease in humans. It is identified by the absence or disruption of the protein dystrophin, which is found in a variety of tissues, most notably skeletal muscle (Anderson et al, 2002)

Extubation is described as the removal of the endotracheal tube from a patient receiving invasive ventilatory support (Savi et al, 2012)

Extubation Failure is described as the need for re-intubation 24 – 72 hours after planned extubation (Frutos-Vivar et al, 2011)

Fibre Cross-sectional Area is defined as the total cross-sectional area of all the muscle fibres at right angles to their long axes (Kawakami et al, 1995)

Functional Residual Capacity is described as the volume of air remaining in the lungs at the end of a normal tidal expiration (Kenyon et al, 2009)

Inspiratory Capacity is described as the maximum volume of air that can be inspired after a normal tidal expiration (Kenyon et al, 2009)

Maximum Inspiratory Pressure (PI_{max}) is identified as a manoeuvre which consists of a maximum inspiratory effort against a closed airway (ATS/ERS Statement on Respiratory Muscle Testing, 2002)

Muscle Fibres are composed of sarcomeres and within each sarcomere are the myofibrillar proteins, actin and myosin. The interaction of the two myofibrillar proteins allows muscles to contract (Scott et al, 2001)

Muscle Mass is defined as a function of the size and number of muscle fibres (Nick et al, 1989)

Phrenic Nerve Stimulation is specific for the diaphragm and is not influenced by the central nervous system (ATS/ERS Statement on Respiratory Muscle Testing, 2002)

Residual Volume is described as the volume of air remaining in the lungs after a maximal expiration (Kenyon et al, 2009)

Sarcomere is defined as the basic unit of force generation in a muscle (Ward et al, 2009)

Thickening Fraction is used as an index of diaphragm efficiency as a pressure generator. The index is calculated as: Thickening Fraction = (Thickness at end inspiration – thickness at end expiration)/thickness at end expiration (Matamis et al, 2013)

Transdiaphragmatic Pressure is defined as the difference between the pleural pressure and the abdominal pressure, measured by the balloon catheter system, and is a specific measure for diaphragm contraction (ATS/ERS Statement on Respiratory Muscle Testing, 2002)

Twitch Transdiaphragmatic Pressure is a useful tool to assess the degree of activation of the diaphragm (ATS/ERS Statement on Respiratory Muscle Testing, 2002)

Total Lung Capacity is described as the total volume of your lungs at the end of a maximal inspiration (Kenyon et al, 2009)

Ultrastructure is defined as the architecture of a muscle. The architecture of a muscle is the arrangement of fibres in a muscle that predicts its functional capacity (Ward et al, 2009)

Zone of Apposition is the area of the chest wall where the abdominal contents reach the lower rib cage (Matamis et al, 2013)

Outline of Thesis

The following thesis will be presented in a ‘**masters by publication**’ format.

CHAPTER 1:

Introduction to the study, a literature overview and the significance of the study

CHAPTER 2:

A scoping review, outlining the current measures of diaphragm function in the healthy and critically ill population

CHAPTER 3:

A descriptive, correlational study to determine **the correlation between diaphragm thickness, diaphragm strength and diaphragm endurance** in young, healthy individuals

CHAPTER 4:

A general discussion

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ADDENDA RELATED TO METHODOLOGY

ADDENDA RELATED TO RESULTS

Chapter 1:

INTRODUCTION

This study reports on the potential for utility of ultrasound imaging as a surrogate measure of diaphragmatic strength and explores the relationship between diaphragm thickness, strength and endurance. This study is an early investigation to inform whether the measurement of diaphragm thickness could be a determinant for identifying patients likely to fail extubation.

This chapter will provide motivation for undertaking this study. The chapter largely focuses on the fibre composition of the normal diaphragm; factors that affect the structure of the diaphragm; and the effect of mechanical ventilation on the structure and function of the diaphragm

1.1 STRUCTURE AND FUNCTION OF THE DIAPHRAGM

The muscle fibres of the adult human diaphragm are constantly active, with slow and fast fibres present in equal proportions (Polla et al, 2004). The structural and functional characteristics of respiratory muscle fibres can change in size in response to demands and convert from one type to another, thereby allowing them to adapt to different functional tasks (Scott et al, 2001).

1.1.1 Fibre types: The estimated fibre distribution comprises ±55% slow twitch fibres; ±21% fast twitch oxidative fibres and ±24% fast twitch glycolytic fibres, thereby reflecting the different functional tasks of the fibres (Polla et al, 2004). Fibres can therefore contribute to the overall force production, by contracting forcefully and rapidly, and increase the overall resistance to fatigue, by maintaining contractions for sustained periods of time. Fibres types of respiratory muscles therefore differ from limb and trunk musculature. The fibres in limb and trunk musculature typically develop a predominance of one fibre type, thereby limiting its function to either that of a mover or a stabilizer (McArdle et al, 2006; Polla et al, 2004).

All skeletal muscles are composed of fibres of different types (Polla et al, 2004). The fibres are identified by the isoform of the myosin heavy chain and expressed as slow type 1, fast type 2A, fast type 2X and fast type 2B (Polla et al, 2004). The slow fibres are resistant to fatigue and have a high oxidative metabolism (Polla et al, 2004). The fast 2X and 2B fibres are easily fatigable and have a more glycolytic metabolism (Polla et al, 2004). The fast 2A fibres have an intermediate resistance to fatigue and an oxidative glycolytic metabolism (Polla et al, 2004). This range of muscle fibre types allows for the wide variety of capabilities that human muscles portray (Scott et al, 2001). The tension developed during isometric contractions is lower in slow fibres than in fast fibres, with both fibre types differing in the time course of contractile responses (Polla et al, 2004). During a contraction, the increased tension will reach a peak and decline faster in fast fibres than in the slow fibres (Polla et al, 2004). Slow fibres are therefore able to maintain their contraction for an increased duration, as they are more resistant to fatigue than the fast fibres. The time parameters of a contraction are determined by the sensitivity of myofibrils to calcium, the rate at which cross bridges are formed between actin and myosin and the rate at which calcium is released and taken up from the sarcoplasmic reticulum (Polla et al, 2004).

Muscle fibre characteristics can be modified in response to several conditions. The conditions include training; disuse; hypoxia; malnutrition; increasing age and pharmacological agents (Polla et al, 2004). For the purpose of this review, we will explore the response of muscle fibre characteristics in response to training and aging.

1.2 FACTORS AFFECTING STRUCTURAL CHANGE

1.2.1 Training: Training comprises two main types of muscle exercise, both resulting in different adaptations of the respiratory muscles (Polla et al, 2004). The two types of exercise include endurance and strength training. Endurance training comprises long sessions of submaximal contractions and strength training comprises short sessions of maximal contractions (Polla et al, 2004). Endurance training does not change the fibre types of respiratory muscles (Polla et al, 2004). However, endurance training does cause a decrease in fibre size and cross sectional area of respiratory muscles, with no change in the muscles contractile force properties (Polla et al, 2004). Endurance training increases fibre fatigue resistance, observed in the costal and crural diaphragm and parasternal and external intercostal muscles (Polla et al, 2004). Strength training of respiratory muscles has the opposite effect to endurance training. Strength training increases the volume of contractile proteins, thereby increasing the contractile force and muscle mass of the fibres (Polla et al, 2004; Scott et al, 2001). However, respiratory muscle fibres do not undergo changes due to training and immobilization in the same way as the fibre types of limb muscles (Polla et al, 2004). In human limb muscles, both strength and endurance training result in fibre type transformation (Polla et al, 2004).

1.2.2 Aging: During the aging process, there is a progressive loss of muscle mass and force production in the respiratory muscles (Scott et al, 2001). The loss of muscle mass is mainly the result of a decrease in the total amount of both slow and fast muscle fibres (Scott et al, 2001). The decrease in force production is due to the transformation between muscle fibre types as well as the preferential atrophy of fast muscle fibres (Polla et al, 2004; Scott et al, 2001). Atrophy of fast fibres contributes to the decreased muscle mass, leading to a larger proportion of slow type fibres in aged muscle (Scott et al, 2001). The shift towards the slow fibre phenotype during aging results in slower contraction and relaxation times (Polla et al, 2004). Age related changes mentioned may therefore result in some loss of function, in both respiratory and limb muscles (Scott et al, 2001).

1.3 EFFECT OF MECHANICAL VENTILATION ON DIAPHRAGM STRUCTURE AND FUNCTION

Mechanical ventilation is one of the most frequently used critical care technologies, but while mechanical ventilation is a life-saving modality, it results in structural and functional changes to the diaphragm (Jaber et al, 2011b).

The development of diaphragmatic injury seems to be an earlier phenomenon than the development of diaphragmatic atrophy (Jaber et al, 2011b). In a study by *Levine et al.* (2008), they reported that long term mechanical ventilation resulted in marked atrophy of both slow and fast fibres of the diaphragm. Mechanisms responsible for injury and atrophy are not identical, although they may be linked (Jaber et al, 2011b). The mechanism for the development of diaphragm injury with inactivity is unclear (Sassoon et al, 2002). However, hypoxemia or ischaemia have been known to cause mitochondrial and myofibrillar injuries, with decreased diaphragm perfusion demonstrated during controlled mandatory ventilation (Sassoon et al, 2002). The effect of intermittent, non-synchronized contractions by the diaphragm during mechanical ventilation can also not be excluded as a cause of diaphragm injury (Sassoon et al, 2002). Both injury and atrophy have been significantly correlated with the duration of mechanical ventilation, but the degree of injury and atrophy do not correlate with one another (Jaber et al, 2011b). These studies suggest that mechanical ventilation has a time-dependent, detrimental effect on the diaphragm, with the degree of diaphragmatic atrophy directly proportional to the duration of mechanical ventilation (Jaber et al, 2011b).

Diaphragmatic biopsies in short and long term mechanically ventilated patients were compared in a study conducted by *Jaber et al.* (2011b). Mechanical ventilation for at least five days, demonstrated an increase in the prevalence of ultra-structural abnormalities within the diaphragm when compared to short term (less than five days) mechanical ventilation. Abnormalities included the disruption of the normal myofibrillar organization and enlarged spaces containing disorganized sarcomeric material (Jaber et al, 2011b). Myofibrillar disorder was significantly correlated with the abnormal force generating capacity of the diaphragm (Jaber et al, 2011b). The researchers also reported an up-regulation of members of the calcium dependant calpain protease system in the diaphragms of long term mechanically ventilated patients. Calpains degrade cytoskeletal proteins in muscle and thereby contribute not only to muscle atrophy, but also to sarcomeric disarray and the development of injury responses (Jaber et al, 2011b). The biochemical changes observed possibly contribute to the abnormal force generating capacity of the diaphragm observed in patients receiving prolonged mechanical ventilation (Jaber et al, 2011b; Petrof et al, 2010).

1.4 EFFECT OF MECHANICAL VENTILATION ON DIAPHRAGM FIBRE SIZE AND CROSS-SECTIONAL AREA

The mean cross sectional area of all diaphragm fibres decreases in long term mechanically ventilated patients (Jaber et al, 2011b). A study by *Jaber et al.* (2011b) reported the decrease to be as high as 39% in long term mechanically ventilated patients, compared with the group of short term (two – three hours) mechanically ventilated patients. The same study showed that a significant relationship exists between the duration of mechanical ventilation and the reductions seen in diaphragm fibre size. In a study by *Jaber et al.* (2011b) on short and long term mechanically ventilated patients, there were no significant alternations in the proportion of slow and fast twitch fibres of the adult human diaphragm between groups.

1.5 EFFECT OF MECHANICAL VENTILATION ON DIAPHRAGM CONTRACTILITY AND ENDURANCE

The relationship between the abnormal force generating capacity of the diaphragm and muscle fibre atrophy is unclear (Jaber et al, 2011b). Mechanical ventilation induced decreases in the force generating capacity of the diaphragm cannot be ascribed to atrophy alone, as the force loss is persistent even after correcting for any reductions in muscle cross sectional area (Jaber et al, 2011b; Petrof et al, 2010). The findings by *Jaber et al.* (2011b) confirm findings by *Sassoon et al.* (2002) that suggest that the two responses can be dissociated from one another.

Muscle endurance and its relationship to the duration of mechanical ventilation have also been described (Chang et al, 2005). Patients who had received mechanical ventilation for more than 48 hours had reduced inspiratory muscle endurance that worsened with the duration of mechanical ventilation (Chang et al, 2005). This suggests that patients who require prolonged mechanical ventilation are at risk of respiratory muscle fatigue (Petrof et al, 2010), which is still present after successful discontinuation of mechanical ventilation (Chang et al, 2005). However, it has been hypothesized that a pre-existing decrease in respiratory muscle endurance may either contribute or be the reason for prolonged periods of mechanical ventilation (Chang et al, 2005).

1.6 SIGNIFICANCE OF THE STUDY

On average, 40% of patients in intensive care units require mechanical ventilation. A significant minority, 20 - 25%, of these patients will encounter difficulties in the discontinuation of mechanical ventilation (Grosu et al, 2012). Although only a significant minority of patients will encounter difficulties in the discontinuation of mechanical ventilation (Grosu et al, 2012), their increased length of stay has a major impact on the use of health care resources (Frutos-Vivar et al, 2011).

A variety of discontinuation strategies have been implemented to assess discontinuation readiness, all with varying degrees of success (Grap et al, 2003). The removal of the endotracheal tube comprises the second stage of the discontinuation process (Savi et al, 2012). However, failure to extubate occurs in 5 - 20% of patients and may occur for reasons different from those resulting in discontinuation failure. The above mentioned range depends on the patient population, with the risk being the highest for medical and neurological patients (Frutos-Vivar et al, 2011).

As mentioned, the structural components of the diaphragm; namely fibre size and cross sectional area, are affected by mechanical ventilation (Jaber et al, 2011b). The functional components of the diaphragm; namely the endurance and the strength of the contraction, are also affected by mechanical ventilation (Jaber et al, 2011b; Petrof et al, 2010; Levine et al, 2008). Therefore, to determine an effective method to measure the thickness, strength and endurance properties of the diaphragm, we may be able to identify patients likely to fail extubation. Another angle would be to correlate the structural and functional components of the diaphragm, to determine if they were related or predictive of one another.

The aim of this study was to investigate the relationships between diaphragm thickness, diaphragm strength and diaphragm endurance in young, healthy individuals

Chapter 2:

LITERATURE REVIEW

2.1 INTRODUCTION

Respiratory muscles have the same structure and function as limb and trunk musculature (Polla et al, 2004), but do not undergo change due to training or inactivity in the same way. In human limb muscles, both strength and endurance training result in fibre type transformation, thereby increasing the predominance of one fibre type (Polla et al, 2004). In respiratory muscles, specifically the diaphragm, the distribution of slow and fast fibres is equal (Polla et al, 2004). When the respiratory muscles are trained, hardly any change is observed in the composition of fibre types (Polla et al, 2004). Instead, respiratory muscles will increase or decrease in fibre size, depending on the training stimulus, but will not shift from one type to another. (Polla et al, 2004)

It is also evident from the literature that the structural and functional features of respiratory muscle fibres are highly specialised and can readily adapt to altered functional requirements (Scott et al, 2001). The literature also emphasises the detrimental effect of mechanical ventilation on the structure and constant function of the diaphragm (Jaber et al, 2011b; Petrof et al, 2010; Levine et al, 2008).

To inform the planning of a primary study, we conducted a scoping review of the literature (Levac et al, 2010). The aim of this review was to determine whether a relationship between diaphragm thickness, diaphragm strength and diaphragm endurance had been established. The objectives were to describe the populations in which the function of the diaphragm had been tested and to establish whether uniform testing procedures have been adhered to.

2.2 MATERIALS AND METHODS

2.2.1 Searching

Seven computerized bibliographic databases were searched, namely Pubmed, PEDRO, Cinahl, Cochrane, MEDLINE, EBSCO Host and Science Direct from inception to April 2013. We conducted a broad search strategy using and combining the key terms “diaphragm strength”, “diaphragm force generating capacity”, “diaphragm endurance”, “diaphragm fatigue”, “diaphragm thickness”, “diaphragm thinning” and “measure,” with limits on articles in English. Additional limits were applied, which differed between the databases. A detailed search strategy is available in Addendum A.

2.2.2 Study Eligibility

We included all papers that reported on diaphragm measures and papers that recruited healthy adult (19 years and older) human participants. We excluded papers that exclusively enrolled neonates, paediatrics and adolescents, as well as papers performed on animals or that were not published in English.

2.2.3 Study Selection and Data Extraction

One reviewer independently screened and evaluated the titles, abstracts and then full texts of all publications returned by the search strategy for potentially relevant publications. Full texts were retrieved by accessing electronic journals, manually searching journals or by contacting the authors via e-mail. Any uncertainty regarding paper selection and data extraction was resolved by consensus and discussion with a supervisor if needed.

The same reviewer independently extracted all relevant data items from the included papers, using an electronic data extraction form. The data form was compiled by the researcher in consultation with supervisors to optimize data synthesis.

2.2.4 Methods of Analysis and Synthesis

Included papers were assessed for homogenous data, such as comparable patient populations; methods of measurement; and equipment specifications for the measurement of diaphragm function. Due to the heterogeneity of the rest of the data, statistical pooling was not appropriate and the results were summarized in a narrative form.

2.3 RESULTS

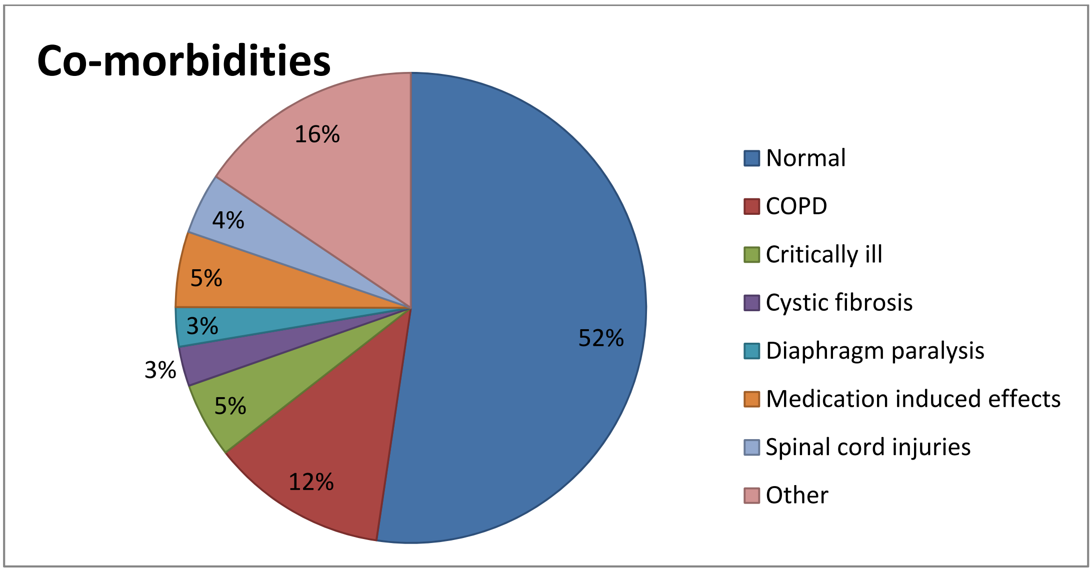
In the results section we discuss the trial publications and include a description of the trials. For the purpose of the review, only a number of the published trials were included and discussed in detail.

2.3.1 Trial Publications

The search strategy yielded a total of 3938 citations. After duplicate elimination (Addendum B), a total of 1468 citations were left. Out of the 1468 titles screened, 787 records were excluded at title level. 681 abstracts were retrieved and screened and 258 records were excluded at abstract level. 405 full texts were retrieved and assessed for inclusion into the review. (Addendum C)

2.3.2 Trial Description

The majority of papers reporting on diaphragm measurements were recorded in healthy subjects (n=137). These papers have been collated into a database, which will be used for future studies.



*Other: Amyotrophic lateral sclerosis, Asbestos related pleural disease, Asthma, Chronic heart failure, Chronic airflow limitation, Guillain-Barre Syndrome, Grave's disease, Healthy vs liver disease, Healthy vs autopsy studies, Idiopathic inflammatory myopathies, Interstitial lung disease, Hypothyroidism, Diabetes Mellitus, Rheumatoid Arthritis, Chronic hyperinflation, Effects of anaesthesia, Multiple Sclerosis, Chronic Hypoxemia, Chronic Abdominal Distention, Effects of caffeine, Lower back pain, Left Ventricular Failure, Malnutrition, Muscular Dystrophy, Myasthenia gravis, Neuralgic Amyotrophy, Neuromuscular Disease, Obstructive Sleep Apnoea, Polio, Restrictive thoracic disease, Systemic Lupus Erythematosus, Spinal Muscular Atrophy, Stroke, Surgery, Sympathetically Mediated Pain

2.3.3 This Review

For a more focused review, additional inclusion criteria were assigned. We included only the "critically ill" and the "healthy" populations. The "healthy" population was included to limit the possible effect of pathology on measurements. The "critically ill" population was included as our end goal is to be able to manage patients in the intensive care unit. ATS guidelines (ATS/ERS Statement on Respiratory Muscle Testing, 2002) for the testing of respiratory muscle function were published in 2002. Papers were limited to those published within the last five years (2008-2013), to allow sufficient time for the uptake of the ATS guidelines (ATS/ERS Statement on Respiratory Muscle Testing, 2002) into the most recent literature published on the diaphragm.

2.3.4 Description of Included Trials

Of the 23 papers included; 6 papers (26%) tested diaphragm strength, 6 papers (26%) tested diaphragm thickness and 11 papers (48%) tested diaphragm endurance.

Five papers (22%) were published in the USA and four papers (17%) were published in Germany. Switzerland and the UK have each published three papers (13%) in this area and two papers (9%) were published by teams in Canada. The remaining six papers (26%) included populations from Spain, Japan, Australia, Greece, France and Turkey.

Of the populations studied, 12 papers (52%) included healthy subjects, 5 papers (22%) included cyclists, 3 papers (13%) included critically ill patients and 1 paper (4%) included scuba divers, police squad members and swimmers.

Seven papers (30%) included in this review were published in 2010. Six (26%) and four (17%) papers were published in 2009 and 2008, respectively. The minority of the papers were published in 2011 and 2012. Two papers (9%) were published in 2011 and three papers (13%) were published in 2012. Only one paper (4%) was published in 2013.

2.3.4.1 Measurement of Diaphragm Thickness

Six papers report on the use of ultrasound in the measurement of the diaphragm thickness. Papers were analysed according to the following sub-headings: Subject demographics, equipment specifications and measurement method. The “measurement method” was further divided into: Testing protocol; probe placement; cycle of breathing; parameters for thickness measurements and data processing.

Subject demographics

Three papers (50%) used this technique in the critical care population to describe changes found in the diaphragm muscle during mechanical ventilation and to assess the accuracy and feasibility of ultrasound in determining the function of the diaphragm (Cartwright et al, 2013; Grosu et al, 2012; Vivier et al, 2012). Three papers (50%) evaluated the use of ultrasound to isolate diaphragm muscle contractions during spontaneous breathing in healthy volunteers (Baldwin et al, 2011; Orozco-Levi et al, 2010; Kaneko et al, 2009). The papers were published in a wide variety of countries, with two papers (33%) published in the USA (Cartwright et al, 2013; Grosu et al, 2012). The six papers were published over a number of years, from 2009 to 2013. Two papers (33%) included males only as part of their sample (Orozco-Levi et al, 2010; Kaneko et al, 2009) and the other four papers (67%) included both males and females (Cartwright et al, 2013; Grosu et al, 2012; Vivier et al, 2012; Baldwin et al, 2011). The mean age was reported in three papers (50%) (Cartwright et al, 2013; Orozco-Levi et al, 2010; Kaneko et al, 2009) and the mean BMI was reported in two papers (33%) (Cartwright et al, 2013; Orozco-Levi et al, 2010). (Table 2.1)

Equipment Specifications

Orozco-Levi et al. (2010) measured the maximum velocity of relaxation of the diaphragm, using a curvilinear transducer with imaging done in M-mode. The remaining four papers (67%) used a linear transducer probe to determine diaphragm thickness measurements (Cartwright et al, 2013; Vivier et al, 2012; Baldwin et al, 2011; Kaneko et al, 2009). *Grosu et al.* (2012) did not mention what probe they used. Each paper used a different frequency to measure diaphragm thickness, with frequencies ranging from 7.5 – 18MHz. Three papers (50%) (Grosu et al, 2012; Orozco-Levi et al, 2010; Kaneko et al, 2009) used B-mode to image the diaphragm and the remaining three papers (50%) did not mention what mode they used for imaging (Cartwright et al, 2013; Vivier et al, 2012; Baldwin et al, 2011). (Table 2.2)

Table 2.1: Summary of papers using ultrasound imaging to measure diaphragm thickness

Study	Country	Study Objective	Gender (% M)	Age (Years) Mean SD	Body Mass Index (kg/m2) Mean SD	Activity Level
						HEALTHY
Orozco-Levi et al 2010	Spain	To use ultrasound to study in autopsy samples To use ultrasound in a static study of the diaphragm to standardised lung volumes To use ultrasound in a dynamic study of the contraction and relaxation of the diaphragm	100% M	34 ± 9	25 ± 3	Healthy, no specific training
Kaneko et al 2009	Japan	To assess the function of respiratory muscles during breathing exercises by ultrasonography To assess if marked recruitment of respiratory muscles could be distinguished using ultrasonography	100% M	21.8 ± 1.9		Healthy
Baldwin et al 2011	Australia	To validate ultrasound measurement of diaphragm and peripheral muscle thickness	46% M			Healthy, Caucasian volunteers
						CRITICALLY ILL
Grosu et al 2012	USA	To quantify the rate and degree of diaphragm thinning during MV using daily sonographic assessments	86% M			Newly intubated patients (Within 2hrs)
Vivier et al 2012	Switzerland	To assess the feasibility and accuracy of ultrasonography to assess diaphragmatic function and its contribution to respiratory workload in critically ill patients under non-invasive ventilation	58% M			Patients eligible for study: Intubated for 48h/more, who tolerated 1h of spontaneous breathing trials after recovery from their acute disease or were extubated
Cartwright at al 2013	USA	To assess the ultrasonographic changes that occur in muscles during ICU hospitalization	44% M	59.3	30	Intubated patients on MV for acute respiratory failure

Measurement method

The method used to measure diaphragm thickness using ultrasound is reported and includes a description of procedure familiarisation; whether instructions were given to subjects before testing; the body position of the subject; and the number of images captured under the heading “testing protocol.” We also describe the exact placement of the ultrasound probe during imaging and report on imaging performed at different points during the cycle of breathing. The measurement method will also cover the parameters used to measure the thickness of the diaphragm and the data processed by the operators.

Testing Protocol

Only *Kaneko et al*. (2009) familiarised subjects with the testing procedure and provided subjects with instructions for testing. Subject testing positions varied. Subjects were seated in two papers (33%) (Grosu et al, 2012; Kaneko et al, 2009) and semi-recumbent in two papers (33%) (Vivier et al, 2012; Baldwin et al, 2011). Subject position was not reported on in the remaining two papers (33%) (Cartwright et al, 2013; Orozco-Levi et al, 2010). The number of diaphragm thickness images captured also varied. Three papers (50%) captured three images (Grosu et al, 2012; Baldwin et al, 2011; Kaneko et al, 2009), one paper (17%) captured more than three images (Vivier et al, 2012) and two papers (33%) did not report on the number of images captured (Cartwright et al, 2013; Orozco-Levi et al, 2010). Measurements of diaphragm thickness appear to be once off measurements (Vivier et al, 2012; Orozco-Levi et al, 2010; Kaneko et al, 2009; Baldwin et al, 2009), as seen in four of the papers (67%), with only two papers (33%) conducting follow up measurements of diaphragm thickness (Cartwright et al, 2013; Grosu et al, 2012). (Table 2.3)

Probe Placement

Some similarities were detected with the probe position when using ultrasound. All papers, except *Cartwright et al.* (2013), measured diaphragm thickness by placing the probe perpendicularly, in the mid/anterior axillary line and imaging the diaphragm through the zone of apposition. Differences were detected with the probe position in terms of which intercostal space was used as a reference level for imaging. Two papers (33%) used the eighth intercostal space (Cartwright et al, 2013; Kaneko et al, 2009), two papers (33%) used the ninth intercostal space (Baldwin et al, 2011; Kaneko et al, 2009) and two papers (33%) used the tenth intercostal space (Vivier et al, 2012; Orozco-Levi et al, 2010). Three papers (50%) performed measurements from the right hand side (Grosu et al, 2012; Vivier et al, 2012; Baldwin et al, 2009). (Table 2.4)

Table 2.2: Summary of the equipment used to measure diaphragm thickness

Study	What measured		Equipment used		Frequency (MHz)						US Mode	
	Tdi	MVRdi	Curvilinear transducer	Linear transducer	7.5	7.0-10.0	7.5-10	10	12	18	B-mode	M-mode
Orozco-Levi et al 2010	Y	Y	Y	N	Y	N	N	N	N	N	Y	Y
Kaneko et al 2009	Y	N	N	Y	N	Y	N	N	N	N	Y	N
Baldwin et al 2011	Y	N	N	Y	N	N	N	Y	N	N		
Grosu et al 2012	Y	N			N	N	Y	N	N	N	Y	N
Vivier et al 2012	Y	N	N	Y	N	N	N	N	Y	N		
Cartwright at al 2013	Y	N	N	Y	N	N	N	N	N	Y		

*Y = Yes; N = No; Blank = Not reported; US = Ultrasound; Tdi = diaphragm thickness; MVRdi = maximum velocity of relaxation of the diaphragm

Table 2.3: Summary of the testing protocol for measurements of diaphragm thickness

Study	Procedure Familiarisation	Instructions to subject	Subject Position		Number of images captured		Once off measurement	Follow up Measurements
			Seated	Semi-recumbent	X 3	>3		
Orozco-Levi et al 2010	N						Y	N
Kaneko et al 2009	Y		Y	N	Y	N	Y	N
Baldwin et al 2011	N	N	N	Y	Y	N	Y	N
Grosu et al 2012	N	N	Y	N	Y	N	N	Y
Vivier et al 2012	N	N	N	Y	N	Y	Y	N
Cartwright at al 2013							N	Y

*Y = Yes; N = No; Blank = Not reported; X3 = 3 Repetitions; >3 = More than 3 repetitions

Cycle of Breathing

Four papers (67%) measured diaphragm thickness at end expiration (Grosu et al, 2012; Vivier et al, 2012; Baldwin et al, 2011; Kaneko et al, 2009), two papers (33%) measured at end inspiration (Vivier et al, 2012; Kaneko et al, 2009) and one paper (17%) measured diaphragm thickness during breathing exercises (Kaneko et al, 2009). *Orozco-Levi et al.* (2010) measured diaphragm thickness from residual volume to total lung capacity at intervals equivalent to 20% of the functional residual capacity. *Baldwin et al.* (2011) measured diaphragm thickness at 25% of the inspiratory capacity and at 50% of the inspiratory capacity. Three papers (50%) measured diaphragm thickness at more than one point during the cycle of breathing (Vivier et al, 2012; Baldwin et al, 2011; Kaneko et al, 2009). (Table 2.4)

Thickness measurement parameters

Diaphragm thickness measurements were based on the structures included in the measurements. The diaphragm is bound by a limiting membrane on either side and these membranes were used as parameters for measurement. Two papers (33%) (Grosu et al, 2012; Orozco-Levi et al, 2010), measured diaphragm thickness as the distance from the middle of the diaphragmatic pleura to the middle of the peritoneal pleura, to the nearest 0.1 mm. Two papers (33%) (Cartwright et al, 2013; Vivier et al, 2012) did not mention the parameters they used to measure diaphragm thickness. Two papers (33%) (Baldwin et al, 2011; Kaneko et al, 2009) defined measurement parameters, but these parameters were unclear and therefore not reproducible.

Data Processing

Three papers (50%) assessed ultrasound measurement reliability (Vivier et al, 2012; Baldwin et al, 2011; Orozco-Levi et al, 2010), and achieved an ICC=0.99 (Baldwin et al, 2011; Orozco-Levi et al, 2010) and an ICC>0.97 (Vivier et al, 2012). Four papers (67%) used an experienced operator for ultrasound measurements of diaphragm thickness (Cartwright et al, 2013; Grosu et al, 2012; Vivier et al, 2012; Baldwin et al, 2011). Four papers (67%) used the mean value of diaphragm thickness measurements (Cartwright et al, 2013; Vivier et al, 2012; Orozco-Levi et al, 2010; Kaneko et al, 2009) and one paper (17%) used the maximum value of diaphragm thickness measurements (Grosu et al, 2012). In papers reviewed, diaphragm thickness ranged from 1.38mm (0.41) at residual volume to 4.37mm (0.97) at end inspiration; depending on when during the breathing cycle measurements were taken. Two papers (33%) (Vivier et al, 2012; Kaneko et al, 2009) calculated the thickening fraction and two papers (33%) assessed the quality of images taken (Vivier et al, 2012; Baldwin et al, 2011). (Table 2.5)

Table 2.4: Summary of probe placement and cycle of breathing

Study	When Measured						Probe Position						
	RV → TLC	During breathing exercise	End Inspiration	End Expiration	25 % IC	50 % IC	Right	Probe held perpendicular	Mid/ant axillary line	Imaged in ZOA	8^{th} I/C space	9^{th} I/C space	10^{th} I/C space
Orozco-Levi et al 2010	Y	N	N	N	N	N		Y	Y	Y	N	N	Y
Kaneko et al 2009	N	Y	Y	Y	N	N		Y	Y	Y	Y	Y	N
Baldwin et al 2011	N	N	N	Y	Y	Y	Y	Y	Y	Y	N	Y	N
Grosu et al 2012	N	N	N	Y	N	N	Y	Y	Y	Y			
Vivier et al 2012	N	N	Y	Y	N	N	Y	Y	Y	Y	N	N	Y
Cartwright at al 2013									Y		Y	N	N

*Y = Yes; N = No; Blank = Not reported; RV = Residual volume, TLC = Total lung capacity; IC = Inspiratory capacity, ZOA = Zone of apposition, I/C = Intercostal space; RV → TLC = Ultrasound images captured from residual volume to total lung capacity, at intervals equivalent to 20% of the forced vital capacity

Table 2.5: Summary of the data processed by operators

Study	Experienced Operator	Value used		Quality of images	Reliability	Calculated Index (TF)
		Mean	Max			
Orozco-Levi et al 2010		Y	N	N	Y	N
Kaneko et al 2009		Y	N	N	N	Y
Baldwin et al 2011	Y	N	N	Y	Y	N
Grosu et al 2012	Y	N	Y	N	Y	N
Vivier et al 2012	Y	Y	N	Y	Y	Y
Cartwright at al 2013	Y	Y	N	N	N	N

*Y = Yes; N = No; Blank = Not reported; TF = Thickening fraction

Current Understanding of Diaphragm Thickness Measurements

The papers included in this review were conducted in healthy and critically ill populations. Similarities and differences were found among both populations, regarding measurements of diaphragm thickness. Factors that could have an impact on the thickness of the diaphragm were not consistently reported. These factors include subject age and BMI (Polla et al, 2004; Scott et al, 2001). The structures included in the measurement of diaphragm thickness were not clearly defined and parameters used for thickness measurements were inconsistent between papers. This may affect future studies, as “normal” values reported for diaphragm thickness will depend on what structures were included in the measurements, as well as when during the breathing cycle the measurements were taken. The imaging frequency and intercostal space probe placement differed among papers. The eighth, ninth and tenth intercostal spaces were used, but this may be due to improved image quality of the diaphragm in the different spaces. Linear probes were most frequently used for imaging, although curvilinear probes can also be used for deeper penetration if necessary. Probe placement, excluding intercostal space probe placement, and subject position were consistent between papers. The exact point during the breathing cycle when images of the diaphragm were captured differed greatly among papers. This may be due to the various study aims. Comparison of diaphragm measurements between papers is not possible.

Recommendations for future studies using ultrasound to image the diaphragm should clearly state their study aim and the exact technique they have chosen to use. If the technique is based on a previous study, this needs to be stated, as well as any deviations from the reproduced technique and the reason for the deviation. Future studies need to define the structures included in thickness measurements of the diaphragm, too help improve generalizability of thickness results in similar populations tested.

The literature reviewed on the measurement of diaphragm thickness will inform the choice of the technique described in Chapter 3 (Section 3.2.5).

2.3.4.2 Measurement of Diaphragm Strength

Six papers report on strength measurements of the diaphragm. Papers included measurements of diaphragm strength using the maximum inspiratory technique (PI_{max}) and the transdiaphragmatic technique. The papers on diaphragm strength were analysed according to the following subheadings: Subject demographics; equipment specifications; and measurement method. The “measurement method” was further divided in to: Instructions prior to testing; testing protocol; and data processing.

Subject demographics

Three papers (50%) tested diaphragm strength in healthy volunteers during exercise performance, in response to kinesiology taping and to assess the reliability of strength testing procedures (Zübeyir et al, 2012; Dimitriadis et al, 2011; Strongoli et al, 2010). The diaphragm strength was further evaluated in scuba divers (17%) (Ray et al, 2010) and police squad members (17%) (Sperlich et al, 2009). One paper (17%) tested the ability of adults, over a wide age range and multiple ethnicities, to perform diaphragm strength tests (Sachs et al, 2009). The papers were published in a wide variety of countries, with three papers (50%) published in the USA (Strongoli et al, 2010; Ray et al, 2010; Sachs et al, 2009). The six papers were published over a number of years, from 2009 to 2012. One paper (17%) included males only as part of their sample (Ray et al, 2010) and five papers (83%) included both males and females (Zübeyir et al, 2012; Dimitriadis et al, 2011; Strongoli et al, 2010; Sachs et al, 2009; Sperlich et al, 2009). The mean age was calculated in all six papers (100%) and the mean BMI was calculated in only two papers (33%) (Dimitriadis et al, 2011; Sperlich et al, 2009) (Table 2.6).

Equipment specifications

All six papers (100%) measured the PI_{max}, with only *Sperlich et al.* (2009) adhering to ATS guidelines (ATS/ERS Statement on Respiratory Muscle Testing, 2002) and *Strongoli et al.* (2010) using the Black and Hyatt technique (Black et al, 1969). *Strongoli et al.* (2010) also measured the “sniff” and the transdiaphragmatic pressure. The transdiaphragmatic pressure was measured using the balloon catheter method. All six papers (100%) measured PI_{max} at residual volume with a mouth pressure manometer. Three papers (50%) ensured that there was a hole in the mouth piece of the manometer (Ray et al, 2010; Strongoli et al, 2010; Sachs et al, 2009) (Refer to Table 2.7). The manometer system requires a small leak, approximately 2mm in internal diameter and 20-30mm in length, to prevent glottic closure during the PI_{max} manoeuvre (ATS/ERS Statement on Respiratory Muscle Testing, 2002).

Measurement Method

The method used to measure the strength of the diaphragm is reported and includes a description of the instructions given to participants prior to the testing day. We also report on procedure familiarisation; whether instructions were given to subjects before testing; subject position; the use of nose-clips; the number of repetitions performed; and when measurements were taken under the heading “testing protocol.” We also describe the data processed by the operators.

Table 2.6: Summary of papers measuring diaphragm strength

Study	Country	Study Objective	Gender (% M)	Age (Years) Mean SD	BMI (kg/m2) Mean SD	Activity Level
						SCUBA DIVERS
Ray et al 2010	USA	To assess whether respiratory muscle training reduces the work of breathing at depth	100% M	30.3 ± 6.0		Certified scuba divers
						WIDE ETHNIC RANGE
Sachs et al 2009	USA	To assess the ability of adults, over a wide age range and multiple ethnicities, to perform PI_{max} tests	49% M	66 (Mean)		Subjects over a wide age range and multiple ethnicities
						POLICE SQUAD
Sperlich et al 2009	Germany	To assess the effects of a 6-week high-intensity respiratory muscle training program on running performance and oxygen uptake (VO_2max)	71% M	24.9 ± 2.5	22.6 ± 2.2	German special forces police squad
						HEALTHY
Strongoli et al 2010	USA	To assess whether core exercises would produce a variety of transdiaphragmatic pressures. To assess whether the easy exercises would produce pressures sufficient for a training stimulus to the diaphragm	50% M	30 (Mean)		Healthy
Zübeyir et al 2012 Group 1 Group 2	Turkey	To assess the effect of kinesiology taping, applied to primary and accessory respiratory muscles, on respiratory muscle strength	61% M 71% M	21.0 ± 1.4 21.0 ± 1.3		University students
Dimitriadis et al 2011	UK	To assess the test/retest reliability of the MicroRPM portable manometer's measurements of PI_{max} and maximum expiratory pressure in the sitting and standing positions. To assess the number of expiratory maneuvers needed with the MicroRPM for reliability in PI_{max} and maximum expiratory pressure measurements	60% M	21.6 ± 1.1	20.3 ± 3.5	Physiotherapy students

Instructions prior to testing day

Only *Sperlich et al*. (2009) restricted food intake and physical exercise of subjects prior to the testing procedure. *Sperlich et al.* (2009) also instructed subjects to maintain a well hydrated state and to continue with their same diet and training regime. *Ray et al.* (2010) instructed subjects to start a new training program prior to testing. The remaining four papers (67%) gave no instructions prior to testing day (Zübeyir et al, 2012; Dimitriadis et al, 2011; Strongoli et al, 2010; Sachs et al, 2009). (Table 2.8)

Testing Protocol

All subjects were familiarised with the testing procedure, except subjects in the *Zübeyir et al.* (2012) study. Four papers (67%) provided the subject with testing instructions (Dimitriadis et al, 2011; Strongoli et al, 2010; Sachs et al, 2009; Sperlich et al, 2009). Subject body position varied between sitting and standing. Three papers (50%) performed tests with subjects seated (Dimitriadis et al, 2011; Sachs et al, 2009; Sperlich et al, 2009) and one paper (17%) had their subjects standing (Dimitriadis et al, 2011). Nose-clips were applied during testing in two of the papers (33%) (Sachs et al, 2009; Sperlich et al, 2009). Repetitions of PI_{max} measurements differed among papers. Three papers (50%) performed pre-intervention and post-intervention measurements of PI_{max} (Zübeyir et al, 2012; Ray et al, 2010; Sperlich et al, 2009), two papers (33%) performed three PI_{max} repetitions (Zübeyir et al, 2012; Strongoli et al, 2010), two papers (33%) performed five PI_{max} repetitions (Sachs et al, 2009; Sperlich et al, 2009) and one paper (17%) performed eighteen PI_{max} repetitions (Dimitriadis et al, 2011). The paper by *Dimitriadis et al.* (2011), performing eighteen repetitions, completed three test sessions, one week apart, with eighteen repetitions performed at each session. Operators allowed a thirty second interval between efforts and three minute intervals between test conditions (Sitting/Standing). In the paper by *Strongoli et al.* (2010), sniff and transdiaphragmatic measurements were repeated three and five times, respectively. Transdiaphragmatic pressure was measured at rest, as well as during measurements of PI_{max}, sniff and during exercise (Strongoli et al, 2010). (Table 2.9)

Data Processing

Four papers (67%) used an experienced operator to perform measurements of diaphragm strength (Zübeyir et al, 2012; Dimitriadis et al, 2011; Strongoli et al, 2010; Sachs et al, 2009). Of the diaphragm strength measurements recorded, one paper (17%) used mean values only (Sperlich et al, 2009), two papers (33%) used maximum values only (Zübeyir et al, 2012; Sachs et al, 2009), two papers (33%) used both the mean and maximum values (Dimitriadis et al, 2011; Strongoli et al, 2010) and one paper (17%) did not specify what was used (Ray et al 2010). Diaphragm strength values measured ranged from $60cmH_20$ to

166cm$H_2$0 between papers. *Strongoli et al.* (2010) used the peak transdiaphragmatic value obtained during each exercise and averaged all peak transdiaphragmatic measurements for analysis. *Sachs et al.* (2009) assessed the quality and reliability of measurements and *Sperlich et al.* (2009) assessed only the quality of measurements taken. (Table 2.10)

Current Understanding of Diaphragm Strength Measurements

The papers included in the diaphragm strength section of this review were done only in the healthy population, with no papers reported for in the critically ill population. Although all papers tested the PI_{max}, only two papers stipulated that they adhered to ATS guidelines (ATS/ERS Statement on Respiratory Muscle Testing, 2002) or used the Black and Hyatt (Black et al, 1969) technique. However, similarities in the testing technique were clear between papers. Similarities noted include PI_{max} measurements taken at residual volume, the use of a mouth pressure manometer and the familiarisation of subjects with the testing procedure. The importance of instructing and familiarising subjects with the testing procedure should not be neglected, as this may help to eliminate the effect of learning error on the overall results (Dimitriadis et al, 2011). Differences noted were the inconsistent use of nose-clips during testing and the variation between the sitting and standing position during testing. One study tested the strength of the diaphragm using transdiaphragmatic pressure. This is the gold-standard technique for diaphragm strength testing, as the diaphragm is isolated during testing. Although the PI_{max} test produces a more global result of inspiratory muscle strength, it seems this technique has been favoured in the recent literature when testinghealthy subjects.

Recommendations for future studies testing the strength of the diaphragm would be to adhere to guidelines already set, either the ATS guidelines (ATS/ERS Statement on Respiratory Muscle Testing, 2002) or the Black and Hyatt technique (Black et al, 1969). These guidelines are readily available and have been used in recent literature. However, if deviations from the guidelines are made, these need to be clearly stated in the literature so that future studies are able to reproduce any new technique used.

The literature reviewed on measuring the strength of the diaphragm will be used to inform the choice of technique described in Chapter 3 (Section 3.2.5). Although measuring the transdiaphragmatic pressure is a more direct measure of diaphragm strength, our study will make use of the PI_{max} technique, as performed in the majority of the papers.

Table 2.7: Summary of equipment used to measure diaphragm strength

Study	What Measured			Measured at RV	Manometer			Hole in manometer mouthpiece	Connected to pressure gauge	Catheter Method	ATS Guidelines	Black and Hyatt Technique
	PI_{max}	Pdi	Sniff		Water	Digital	Handheld					
Ray et al 2010	Y	N	N	Y	Y	N	N	Y	N	N	N	N
Sachs et al 2009	Y	N	N	Y				Y	Y	N	N	N
Sperlich et al 2009	Y	N	N	Y	N	Y				N	Y	N
Strongoli et al 2010	Y	Y	Y	Y				Y	Y	Y	N	Y
Zübeyir et al 2012	Y	N	N	Y	N	Y	Y		N	N	N	N
Dimitriadis et al 2011	Y	N	N	Y	N	Y	Y		N	N	N	N

*Y = Yes; N = No; Blank = Not reported; PImax = Maximum inspiratory pressure; Pdi = Transdiaphragmatic pressure; RV = Residual volume; ATS = American thoracic society

Table 2.8: Summary of instructions given to subjects prior to the testing day

Study	Restricted food intake 2h prior to testing	Restricted physical exercise 24h prior to testing	Maintain a well hydrated state	Continue with same diet/training regime	Start new training program prior to testing
Ray et al 2010	N	N	N	N	Y
Sachs et al 2009	N	N	N	N	N
Sperlich et al 2009	Y	Y	Y	Y	N
Strongoli et al 2010	N	N	N	N	N
Zübeyir et al 2012	N	N	N	N	N
Dimitriadis et al 2011	N	N	N	N	N

*Y = Yes; N = No; Blank = Not reported

Table 2.9: Summary of the testing protocol for measurements of diaphragm strength

Study	Procedure Familiarisation	Instructions to patient	Patient Position		Nose-clips	Reps						Pdi: When measured			
						PI_{max}				Sniff	Pdi	During Pimax	During Sniff	**During exercises** (5 reps each exercise)	Resting Pdi
			Seated	Standing		Pre-post	X 3	X 5	X18	X3	X5				
Ray et al 2010	Y	N				Y	N	N	N	N	N	N	N	N	N
Sachs et al 2009	Y	Y	Y	N	Y	N	N	Y	N	N	N	N	N	N	N
Sperlich et al 2009	Y	Y	Y	N	Y	Y	N	Y	N	N	N	N	N	N	N
Strongoli et al 2010	Y	Y				N	Y	N	N	Y	Y	Y	Y	Y	Y
Zübeyir et al 2012						Y	Y	N	N	N	N	N	N	N	N
Dimitriadis et al 2011	Y	Y	Y	Y		N	N	N	Y	N	N	N	N	N	N

*Y = Yes; N = No; Blank = Not reported; PI_{max} = Maximum inspiratory pressure; Pdi = Transdiaphragmatic pressure

Table 2.10: Summary of the data processed by operators

Study	Experienced Operator	Value used		Quality of measurements	Reliability
		Mean	Max		
Ray et al 2010					
Sachs et al 2009	Y	N	Y	Y	Y
Sperlich et al 2009		Y	N	Y	N
Strongoli et al 2010	Y	Y	Y	N	N
Zübeyir et al 2012	Y	N	Y	N	N
Dimitriadis et al 2011	Y	Y	Y	N	N

*Y = Yes; N = No; Blank = Not reported

2.3.4.3 Measurement of Diaphragm Endurance

11 papers report on the measurement of diaphragm endurance. Papers included measure the endurance of the diaphragm using the magnetic coil method, bilateral anterior magnetic phrenic stimulation and PI_{max}. Papers on diaphragm endurance were analysed according to the following subheadings: Subject demographics, equipment specifications and measurement method. The "measurement method" was further divided in to: Instructions prior to the testing day, testing protocol, stimulation specifics, timing of measurement and data processing.

Subject demographics

Five papers (45%) included the cycling population to describe exercise induced diaphragmatic fatigue and to determine whether there are sex differences in the prevalence and severity of exercise induced diaphragmatic fatigue (Guenette et al, 2010; Kabitz et al, 2010; Vogiatzis et al, 2008; Kabitz et al, 2008a; Kabitz et al, 2008b). Five papers (45%) evaluated the diaphragm endurance of healthy volunteers to describe the development of diaphragmatic fatigue and the effect of chest wall restriction on fatigue (Tomczak et al, 2010; Verges et al, 2010; Taylor et al, 2009; Verges et al, 2009; Renggli et al, 2008). One paper (9%) included swimmers, to determine the effect of different breathing frequencies on the fatigue of the diaphragm (Jakovljevic et al, 2009). The papers were published in a wide variety of countries, with three papers (27%) published in Germany (Kabitz et al, 2008a; Kabitz et al, 2010; Kabitz et al, 2008b). The 11 papers were published over a number of years, from 2008 to 2010. Ten papers (91%) included males only as part of their sample and one study (9%) included both males and females (Guenette et al, 2010). The mean age was calculated in all eleven papers (100%) and the mean BMI was calculated in three papers (27%) (Kabitz et al, 2010; Kabitz et al, 2008a; Kabitz et al, 2008b). (Table 2.11)

Equipment Specifications

Ten papers (91%) measured twitch transdiaphragmatic pressure using the double balloon catheter method, with balloons connected to the pressure transducer. Five papers (45%) (Kabitz et al, 2010; Guenette et al, 2010; Taylor et al, 2009; Kabitz et al, 2008a; Kabitz et al, 2008b;) used air-filled balloons and two papers (27%) (Guenette et al, 2010; Taylor et al, 2009) used the occlusion technique to assist in oesophageal catheter placement. Five papers (45%) did not report on air-filled balloons and nine papers (82%) did not report on the use of the occlusion technique. Three papers (27%) adhered to the ATS guidelines (ATS/ERS Statement on Respiratory Muscle Testing, 2002) for testing (Kabitz et al, 2010; Kabitz et al, 2008a; Kabitz et al, 2008b). *Taylor et al.* (2009) measured the maximal transdiaphragmatic pressure, in addition to twitch transdiaphragmatic pressure, using the double balloon catheter method, with balloons connected to a pressure transducer and filled with air. The occlusion technique was used by *Taylor et al.* (2009) to place the oesophageal catheter for maximal transdiaphragmatic pressure measurements. *Jakovljevic et al.* (2009) measured PI_{max} only, using a mouth pressure manometer. *Verges et al.* (2009) measured PI_{max}, in addition to twitch transdiaphragmatic pressure, using a mouth pressure manometer. (Table 2.12)

Measurement Method

The method used to measure the endurance of the diaphragm is reported and includes a description of the instructions given to participants prior to the testing day. We also report on subject position, the use of nose-clips, whether the mouth was closed or not and whether the subjects were given instructions prior to the test under the heading "testing protocol." We describe the use of the magnetic coil method for cervical and thoracic magnetic stimulation, whether supra-maximal stimulation was achieved, the use of bilateral anterior magnetic phrenic stimulation and whether the potentiated or un-potentiated method was used under the heading "stimulation specifics." Lastly we report on when the twitch transdiaphragmatic pressure was measured, the use of automated announcements and initial delays before stimulation as well as how many repetitions were performed under the heading "timing of measurements."

Instructions prior to testing day

All papers (82%), except *Vogiatzis et al,* (2008) and *Tomczak et al.* (2010), restricted either/all physical exercise; food intake and caffeinated beverages. Caffeinated beverages were restricted as they enhance inspiratory muscle endurance. Eight papers (73%) restricted physical exercise and food intake prior to testing (Verges et al, 2010; Jakovljevic et al, 2010; Kabitz et al, 2010; Taylor et al, 2009; Verges et al, 2009; Kabitz et al, 2008a; Kabitz et al, 2008b; Renggli et al, 2008). Three papers (27%) restricted caffeinated beverages on the same day as testing (Verges et al, 2010; Verges et al, 2009; Kabitz et al, 2008a) and two papers (18%) restricted caffeine intake as early as twenty four hours before testing (Kabitz et al, 2008b; Renggli et al, 2008). All subjects were familiarized with testing procedures, except subjects in the paper by *Vogiatzis et al*. (2008). (Table 2.13)

Table 2.11: Summary of papers measuring diaphragm endurance

Study	Country	Study Objective	Gender (% M)	Age (Years) Mean SD	BMI (kg/m2) Mean SD	Activity Level
						CYCLISTS
Vogiatzis et al 2008	Greece	To determine the contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists	100% M	31 ± 5		Competitive male cyclists
Kabitz et al 2008a	Germany	To assess whether diaphragmatic force-generation undergoes similar regulations during either whole-body-exercise or controlled hyperventilation, but differs during recovery	100% M	27 ± 2.5	BMI: 21.8 ± 1.3	Trained, amateur cyclists
Kabitz et al 2010	Germany	To assess diaphragmatic contractility and diaphragmatic fatigue during self-paced long-term exhaustive exercise at maximally tolerated loading by the use of supra-maximal twitch transdiaphragmatic pressure (TwPdi)	100% M	27.7 ± 1.9	BMI: 23.3 ± 1.9	Trained, amateur cyclists
Kabitz et al 2008b	Germany	To assess whether both diaphragmatic force-generation and diaphragmatic fatigue remain unchanged during consecutive exercise-trials	100% M	27.6 ± 2.5	BMI: 22.6 ± 2.3	Trained, amateur cyclists
Guenette et al 2010 Male Female	Canada	To determine whether there are sex differences in the prevalence and severity of exercise-induced diaphragmatic fatigue	52% M 43% M	27 ± 1 28 ± 1		Competitive, endurance trained cyclists Non-competitive endurance trained

						SWIMMERS
Jakovljevic et al 2009	UK	To assess the influence of different breathing frequencies on the severity of inspiratory muscle fatigue induced by high-intensity front crawl swimming	100% M	21.2 ± 1.9		Collegiate swimmers
						HEALTHY
Renggli et al 2008	Switzerland	To assess the development of inspiratory and expiratory muscle fatigue during normocapnic hyperpnoea	100% M	28 ± 6		Healthy, non-smoking
Tomczak et al 2010	Canada	To assess whether submaximal exercise with chest wall restriction, as a model of restrictive pulmonary disease, would result in diaphragm fatigue	100% M	30 ± 7		Healthy
Verges et al 2010	France	To assess the effect of different blood oxygenation levels on isolated hyperpnoea-induced respiratory muscle fatigue	100% M	31.8 ± 9.5		Healthy, non-smoking
Taylor et al 2009	UK	To assess whether expiratory resistive loading elicits inspiratory as well as expiratory muscle fatigue	100% M	29 ± 6		Healthy, non-smoking, recreationally active
Verges et al 2009 RMET IMT SHAM	Switzerland	To compare the effects of different respiratory muscle training regimes on fatigue-related variables during volitional hyperpnoea	100% M	28 ± 4 26 ± 6 26 ± 6		Moderately trained, non-smoking

*Table 2.11 continued

Table 2.12: Summary of equipment used to measure diaphragm endurance

Study	What Measured			Mouth pressure manometer	Double balloon catheter method	Balloons connected to pressure transducer	Air-filled balloons	Occlusion technique	ATS Guidelines
	Pdi,tw	Pdi,max	PI_{max}						
Vogiatzis et al 2008	Y	N	N	N	Y	Y			N
Kabitz et al 2008a	Y	N	N	N	Y	Y	Y		Y
Kabitz et al 2010	Y	N	N	N	Y	Y	Y		Y
Kabitz et al 2008b	Y	N	N	N	Y	Y	Y		Y
Guenette et al 2010	Y	N	N	N	Y	Y	Y	Y	N
Jakovljevic et al 2009	N	N	Y	Y	N	N	N	N	N
Renggli et al 2008	Y	N	N	N	Y	Y			N
Tomczak et al 2010	Y	N	N	N	Y	Y			N
Verges et al 2010	Y	N	N	N	Y	Y			N
Taylor et al 2009	Y	Y	N	N	Y	Y	Y	Y	N
Verges et al 2009	Y	N	Y	Y	Y	Y			N

*Y = Yes; N = No; Blank = Not reported; Pdi,tw = Twitch transdiaphragmatic pressure; Pdi,max = Maximum transdiaphragmatic pressure; PI_{max} = Maximum inspiratory pressure; ATS = American thoracic society

Table 2.13: Summary of instructions given to subjects prior to the testing day

Study	Restricted physical exercise			Restricted food intake		Restricted caffeinated beverages				Procedure Familiarization
	Same day	24h before	48h before	2h before	3h before	Sam e day	6h before	12h before	24h before	
Vogiatzis et al 2008	N	N	N	N	N	N	N	N	N	N
Kabitz et al 2008a	N	Y	N	Y	N	Y	N	N	N	Y
Kabitz et al 2010	N	Y	N	Y	N	N	Y	N	N	Y
Kabitz et al 2008b	N	Y	N	Y	N	N	N	N	Y	Y
Guenette et al 2010	N	N	N	N	N	N	N	N	N	Y
Jakovljevic et al 2009	Y	Y	N	Y	N	N	N	N	N	Y
Renggli et al 2008	N	Y	Y	Y	N	N	N	N	Y	Y
Tomczak et al 2010	N	N	N	N	N	N	N	N	N	Y
Verges et al 2010	N	Y	Y	Y	N	Y	N	N	N	Y
Taylor et al 2009	N	N	Y	N	Y	N	N	Y	N	Y
Verges et al 2009	N	Y	Y	Y	N	Y	N	N	N	Y

*Y = Yes; N = No; Blank = Not reported

Table 2.14: Summary of the testing protocol used to measure diaphragm endurance

Study	Position				Nose-clips used	Mouth Closed	Instructions to patient
	Prone	Seated	Upright	Neck Flexed			
Vogiatzis et al 2008	N	Y	N	N	Y	Y	Y
Kabitz et al 2008a	N	Y	N	N	Y	Y	Y
Kabitz et al 2010	N	Y	N	N	Y	Y	Y
Kabitz et al 2008b	N	Y	N	N	Y	Y	Y
Guenette et al 2010	N	Y	N	Y	N	N	Y
Jakovljevic et al 2009	N	N	Y	N		N	Y
Renggli et al 2008	Y	Y	N	N	Y		Y
Tomczak et al 2010	N	Y	N	Y			Y
Verges et al 2010	Y	Y	N	N			Y
Taylor et al 2009	N	Y	N	Y			Y
Verges et al 2009	Y	Y	N	N			Y

*Y = Yes; N = No; Blank = Not reported

Testing Protocol

All subjects were informed regarding testing procedures. For thoracic magnetic stimulation measurements, subjects were positioned in prone in three out of the four papers (75%) in which thoracic magnetic stimulation was performed (Verges et al, 2010; Verges et al, 2009; Renggli et al, 2008). For cervical magnetic stimulation measurements, subjects were seated in all six papers (100%) in which cervical magnetic stimulation was performed (Guenette et al, 2010; Tomczak et al, 2010; Verges et al, 2010; Taylor et al, 2009; Verges et al, 2009; Renggli et al, 2008). Three out of the six papers (50%) in which cervical magnetic stimulation was performed had their subjects seated, with their necks flexed (Guenette et al, 2010; Tomczak et al, 2010; Taylor et al, 2009). In the four papers in which bilateral anterior magnetic phrenic stimulation was conducted, subjects were seated and nose-clips were used (Kabitz et al, 2010; Vogiatzis et al, 2008; Kabitz et al, 2008a; Kabitz et al, 2008b). The upright position was only used in one paper (9%), in which PI_{max} was calculated as a measure of diaphragm endurance (Jakovljevic et al, 2009). (Table 2.14)

Stimulation specifics

The magnetic stimulation coil method was used to perform cervical magnetic stimulation in six papers (55%) (Guenette et al, 2010; Tomczak et al, 2010; Verges et al, 2010; Taylor et al, 2009; Verges et al, 2009; Renggli et al, 2008) and thoracic magnetic stimulation in four papers (36%) (Guenette et al, 2010; Verges et al, 2010; Verges et al, 2009; Renggli et al, 2008). In the remaining four papers (36%), bilateral anterior magnetic phrenic stimulation at 100% power output was used (Kabitz et al, 2010; Vogiatzis et al, 2008; Kabitz et al, 2008a; Kabitz et al, 2008b). Stimulation sites varied between papers. Six papers (55%) stimulated the phrenic nerve at the neck or, more specifically in three papers (27%), at the level of C7 (Verges et al, 2010; Verges et al, 2009; Renggli et al, 2008). Other stimulation sites include the posterior border of sternocleidomastoid used in one paper (9%) (Vogiatzis et al, 2008) or at the site where the highest twitch transdiaphragmatic pressure was generated, used in three papers (27%) (Guenette et al, 2010; Tomczak et al, 2010; Taylor et al, 2009). Supra-maximal stimulation was achieved in nine papers (82%) (Guenette et al, 2010; Kabitz et al, 2010; Tomczak et al, 2010; Verges et al, 2010; Taylor et al, 2009; Verges et al, 2009; Kabitz et al, 2008a; Kabitz et al, 2008b; Renggli et al, 2008). The un-potentiated measurement method was performed in eight papers (73%) (Guenette et al, 2010; Kabitz et al, 2010; Tomczak et al, 2010; Verges et al, 2010; Verges et al, 2009; Kabitz et al, 2008a; Kabitz et al, 2008b; Renggli et al, 2008) and the potentiated method was performed in two papers (15%) (Tomczak et al, 2010; Taylor et al, 2009). (Table 2.15)

Timing of measurement

Ten papers (91%) measured twitch transdiaphragmatic pressure before the task and immediately after task completion. One paper (9%) measured twitch transdiaphragmatic pressure before the task and at ten minutes after task completion (Guenette et al, 2010). Five papers (45%) measured twitch transdiaphragmatic pressure during the task (Kabitz et al, 2010; Verges et al, 2009; Kabitz et al, 2008a; Kabitz et al, 2008b; Renggli et al, 2008), two papers (18%) at task failure (Verges et al, 2009; Renggli et al, 2008), two papers (18%) at one minute after the task (Taylor et al, 2009; Renggli et al, 2008) and three papers at ten minutes after the task (Guenette et al, 2010; Tomczak et al, 2010; Vogiatzis et al, 2008). One paper (9%) measured twitch transdiaphragmatic pressure at fifteen minutes after the task (Taylor et al, 2009), seven papers (64%) at thirty minutes after the task (Guenette et al, 2010; Tomczak et al, 2010; Verges et al, 2010; Taylor et al, 2009; Verges et al, 2009; Vogiatzis et al, 2008; Renggli et al, 2008) and four papers (36%) at sixty minutes after the task (Guenette et al, 2010; Verges et al, 2009; Vogiatzis et al, 2008; Renggli et al, 2008). Three papers (27%) made use of automated announcements during measurements (Kabitz et al, 2010; Kabitz et al, 2008a; Kabitz et al, 2008b). Two papers had an initial fifteen second delay before automated announcements (Kabitz et al, 2008a; Kabitz et al, 2008b) and one paper (9%) had an initial delay of twenty two and a half seconds before automated announcements (Kabitz et al, 2010). Four papers (36%) had twitch stimulations every thirty seconds (Taylor et al, 2009; Vogiatzis et al, 2008; Kabitz et al, 2008a; Kabitz et al, 2008b) and one paper (9%) had twitch stimulations every forty five seconds (Kabitz et al, 2010). The total amount of twitch stimulations varied greatly between papers. *Jakovljevic et al.* (2009) measured PI_{max} before the task, directly after the task and again at one minute after the task as a measure of diaphragm endurance (Table 2.16)

Data Processing

Seven papers (64%) used an experienced operator to conduct the tests (Kabitz et al, 2010; Verges et al, 2010; Verges et al, 2009; Vogiatzis et al, 2008; Kabitz et al, 2008a; Kabitz et al, 2008b; Renggli et al, 2008). The remaining four papers (36%) did not report on who performed the measurements (Guenette et al, 2010; Tomczak et al, 2010; Jakovljevic et al, 2009; Taylor et al, 2009). All 11 papers used the mean value and 10 papers (91%) considered the quality of measurements taken. The different aims of the study make it difficult to establish a range for diaphragm endurance values. Four papers (36%) calculated relative reduction indexes for final measurements (Taylor et al, 2009; Verges et al, 2009; Jakovljevic et al, 2009; Renggli et al, 2008). (Table 2.17)

Table 2.15: Summary of the stimulation specifics when measuring diaphragm endurance

Study	Magnetic Stimulation coil method	Stimulation sites					Supra-maximal Stimulation	BAMPS @ 100% power output	CMS	TMS	Potentiated Method	Un-potentiated Method
		Posterior border SCM	At the neck	C 7	T10	Site of highest Pdi,tw						
Vogiatzis et al 2008	N	Y	N	N	N	N	N	Y	N	N	?	?
Kabitz et al 2008a	N	N	Y	N	N	N	Y	Y	N	N	N	Y
Kabitz et al 2010	N	N	Y	N	N	N	Y	Y	N	N	N	Y
Kabitz et al 2008b	N	N	Y	N	N	N	Y	Y	N	N	N	Y
Guenette et al 2010	Y	N	N	N	N	Y	Y	N	Y	Y	N	Y
Jakovljevic et al 2009	N	N	N	N	N	N	N	N	N	N	N	N
Renggli et al 2008	Y	N	N	Y	Y	N	Y	N	Y	Y	N	Y
Tomczak et al 2010	Y	N	N	N	N	Y	Y	N	Y	N	Y	Y
Verges et al 2010	Y	N	N	Y	Y	N	Y	N	Y	Y	N	Y
Taylor et al 2009	Y	N	N	N	N	Y	Y	N	Y	N	Y	N
Verges et al 2009	Y	N	N	Y	Y	N	Y	N	Y	Y	N	Y

*Y = Yes; N = No; Blank = Not reported; SCM = Sternocleidomastoid; Pdi,tw = Twitch transdiaphragmatic pressure; BAMPS = Bilateral anterior magnetic phrenic stimulation; CMS = Cervical magnetic stimulation; TMS = Thoracic magnetic stimulation

Table 2.16: Summary of the timing of measurements

Study	Pdi, tw measured									Twitch stimulations					
	Before task	During task	After task	Task Failure	1min after	10mins after	15mins after	30mins after	60mins after	Automated announcements	Initial Delay		Reps		Total
											15s	22.5s	Every30s	Every45s	
Vogiatzis et al 2008	Y	N	Y	N	N	Y	N	Y	Y	N	N	N	Y	N	8-10 per time point
Kabitz et al 2008a	Y	Y	Y	N						Y	Y	N	Y	N	30
Kabitz et al 2010	Y	Y	Y	N						Y	N	Y	N	Y	82
Kabitz et al 2008b	Y	Y	Y	N						Y	Y	N	Y	N	93
Guenette et al 2010	Y	N	N	N	N	Y	N	Y	Y	N	N	N	N	N	5 per time point
Jakovljevic et al 2009	Y	N	Y	N	Y	N	N	N	N	N	N	N	N	N	Pre: x5 Post:x2
Renggli et al 2008	Y	Y	Y	Y	Y	N	N	Y	Y	N	N	N	N	N	9 per time point at FRC
Tomczak et al 2010	Y	N	Y	N	N	Y	N	Y	N	N	N	N	N	N	NP: X8 P: X5
Verges et al 2010	Y	N	Y	N	N	N	N	Y	N	N	N	N	N	N	X6 per time point
Taylor et al 2009	Y	N	Y	N	Y	N	Y	Y	N	N	N	N	Y	N	P: x6
Verges et al 2009	Y	Y	Y	Y	N	N	N	Y	Y	N	N	N	N	N	9 per time point at FRC

*Y = Yes; N = No; Blank = Not reported; Pdi,tw = Twitch transdiaphragmatic pressure; FRC = Functional Residual Capacity; NP = Non-potentiated; P=Potentiated

Table 2.17: Summary of data processed by the operators

Study	Experienced Operator	Values used		Quality of measurement considered	Calculated index/relative reduction pre-post
		Mean	Maximum		
Vogiatzis et al 2008	Y	Y	Y (Average value of the 3x measurements with highest Pdi,tw)	Y	N
Kabitz et al 2008a	Y	Y	N	Y	N
Kabitz et al 2010	Y	Y	N	Y	N
Kabitz et al 2008b	Y	Y	N	Y	N
Guenette et al 2010		Y	N	Y	N
Jakovljevic et al 2009		Y	N	Y	Y
Renggli et al 2008	Y	Y	N	N	Y
Tomczak et al 2010		Y	N	Y	N
Verges et al 2010	Y	Y	N	Y	N
Taylor et al 2009		Y	N	Y	Y
Verges et al 2009	Y	Y	N	Y	Y

*Y = Yes; N = No; Blank = Not reported; Pdi, tw = Twitch transdiaphragmatic pressure

Current Understanding of Diaphragm Endurance Measurements

The papers included in the diaphragm endurance section of this review were done only in the healthy population, with no papers reported for in the critically ill population. Similarities and differences were found among papers. All papers tested the twitch transdiaphragmatic pressure, but only three papers stipulated adhering to the ATS guidelines, despite numerous similarities found between papers. This suggests that guidelines may have been adhered to, but were not stated. The majority of the papers made use of the cervical magnetic stimulation method as well as bilateral anterior magnetic phrenic stimulation to measure the twitch transdiaphragmatic pressure. However, the PI_{max} and transdiaphragmatic pressure were also used to calculate the endurance of the diaphragm. These techniques can be found in the ATS guidelines and have been implemented in recent literature. Differences noted were the inconsistent use of air filled balloons, the use of the occlusion technique to assist in the placement of the oesophageal balloon, the use of nose-clips and the need for the subject to close their mouth. Further discrepancies were noted in the total number of twitch stimulations performed and exactly when during the task these stimulations need to be performed. These discrepancies, however, may be due to the overall aim of the study. Papers were consistent with regards to instructions to participants prior to the testing day, familiarising subjects with the testing technique, subject position and the data processed by operators. The quality of measurements was also taken into account. All papers that mentioned the operator involved in the testing, stipulated that they be skilled and experienced.

Recommendations for future studies testing the endurance of the diaphragm should state the aim of their study clearly and the exact technique they have used. Papers need to include whether they have incorporated any of the readily available guidelines into their study or if they based their techniques on previous studies done. This is important to assist future research in this area.

The literature reviewed on measurements of diaphragm endurance will be used to inform the choice of technique described in Chapter 3 (Section 3.2.5). The majority of the papers used the twitch transdiaphragmatic pressure to evaluate the endurance of the diaphragm. Due to the complex and highly specialized nature of the twitch transdiaphragmatic technique, a skilled operator is required. One paper in the review did, however, measure the PI_{max} before and after a fatiguing task to determine the endurance of the diaphragm. A similar technique will be used in the main study.

2.4 CONCLUSION

No studies were found investigating the relationship between diaphragm thickness, diaphragm strength and diaphragm endurance. This review identified that guidelines for the measurement of diaphragm function do exist in the healthy and critically ill population, but that they are not being adhered to by the majority of papers. Procedures for the measurement of diaphragm function are not fully described in all papers, thereby affecting the reproducibility of techniques and interpretation of the measurements.

Chapter 3:

RESEARCH MANUSCRIPT

This chapter was prepared as a manuscript for submission to *BMC Critical Care* under the title **"An Investigation into the Use of Ultrasound as a Surrogate Measure of Diaphragm Strength."** (Addendum N)

3.1 INTRODUCTION

The structural and functional characteristics of respiratory muscle fibres change in response to demands (Scott et al, 2001). Typically the diaphragm has equal distribution of fast and slow twitch muscles fibres due to its function (Polla et al, 2004). Slow fibres are resistant to fatigue and have a high oxidative metabolism, whereas fast fibres are easily fatigable and have a more glycolytic metabolism (Polla et al, 2004). The resistance to fatigue depends on the balance between energy production and utilisation (Polla et al, 2004). The metabolic and contractile plasticity of respiratory muscle fibres in response to stimuli allows for adaptation to different functional tasks (Scott et al, 2001). Several conditions have been identified as a stimulus for modification of muscle fibres. The conditions include training;-disuse;-adaptation to hypoxia; -malnutrition; -age related changes; -pharmacological agents and ventilatory support (Jaber et al, 2011b; Polla et al, 2004).

In the intensive care unit population, approximately 40% of patients require mechanical ventilation and 20-25% of these patients will encounter difficulties in the discontinuation of mechanical ventilation (Grosu et al, 2012). Age above 70 years, higher illness severity at discontinuation onset and a longer duration of mechanical ventilation prior to extubation, are some predisposing factors to extubation failure (Thille et al, 2011; Sohl et al, 2004). Drug administration, nutritional status and the presence of co-morbidities have also been reported to affect successful discontinuation of mechanical ventilation (Jaber et al, 2011b). Mechanical ventilation may have harmful effects on the diaphragm (Jaber et al, 2011b). Animal and human studies have shown diaphragmatic inactivity associated with mechanical ventilation, a condition referred to as ventilator induced diaphragmatic dysfunction (Jaber et al, 2011a; Jaber et al, 2011b, Petrof et al, 2010). Ventilator induced diaphragmatic dysfunction is defined as a reduction in the force generating capacity of the diaphragm (Grosu et al, 2012). Muscle fibre atrophy and muscle fibre injury have also been documented (Jaber et al, 2011b, Petrof et al, 2010).

The relationship between the decreased force generating capacity of the diaphragm and muscle fibre atrophy is unclear (Jaber et al, 2011b). *Jaber et al.* (2011b) found that the mechanical ventilation induced decreases in the force generating capacity of the diaphragm could not be ascribed to atrophy alone. The force loss was persistent even after correcting for any reductions in muscle cross sectional area (Jaber et al, 2011b; Petrof et al, 2010). The findings by *Jaber et al.* (2011b) confirm findings by *Sassoon et al.* (2002) that suggest that the two responses can be dissociated from one another.

Muscle endurance and its relationship to the duration of mechanical ventilation have also been described (Chang et al, 2005). Patients who had received mechanical ventilation for more than 48 hours had reduced inspiratory muscle endurance that worsened with the duration of mechanical ventilation (Chang et al, 2005). This suggests that patients who require prolonged mechanical ventilation are at risk of respiratory muscle fatigue (Petrof et al, 2010), which is still present after successful discontinuation of mechanical ventilation (Chang et al, 2005).

A major challenge in determining ventilator induced diaphragmatic dysfunction in humans is the ability to accurately evaluate respiratory muscle function in critically ill mechanically ventilated patients (Jaber et al, 2011a). Diaphragm muscle strength has been measured using a variety of methods (ATS/ERS Statement on Respiratory Muscle Testing, 2002). Volitional tests of respiratory muscle strength give an estimate of inspiratory and expiratory muscle strength, are relatively easy to perform and are often well tolerated by patients (ATS/ERS Statement on Respiratory Muscle Testing, 2002). However, a disadvantage of volitional tests is that they require full patient co-operation and are poorly reproducible, particularly in the intubated, critically ill population (Grosu et al 2012; ATS/ERS Statement on Respiratory Muscle Testing, 2002). Non-volitional tests of diaphragm muscle strength include phrenic nerve stimulation (ATS/ERS Statement on Respiratory Muscle Testing, 2002 et al 2002). Phrenic nerve stimulation is an invasive test specific to the diaphragm, but the technique requires considerable skill, is uncomfortable for the patient and difficult to achieve in clinical settings, such as the intensive care unit (ATS/ERS Statement on Respiratory Muscle Testing, 2002).

Measurements of diaphragm thickness using ultrasound imaging may be a suitable alternative for interpreting diaphragm function (Matamis et al, 2013; Grosu et al, 2012; Unger, 2012; Vivier et al, 2012; Moreau et al, 2010; Cohn et al, 1997; De Bruin et al, 1997, McCool et al, 1997; Ueki et al, 1995). The relationship between muscle thickness measurements and function has been investigated in selected populations (Unger, 2012; Moreau et al, 2010). In children with cerebral palsy, thickness measurements of vastus lateralis muscle thickness were predictive of the strength (Moreau et al, 2010), while transverse abdominus muscle thickness correlated significantly with the ability to execute sit-ups (Unger, 2012). In the ICU setting *Grosu et al.* (2012) also used ultrasound to measure the thickness of the diaphragm and found that thinning of the diaphragm begins within 48 hours after the initiation of mechanical ventilation. However, uncertainty exists with regards to any existing correlation between dimension and force of the diaphragm. A study on maximal inspiratory pressures and dimensions of the diaphragm in healthy individuals and weightlifters found that the weightlifters had greater pressures and thicker diaphragms than adults of similar stature who had not trained with weights (McCool et al, 1997). A study by *De Bruin et al.* (1997) on diaphragm thickness and inspiratory muscle strength in patients with Duchenne muscular dystrophy found that the resting diaphragm thickness, measured using ultrasound, was increased in patients with Duchenne muscular dystrophy and accompanied by impaired respiratory muscle force (De Bruin et al, 1997). The correlation between diaphragm strength and thickness remains unclear (Grosu et al, 2012).

To inform future research in this area, the correlation between the variables; thickness, strength and endurance, must be established. The primary aim of this paper is to investigate the correlation between diaphragm thickness, strength and endurance in young, healthy individuals.

3.2 MATERIALS AND METHODS

3.2.1 Trial design

Descriptive, correlational trial design.

3.2.2 Research setting

University in the Cape Metropole, Western Cape, South Africa

3.2.3 Ethical considerations

Institutional approval was granted (S13/05/111) and all participants provided written informed consent (Addendum D)

3.2.4 Sample

A sample of convenience was used. We recruited university students between the ages of 19 – 24 years during September 2013 – October 2013

University students were enrolled if they were the correct age, healthy and took part at a university level in endurance type sports; strength type sports or lead a sedentary lifestyle.

Students that participated at a university sporting level followed a structured training regime as per described by the respective coaches. Students at a sedentary level followed no structured training regime. Students in each category were matched for age and BMI. Students were excluded if they had any type of surgical procedure within 12 months prior to the study; on any medication or were suffering from any type of neurological condition (e.g. Myasthenia Gravis); respiratory condition (e.g. Cystic Fibrosis, Asthma) or orthopaedic condition (e.g. Ankylosing Spondylitis) affecting mobility of the thorax or hindering respiratory function.

3.2.5 Measurements

Procedure: Letters (Addendum E), together with the research proposal, were sent to the sporting club managers. Each manager then selected 15 participants each, based on the inclusion criteria, for recruitment into the study. The sedentary group was recruited by the PI from the third year physiotherapy class at the university. The primary investigator scheduled two separate meetings with each of the groups. At the first meeting, aims and objectives of the study were explained and informed consent was obtained (Addendum F). Subjects were asked to refrain from strenuous physical activity and food intake two hours prior to testing to ensure maximal comfort.

At the second meeting, baseline measurements (height, weight, age, activity level) were recorded and diaphragm thickness, strength and endurance were measured (Addendum G). Measurements were completed in the same order by the primary investigator. Inter-rater reliability (ICC agreement = 0.99) was established in the pilot study prior to the commencement of the main study (Addendum H & I). The laboratory temperature and equipment set-up remained standardized and all equipment was calibrated daily prior to testing.

Diaphragm thickness: Diaphragm muscle thickness was measured using a combination of techniques previously described. Imaging was performed using the Acuson P300 ultrasound system (Siemens), with a 12mHz high resolution linear transducer probe (Matamis et al 2013; Vivier et al, 2012) in two-dimensional B-mode (Grosu et al, 2012; Cohn et al, 1997). Subjects were in a supine position during measurements, with the arms relaxed at their sides, and instructed to relax and breathe quietly. The probe was placed in the right mid-axillary line at the level of the right eighth or ninth intercostal space, whichever gave the clearest image (Grosu et al, 2012; Cohn et al 1997), and the diaphragm was imaged through the zone of apposition (Grosu et al, 2012; Vivier et al, 2012; Cohn et al, 1997) (Figure 3.1). The right diaphragm (Ueki et al, 1995) was identified as a structure made of three distinct layers: A non-echogenic central muscular layer bound by echogenic membranes, the peritoneum and the diaphragmatic pleura. The ultrasound beam was directed perpendicular to the diaphragm (Vivier et al, 2012; Ueki et al, 1995) and a sine-loop of three consecutive breathing cycles were recorded, with emphasis on end expiration (Grosu et al, 2012; McCool et al, 1997). Images were coded and stored. After collecting all the images, the primary investigator measured the coded images.

Three still images were obtained at end expiration, to establish reproducibility of the measurements. Diaphragm thickness was measured at the point where the limiting membranes were most parallel (Baldwin et al, 2011). Diaphragm thickness was further defined as the distance from inner edge of the diaphragmatic pleura to the inner edge of the peritoneal pleura, to the nearest 0.1mm-, was then measured. The mean value of the three captured images was used as the final measurement of diaphragm thickness (Ueki et al, 1995).

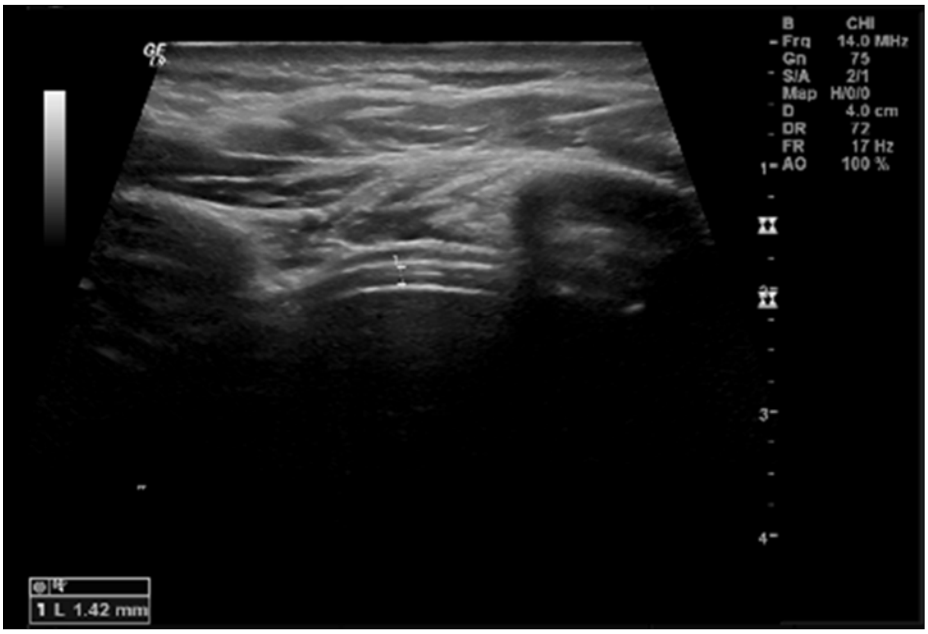


Figure 3.1: Measurement of diaphragm thickness using ultrasound

Diaphragm Strength: The strength of the diaphragm was measured using the POWERbreathe KH1 device. The validity of the POWERbreathe device has been established (Langer et al, 2013). The POWERbreathe KH1 is a small, hand-held, non-invasive mouth-pressure manometer with a rubber flanged mouth piece and a small monitor that digitally displays the test results in cmH20. Participants were instructed to sit (Dimitriadis et al, 2011) comfortably on the standard chair provided, backs firmly positioned against the backrest and feet placed firmly on the floor. Nose clips were applied to avoid nasal air leak (Dimitriadis et al, 2011). Participants were then instructed to hold the gauge with both hands, close their lips firmly around the flanged mouthpiece, exhale "until their lungs are empty" and then to inhale maximally for two seconds. Each participant performed a 5-breath maximum effort warm-up to familiarize themselves with the procedure (Dimitriadis et al, 2011). Immediately after the warm-up, the participant performed two PI_{max} manoeuvers and the maximum value was recorded as the final measurement of diaphragm strength. This maximum value was also used as the PI_{max} initial value when testing the endurance of the diaphragm (Janssens et al, 2013; Chang et al, 2005).

Diaphragm Endurance: The endurance of the diaphragm was measured using the POWERbreathe pressure threshold device. 60% of the participant's PI_{max} initial value was calculated and the pressure threshold device was set at this resistance (Janssens et al, 2013). The participant was seated during the testing procedure, with nose-clips applied, as described in *"diaphragm strength"*. Participants were instructed to place their lips firmly around the mouth piece and relax both their arms. Participants could choose their own breathing frequency, but were instructed to perform forceful and deep inspirations against a resistive load, followed by complete expirations, until task failure (Charususin et al, 2013). Task failure was defined as the duration from the onset of the task to the point at which the participant removed the mouthpiece (McConnell et al, 2010). A defined time limit of fifteen minutes was also set, of which the participants were not informed (Janssens et al, 2013). If the participants did not reach task failure before fifteen minutes, then the endurance task was stopped (Janssens et al, 2013). At task failure or the defined time limit, the participant was instructed to remove the mouthpiece and to perform another PI_{max} maneuver (PI_{max} final) using the POWERbreathe KH1 device. The fatigue resistance index (FRI = PI_{max} final/PI_{max} initial) was then calculated and the time to task failure was documented (Chang et al, 2005). No encouragement was provided during the task and no indication of the time of task performance was given (Charususin et al, 2013)

3.2.6 Data Processing and Statistical Analysis

All data was captured by the PI from the data sheets and checked at random. The summary statistics for descriptive purposes included the means and standard deviation when the data was distributed normally, and the medians and interquartile ranges for skewed data. The groups were compared using repeated measures ANOVA. Independent associations were determined using ANCOVA. Relationships between the dependant variables were tested using Pearson (or Spearman depending on the nature of the data) correlations. A 5% significance level ($P < 0.05$) was used as a guideline for determining significant correlations. Data was analysed using Statistica Version 9 (Statsoft, Tulsa, Oklahoma, USA) in consultation with a statistician. Correlations were interpreted as strong (r = 0.68 to 1.0), moderate (r = 0.36 to 0.67) and weak (r ≤ 0.35) (Taylor, 1990)

3.3 RESULTS

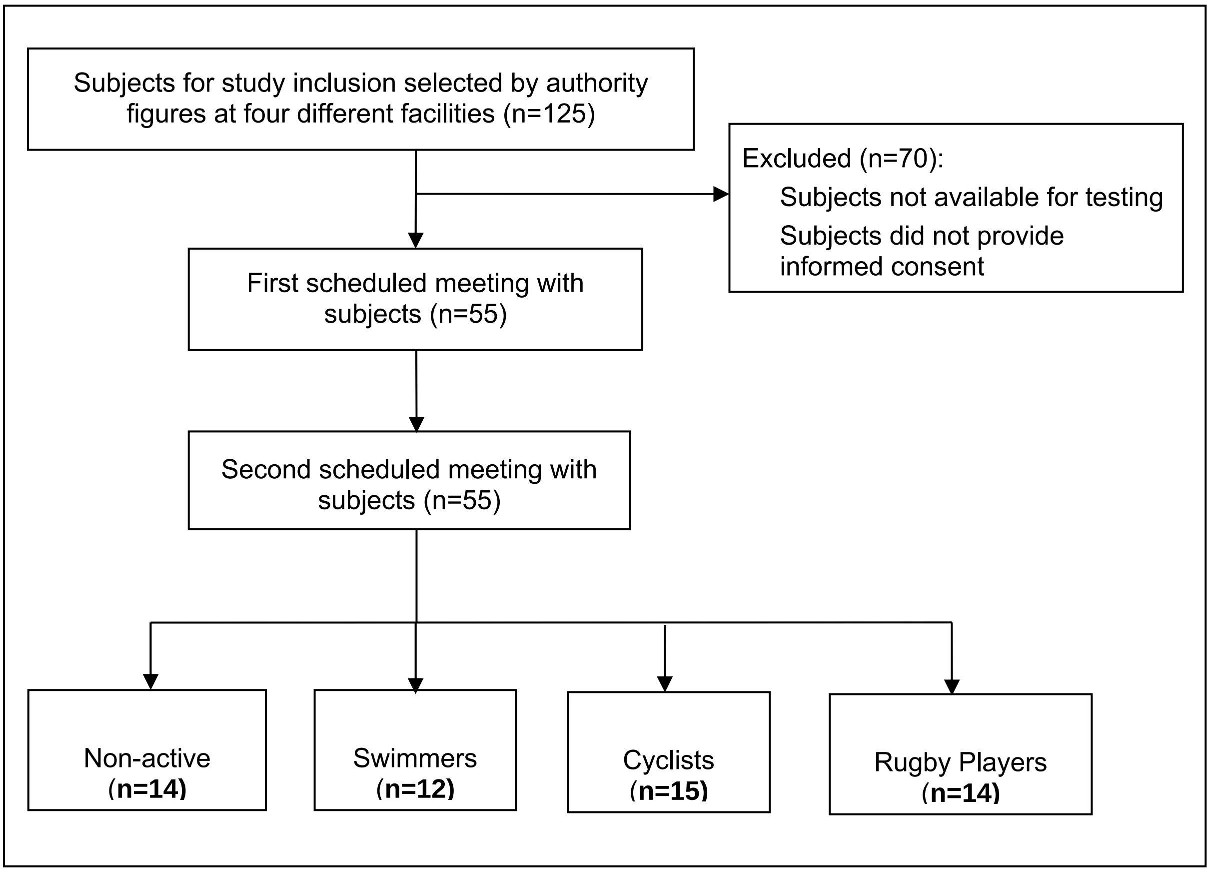


Figure 3.1: CONSORT flow diagram showing inclusion and allocation of study participants

Fifty-five university subjects (34 males, 21 females) were recruited for the study (Figure 3.1). The mean age of the sample was 21.16 (SD = 1.55) years, with a mean BMI of 25.43 (SD = 3.70). (Table 3.1)

Table 3.1: Baseline data

Characteristics	Non-active	Cycling	Swimming	Rugby	Total
Gender Male (%)	14 (25.5%)	15 (27%)	12 (22%)	14 (25.5%)	55
Age Mean (SD)	21.43 (1.34)	21.67 (1.11)	20.17 (1.90)	21.21 (1.58)	21.16 (1.55)
BMI Mean (SD)	25.27 (3.11)	23.24 (2.43)	23.54 (1.49)	29.57 (3.43)	25.43 (3.70)

3.3.1 Diaphragm Thickness

The mean (SD) diaphragm thickness was 1.92mm (0.61) (Table 3.2).

Table 3.2: Diaphragm measurements for whole sample

Variables	Mean	SD	-95% CI	+95% CI
Diaphragm Thickness (mm)	1.92	0.61	1.75	2.08
PI_{max} (cmH_20)	128.44	35.35	118.88	137.99
Fatigue Resistance Index	0.93	0.13	0.89	0.98

A moderate positive correlation was established between diaphragm thickness and diaphragm strength measurements ($r = 0.52$; $r^2 = 0.27$, $p < 0.01$) (Figure 3.3). This suggests that only 27% of the variation observed in diaphragm thickness measurements can be attributed to strength.

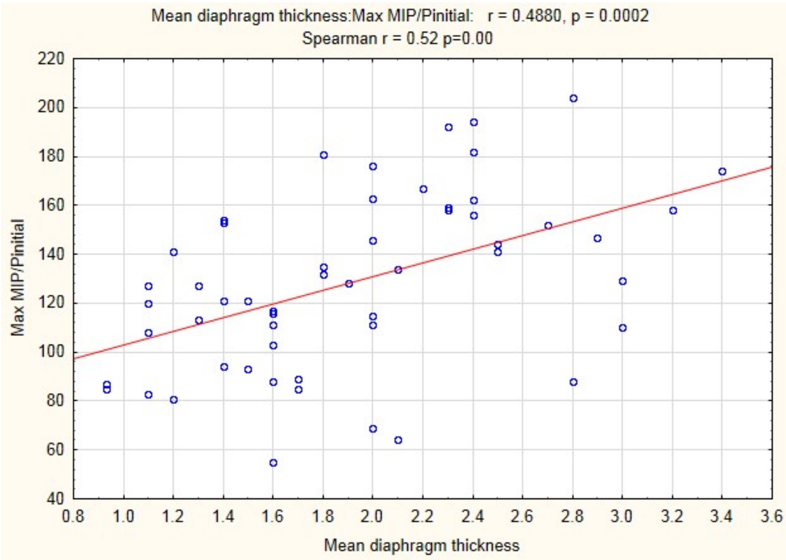


Figure 3.3: Whole sample mean diaphragm strength (PI_{max}) vs mean diaphragm thickness

BMI was the other variable that influenced diaphragm thickness measurements ($r = 0.57$; $r^2 = 0.33$; $p < 0.01$) (Figure 3.4). This suggests that only 33% of the variation noted in diaphragm thickness can be attributed to subject BMI, therefore subjects with a higher BMI will present with a thicker diaphragm. Gender was independently correlated with diaphragm thickness ($p = 0.03$).

No relationship was found between the thickness of the diaphragm and the endurance of the diaphragm ($r = -0.15$; $r^2 = 0.02$; $p = 0.29$) (Figure 3.5).

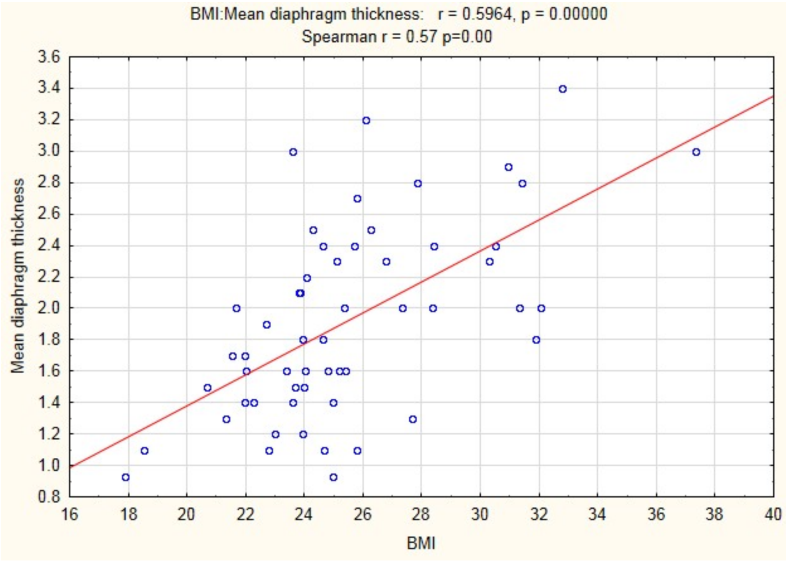


Figure 3.4: Whole sample mean diaphragm thickness vs mean BMI

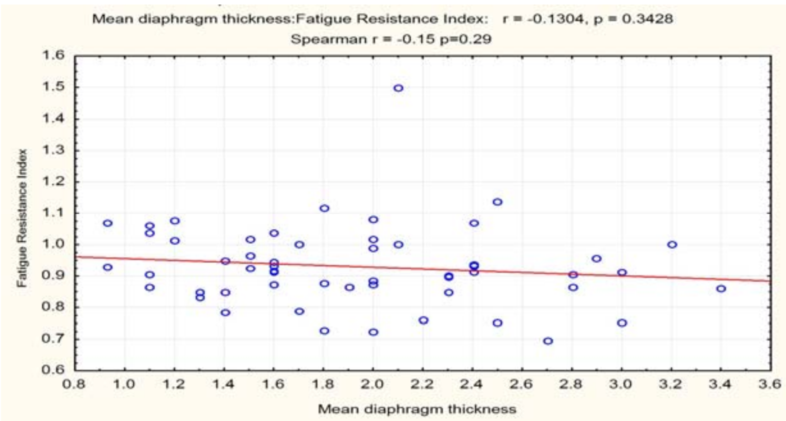


Figure 3.5: Whole sample mean fatigue resistance index (FRI) vs mean diaphragm thickness

3.3.2 Diaphragm Strength

The mean (SD) diaphragm strength was 128.44cm$H_2$0 (35.35) (Table 3.2). Interesting to note that there was no association between student activity levels and diaphragm strength (p=0.18). Although age (r = 0.34; r^2 = 0.12; p = 0.01) and BMI (r = 0.37; r^2 = 0.14; p = 0.01) are independently correlated with strength, the correlation is weak. Subject gender was also independently correlated with diaphragm strength (p = 0.03). No relationship was found between the strength of the diaphragm and the endurance of the diaphragm (r = -0.19, r^2 = 0.04; p = 0.16).

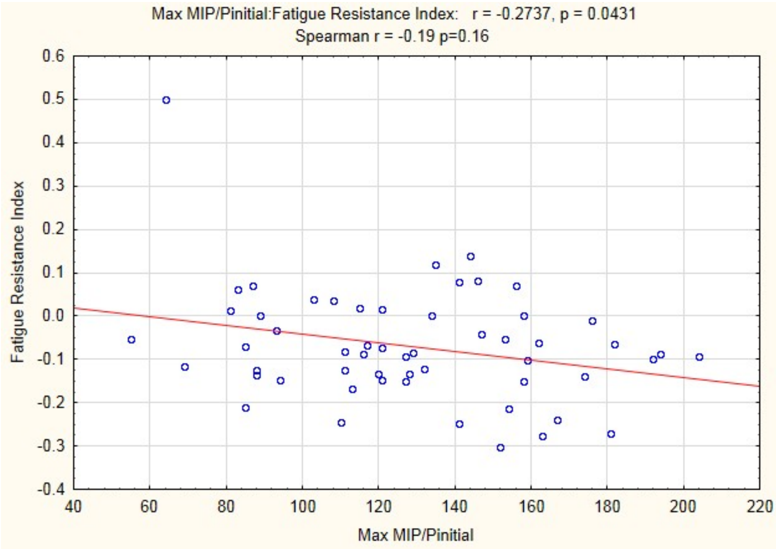


Figure 3.6: Whole sample mean fatigue resistance index (FRI) vs mean diaphragm strength

3.3.3 Diaphragm Endurance

The mean (SD) fatigue resistance index was 0.93 (0.13) (Refer to Table 3.2). While the overall activity level of students was not associated with the diaphragm endurance (p=0.08) (Figure 3.7), a significant difference was observed in the endurance abilities of cycling and the rugby sub-groups (p = 0.009) (Addenda J - M). Age (r = 0.17; r^2 = 0.03; p = 0.21), BMI (r = -0.14; r^2 = 0.02; p = 0.31) and subject gender (p = 0.55) were not independently correlated with the endurance of the diaphragm. There was a significant difference between mean PI_{max} initial (128.44 ± 35.35) and mean PI_{max} final (118.27 ± 32.49) values obtained during testing (p < 0.01). This indicates a decline in inspiratory muscle function (Janssens et al, 2013).

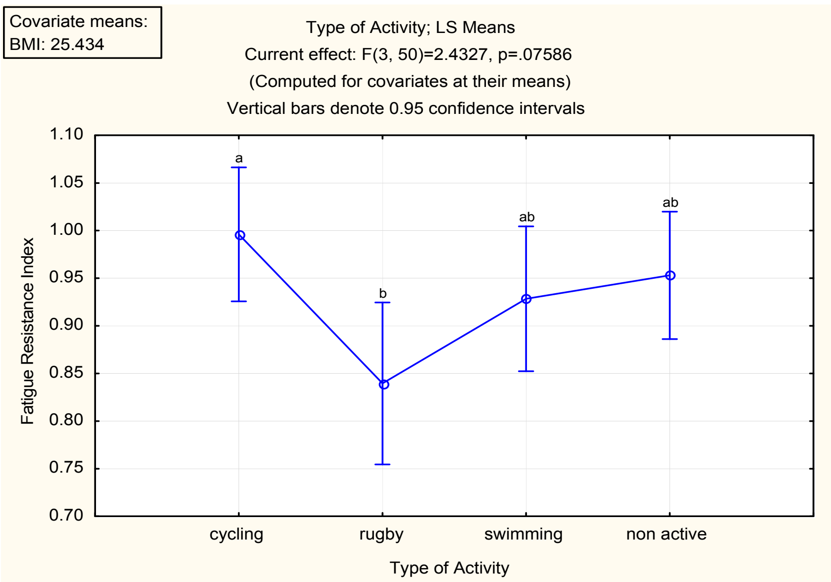


Figure 3.7: Whole sample mean fatigue resistance index (FRI) vs Type of Activity

3.4 DISCUSSION

To our knowledge, this is the first study to investigate relationships between measurements of diaphragm thickness, diaphragm strength and diaphragm endurance. The findings suggest that diaphragm thickness and diaphragm strength are related, No correlation between diaphragm endurance and diaphragm thickness was found. The relationship between diaphragm endurance and strength also showed no correlation.

The hypothesis that a correlation exists between diaphragm thickness and diaphragm strength was reported in the weightlifting (McCool et al, 1997) and cerebral palsy populations (Moreau et al, 2010). While our results affirm previous studies, they also highlight the strength of the correlation between diaphragm thickness and diaphragm strength, and the important effect that gender and BMI have on diaphragm thickness measurements. This has not been reported previously. In the Duchenne muscular dystrophy population, results by *De Bruin et al.* (1997) report that the resting diaphragm thickness, measured using ultrasound, was increased in young patients with the disease. The increase in resting diaphragm thickness was accompanied by impaired respiratory muscle force. However, the finding could be explained by the pseudo-hypertrophy that occurs in some muscles during the course of the disease (De Bruin et al, 1997).

It was hypothesised that a leaner diaphragm may be more fatigue resistance than a thicker diaphragm (Polla et al, 2004). This was, however, not the case. Either no relationship exists between diaphragm thickness and diaphragm endurance or measurement error was the problem. During the fatiguing task, subjects were allowed to set their own breathing frequency, they were not motivated at all during the task and they were not informed of the defined time limit set. Our study failed to include a warm up session, so subjects were unaware of what to expect with regards to the set resistance. A warm up is indicated since it creates narrower limits of agreement for the outcome measure and maximises the magnitude of inspiratory muscle fatigue (Janssens et al, 2013). The saliva build up in the pressure threshold device caused a few subjects to reach task failure quicker than other subjects. However, as the fatigue resistance index was the main outcome measure for this task and not time to task failure, the saliva build up and failure to adapt to the set resistance should not have had an influence on the index calculated, and thereby the results. The fatigue resistance index may, however, not be sensitive enough to determine the endurance of the diaphragm. However, the possibility still exists that there is no correlation between diaphragm thickness and diaphragm endurance and the relationship needs to be confirmed in future studies.

The precision of the measurements indicate accurate sampling (Table 3.2). Sampling was done in such a way that we stratified for gender, age, BMI and type of activity. This was done to ensure that we included different body sizes and activity levels as part of our study sample, thereby limiting the possibility of skewed data.

The study was further strengthened by the use of a standardized ultrasound technique for the measurement of diaphragm thickness (Section 3.2.5). We used ultrasound to image the diaphragm at end expiration, where the diaphragm is at its thinnest (Grosu et al, 2012). Measurements were slightly more challenging in the front row rugby player participants, , due to the depth of the diaphragm compared to leaner subjects. Another challenge was the large displacement of the chest wall in some subjects during inspiration. The thickening fraction of the diaphragm may have been a better calculation to consider, as it provides information on the efficacy of the diaphragm as a pressure generator (Matamis et al, 2013; Vivier et al, 2012; Ueki et al, 1995). In children with Duchenne muscular dystrophy, it was found that despite a greater diaphragm thickness at functional residual capacity, the thickening fraction was less than that of controls during maximum inspiratory efforts (De Bruin et al, 1997).

We acknowledge that PI_{max} assesses the overall force produced by the inspiratory muscles, and is therefore not a direct measure of diaphragm strength. Transdiaphragmatic pressure is another, more direct method, used to measure the strength of the diaphragm (Section 2.3.4.2). The transdiaphragmatic pressure and PI_{max} were strongly correlated with one another in a recent study (McCool et al, 1997). The transdiaphragmatic pressure did, however, have a stronger correlation with the dimensions of the diaphragm than PI_{max} did (McCool et al, 1997). We are also aware that PI_{max} testing requires full patient co-operation and that a low result may therefore not necessarily indicate reduced inspiratory muscle strength, but rather a lack of patient motivation. However, in healthy subjects, maximal effort during the PI_{max} manoeuvre can be controlled, thereby achieving maximum activation of the diaphragm (De Bruin et al, 1997; Ueki et al, 1995). To account for subject learning error, we allowed five PI_{max} practice tests before the two final recordings (Dimitriadis et al, 2011). We encouraged each subject through each PI_{max} attempt, including the practice tests. Subject position remained unchanged and the same type of mouth piece was used for each subject. All testing factors were therefore controlled and the only shortcoming possible was patient effort, which could only be monitored subjectively.

3.5 CONCLUSION

We conclude from this study that a moderate correlation exists between the thickness of the diaphragm and the strength of the diaphragm. However, BMI and gender are important variables to consider when measuring the thickness and strength of the diaphragm. No correlation between diaphragm endurance and diaphragm thickness was found. The relationship between diaphragm endurance and strength also showed no correlation

The use of ultrasound imaging to determine diaphragm thickness proved to be a feasible and reliable measurement tool. As diaphragm thickness measurements are a moderate indicator of the strength of the diaphragm, ultrasound may have the potential to be used as a surrogate measure for diaphragm strength. Ultrasound may also help clinicians to detect and monitor dysfunction of the diaphragm in the early stages of admission to the acute setting. However, the clinical relevance of diaphragm monitoring is yet to be established. Future studies also need to determine whether changes in diaphragm thickness are related to extubation outcome.

Chapter 4:

GENERAL DISCUSSION

The aim of this thesis was to investigate the structure and function of the diaphragm. The aim of the scoping review was to determine whether a correlation between diaphragm thickness, strength and endurance had been established. Another aim of the scoping review was to identify the current measurement methods of diaphragm function in various populations and to describe whether uniform testing procedures have been adhered to. No correlation was found in the scoping review. The aim of the primary study was to investigate the relationship between diaphragm thickness, strength and endurance

4.1 CURRENT UNDERSTANDING OF LITERATURE

The function of the diaphragm has been evaluated in a variety of populations. It has been identified that the ATS guidelines, (ATS/ERS Statement on Respiratory Muscle Testing, 2002 et al 2002) reporting on strength and endurance testing of the diaphragm, are well developed and should be implemented in future studies. Discrepancies acknowledged in the review could be addressed and corrected for by adhering to the guidelines. It was also identified that ultrasound measurements are a fairly new field of interest, as no standardized protocol has been developed yet. The lack of a standardizing protocol complicates the comparison of results between studies and future studies need to standardize the ultrasound measurement method.

4.2 PRELIMINARY HYPOTHESIS

Our **first preliminary hypothesis** was that there would be a correlation between the diaphragm thickness and the diaphragm strength, the thicker the diaphragm the stronger it would be. This hypothesis was shown to be correct. We also discovered that although diaphragm thickness and diaphragm strength are correlated, subject age, gender and BMI are also correlated with the thickness of the diaphragm and need to be taken into account during the sampling and measuring process.

The use of PI_{max} as a measure of diaphragm strength was based on the recent studies (Zübeyir et al, 2012; Dimitriadis et al, 2011; Strongoli et al, 2010; Ray et al, 2010; Sachs et al, 2009; Sperlich et al, 2009) done in healthy participants. We acknowledge that this technique is not the gold standard when testing the strength of the diaphragm. However, it has been strongly correlated with the transdiaphragmatic pressure, which is the gold standard. We also acknowledge that measuring the PI_{max} is a volitional test and that full subject co-operation is needed for a reliable result. Subjects were therefore constantly motivated through each maximum inspiratory pressure attempt and learning error was also controlled for by familiarising subjects with the testing procedure.

Our **second preliminary hypothesis** was that there would be a correlation between diaphragm thickness and diaphragm endurance, therefore the thinner the diaphragm the more fatigue resistant it would be. This hypothesis was proven incorrect. No correlation was found between the thickness of the diaphragm and the endurance of the diaphragm. The possible reason for no correlation may be due to our chosen technique to measure diaphragm endurance (Section 3.2.5). We tested the PI_{max} of subjects before and after a fatiguing task, as described by one study assessed in the review (Chapter 2), and calculated the fatigue resistance index. We acknowledge that the fatigue resistance index may not have been sensitive enough to detect a correlation between diaphragm endurance and diaphragm thickness, should one exist.

The method described above is an alternative method of measuring the endurance of the diaphragm, when the twitch transdiaphragmatic pressure is not possible to assess. We did not assess the twitch transdiaphragmatic pressure in subjects, although it was the preferred methods in the majority of recent studies, due to the invasiveness of the technique and lack of skill of the chosen operator.

The setting on the pressure threshold device, to 60% of the subject's PI_{max} initial, was initially a concern, due to the large increments on the device and possible inconsistent setting. This was queried due to the fact that total inspiratory muscle work and the amount of work per breath are maximized at 60% of the maximum inspiratory pressure and decline at higher and lower loads (Sheel et al, 2002). When calculating the difference between our PI_{max} initial and PI_{max} final values, the difference was significant, thereby confirming a decline in inspiratory muscle function (Janssens et al, 2013). However, it is not yet known whether a minimum percentage change is required in order for this to be indicative of inspiratory muscle fatigue (Janssens et al, 2013). The pressure threshold device was therefore not a contributing factor to the rejection of the null hypothesis. The other possibility is that there is, in fact, no correlation between diaphragm thickness and diaphragm endurance, but this correlation warrants further research.

4.3 ACHIEVEMENT OF STUDY AIMS

Both the primary and secondary aims of this study were achieved. The primary aim of the study was to determine the correlation between diaphragm thickness, diaphragm strength and diaphragm and endurance. This aim was achieved in the main study (Chapter 3).

The secondary aim of the study was to determine the intra-rater reliability when using ultrasound to measure diaphragm thickness. This aim was achieved in the pilot study (Addendum I) and the main study (Chapter 3). We conclude, due to high intra-rater reliability, that the use of ultrasound is a useful tool to monitor the function of the diaphragm and to measure diaphragm thickness in young healthy individuals. . Ultrasound is a valuable skill and also a skill that is easily learnt. The ultrasound technique described in this thesis was based on recommendations of previous studies done and can be used by clinicians performing future research in this area. It is important to remember that the ultrasound technique can change depending on the aim of the study and the different populations tested. These changes need to be accurately documented and described so that future studies may use further adapted techniques to their advantage.

4.4 LIMITATIONS

The impact of limitations on the measuring the function of the diaphragm are discussed in Chapter 3. These include:

- Using the fatigue resistance index as a measure of diaphragm endurance. The fatigue resistance index is not a direct measure and it is not the gold standard of diaphragm endurance testing
- No warm-up session prior to the endurance test
- Saliva build up in the pressure threshold device during the endurance test
- PI_{max} as a measure of diaphragm strength. PI_{max} is not a direct measure, although it has been correlated with the gold standard
- Measurement of diaphragm thickness at the end of expiration, when thickening fraction may have been a better choice
- Time limit on the scoping review for the last five years. Although only the last five years were analysed in the review, all the articles have been identified and are available for use

4.5 FUTURE RESEARCH

- Investigation of a surrogate measure for diaphragm function remains an important area of research
- Determine the relationship between the thickness of the diaphragm and its overall displacement and then compare these two variables with the strength of the diaphragm
- Determine the correlation between diaphragm thickness and extubation outcome in the intensive care unit
- Investigate the link between nutritional status and extubation outcome in the intensive care unit and how they may be linked to one another or to the thickness of the diaphragm
- Determine the validity of the fatigue resistance index and how the fatigue resistance index compares to the gold standard for measuring diaphragm endurance

4.6 TAKE HOME MESSAGE

- Guidelines should be adhered to for the testing of diaphragm strength and diaphragm endurance, to ensure that the comparison of results between studies is possible
- The important effect of gender and BMI on the thickness and strength of the diaphragm and the need to consider these variables during measurements
- Ultrasound is a non-invasive, reliable and easily learned skill
- Ultrasound has the potential to be used as a surrogate marker for diaphragm strength in the intensive care unit population
- The protocol established (Section 3.2.5) for the measurement of diaphragm thickness using ultrasound can be used by clinicians in this field

4.7 FINAL CONCLUSION

Guidelines for the measurement of diaphragm function do exist, but they are not adhered to by the majority of studies. Study procedures are inconsistently reported and this may affect the reproducibility of techniques in future studies. We further conclude that a correlation exists between diaphragm thickness and diaphragm strength. The use of ultrasound to measure diaphragm thickness proved to be a reliable technology and gave a moderate indication of the strength of the diaphragm. This technology may help clinicians to detect and monitor dysfunction of the diaphragm in the early stages of admission to the acute setting.

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ADDENDA RELATED TO METHODOLOGY

ADDENDUM A

Search Strategy: Databases searched

Pubmed

Limits applied to database:

Species: Humans

Age: Adult (19+ years)

Language: English

Search Terms	Hits
1. Diaphragm AND strength	220
2. Diaphragm AND force generating capacity	8
3. #1 AND measure*	130
4. #2 AND measure*	3
5. #3 AND instrument*	5
6. #1 AND invasive test*	3
7. Diaphragm AND endurance	90
8. Diaphragm AND fatigue	213
9. #7 AND measure*	43
10. #8 AND measure*	109
11. #9 AND instrument*	2
12. #10 AND instrument*	3
13. Diaphragm AND thickness	162
14. Diaphragm AND thinning	6
15. #13 AND measure*	77
16. #14 AND measure*	1
	1075

PEDro

Search Terms	Hits
1. Diaphragm AND strength	13
2. #1 AND measure	8
3. Diaphragm AND endurance	8

4. Diaphragm AND fatigue	4
5. #3 AND measure	3
6. #4 AND measure	2
7. Diaphragm AND thickness	1
8. #7 AND measure	1
	40

Cinahl

Limits applied to database:

Species: Humans

Age: Adult (19 - 44 years), Middle Aged (45 - 64), Aged (65+ years), Aged 80 and over

Language: English

Search Terms	Hits
1. Diaphragm AND strength	18
2. Diaphragm AND force generating capacity	1
3. Diaphragm AND endurance	9
4. Diaphragm AND fatigue	13
5. #3 AND measure	1
6. #4 AND measure	1
7. Diaphragm AND thickness	8
8. Diaphragm AND thinning	1
	52

Cochrane

Search Terms	Hits
1. Diaphragm AND strength	30
2. Diaphragm AND force generating capacity	20
3. #1 AND measure	30
4. #2 AND measure	20
5. #3 AND instrument	7
6. #4 AND instrument	3
7. #1 AND invasive test	10

8. Diaphragm AND endurance	10
9. Diaphragm AND fatigue	25
10. #8 AND measure	10
11. #9 AND measure	25
12. #10 AND instrument	2
13. #11 AND instrument	6
14. Diaphragm AND thickness	7
15. Diaphragm AND thinning	6
16. #14 AND measure	7
17. #15 AND measure	6
	224

MEDLINE

Limits applied to database:

Species: Humans

Age: Adult (19 - 44 years), Middle Aged (45 - 64), Aged (65+ years), Aged (80 and over, 80+ years)

Language: English

Search Terms	Hits
1. Diaphragm strength NOT ophthalmology NOT gynaecology	207
2. Diaphragm force generating capacity NOT ophthalmology NOT gynaecology	8
3. #1 AND measure	20
4. #1 AND invasive test	6
5. Diaphragm endurance NOT ophthalmology NOT gynaecology	90
6. Diaphragm fatigue NOT ophthalmology NOT gynaecology	211
7. #5 AND measure	6
8. #6 AND measure	13
9. Diaphragm thickness NOT ophthalmology NOT gynaecology	121
10. Diaphragm thinning NOT ophthalmology NOT gynaecology	4
11. #9 AND measure	9
	695

EBSCO Host

Limits applied to database:

Language: English

Search Terms	Hits
1. Diaphragm strength NOT engineering NOT flooring	25
2. Diaphragm force generating capacity NOT engineering NOT flooring NOT animals	3
3. #1 AND measure	2
4. #2 AND measure	25
5. #1 AND invasive test	1
6. Diaphragm endurance NOT engineering NOT flooring	6
7. Diaphragm fatigue NOT engineering NOT flooring NOT animals	23
8. #6 AND measure	6
9. #7 AND measure	2
10. Diaphragm thickness NOT engineering NOT flooring NOT animals	41
11. Diaphragm thinning NOT engineering NOT flooring NOT animals	2
12. #10 AND measure	4
	140

Science Direct

Limits applied to database: Medicine and Dentistry, Neuroscience, Nursing and Health Professions, Pharmacology, Toxicology and Pharmaceutical Science

Search Terms	Hits
1. Diaphragm AND strength AND humans	173
2. Diaphragm AND force generating capacity	6
3. #1 AND measure	121
4. #2 AND measure	3
5. #3 AND instrument	39
6. #1 AND invasive test	56
7. Diaphragm AND endurance AND humans	7
8. Diaphragm AND fatigue AND humans	236
9. #7 AND measure	3

10. #8 AND measure	167
11. #9 AND instrument	1
12. #10 AND instrument	45
13. Diaphragm thickness AND humans	44
14. Diaphragm thinning AND humans	1
15. #13 AND measure	36
	938

Web of Science

Search Terms	Hits
1. Diaphragm strength AND measure	211
2. Diaphragm force generating capacity AND measure	27
3. #3 AND instrument	4
4. #1 AND invasive test	12
5. Diaphragm endurance AND measure	112
6. Diaphragm fatigue AND measure	344
7. #5 AND instrument	2
8. #6 AND instrument	4
9. Diaphragm thickness AND humans	42
10. Diaphragm thinning AND humans	28
11. #9 AND measure	29
12. #10 AND measure	6
	821

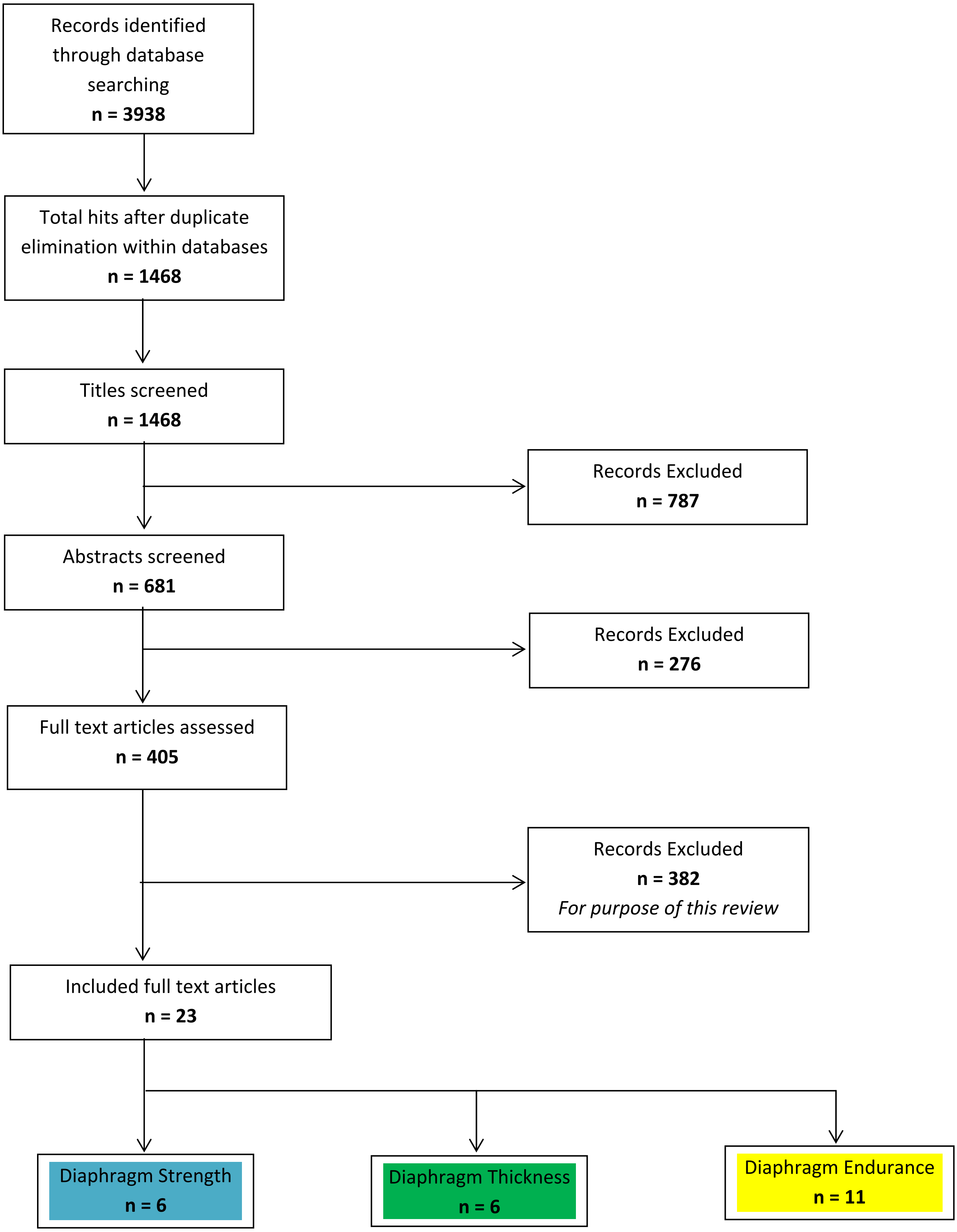
ADDENDUM B

Duplicate Elimination

Database	Total Hits	Total hits after duplicate elimination
Pubmed	1075	1468
PEDro	40	
Cinahl	49	
Cochrane	216	
Medline	659	
EBSCO Host	140	
Science Direct	938	
Web of Science	821	
Total	3938	

ADDENDUM C

Flowchart exclusion Process



ADDENDUM D

Ethics Approval

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot • your knowledge partner

Approval Notice
New Application

06-Aug-2013
Orrey, Samantha ST

Ethics Reference #: S13/05/111

Title: Is there a correlation between diaphragm strength, diaphragm endurance and diaphragm thickness in young, healthy individuals?

Dear Miss Samantha Orrey,

The New Application received on **03-Jun-2013**, was reviewed by members of **Health Research Ethics Committee 1** via Expedited review procedures on **30-Jul-2013** and was approved.
Please note the following information about your approved research protocol:

Protocol Approval Period: **30-Jul-2013 -30-Jul-2014**

Please remember to use your **protocol number** (**S13/05/111**) on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review:
Please note a template of the progress report is obtainable on www.sun.ac.za/rds and should be submitted to the Committee before the year has expired.
The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.
Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Helene Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.
For standard HREC forms and documents please visit: www.sun.ac.za/rds

If you have any questions or need further assistance, please contact the HREC office at 0219389657.

Included Documents:
cv unger
cv hanekom
applic form
checklist
synopsis
protocol
cv orrey
dec letters

Sincerely,

Franklin Weber
HREC Coordinator
Health Research Ethics Committee 1

ADDENDUM E

Letter to Authority

PO Box 19063
Tygerberg
7505
Cape Town
South Africa

<u>1A: REQUEST FOR PERMISSION TO CONDUCT RESEARCH AT UNIVERSITY SPORTING GROUNDS</u>

Dear Sir / Madam

My name is Samantha Orrey and I am a Masters Physiotherapy student at the University of Stellenbosch in the Western Cape. The research I wish to conduct for my Master's thesis aims to determine whether a correlation exists between diaphragm strength, diaphragm endurance and diaphragm thickness in young, healthy individuals. This project will be conducted under the supervision of Dr S Hanekom (PhD Physiotherapy, US) and Dr M Unger (PhD Physiotherapy, US)

I am hereby seeking your consent to approach the athletes, who under your authority, participate in sport at a university level. I would like to include these athletes as participants in my study. Requirements include one meeting with all athletes on a scheduled meeting day where aims of the study will be discussed, written informed consent obtained from each athlete as well as measurements of height and weight. I estimate this meeting will take a maximum of 1-2 hours of your time. At this meeting, we will schedule the next and last meeting where measurements of the participant's diaphragm strength, thickness and endurance will be taken.

I have provided you with a copy of my thesis proposal, which includes copies of the informed consent forms to be used in the research process, as well as a copy of the approval letter which I received from the University of Stellenbosch Research Ethics Committee.

The researcher aims to publish the results of the study in an accredited peer reviewed journal. Participant's personal information will be handled confidentially and participants will remain anonymous to everybody except the researcher herself, the research assistant and the statistician.

Thank you for your time and co-operation.

Yours sincerely,
Samantha Orrey
BSc Physiotherapy (University of Stellenbosch)

PO Box 19063
Tygerberg
7505
Cape Town
South Africa

1B: REQUEST FOR PERMISSION TO CONDUCT RESEARCH AT UNIVERSITY: INSTITUTIONAL PLANNING

Dear Sir / Madam

My name is Samantha Orrey and I am a Masters Physiotherapy student at the University of Stellenbosch in the Western Cape. The research I wish to conduct for my Master's thesis aims to determine whether a correlation exists between diaphragm strength, diaphragm endurance and diaphragm thickness in young, healthy individuals. This project will be conducted under the supervision of Dr S Hanekom (PhD Physiotherapy, US) and Dr M Unger (PhD Physiotherapy, US)

I am hereby seeking your consent to approach the students at Tygerberg Campus, who lead a sedentary or non-active lifestyle at University. I would like to include these students as participants in my study. Requirements include one meeting with these students on a scheduled meeting day where aims of the study will be discussed, written informed consent obtained from each student as well as measurements of height and weight. I estimate this meeting will take a maximum of 1-2 hours of your time. At this meeting, we will schedule the next and last meeting where measurements of the participant's diaphragm strength, thickness and endurance will be taken.

I have provided you with a copy of my thesis proposal, which includes copies of the informed consent forms to be used in the research process, as well as a copy of the approval letter which I received from the University of Stellenbosch Research Ethics Committee.

The researcher aims to publish the results of the study in an accredited peer reviewed journal. Participant's personal information will be handled confidentially and participants will remain anonymous to everybody except the researcher herself, the research assistant and the statistician.

Thank you for your time and co-operation.

Yours sincerely,
Samantha Orrey
BSc Physiotherapy (University of Stellenbosch)

ADDENDUM F

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF RESEARCH PROJECT:

Is there a correlation between diaphragm strength, diaphragm endurance and diaphragm thickness in young, healthy individuals?

PRINCIPAL INVESTIGATOR: Samantha Orrey

ADDRESS: Department of Interdisciplinary Health Sciences, Stellenbosch University

CONTACT NUMBER: 084 324 0249

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the primary researcher or study staff any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied and that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

Setting:

This study will be conducted in the Cape Metropolitan area as well as in Stellenbosch, in the Western Cape. The Stellenbosch study settings will include the rugby, swimming and cycling university training facilities, where the researcher aims to recruit an equal number of participants partaking in endurance and strength type sports. The Cape Metropolitan study setting will include Tygerberg Campus, where the researcher aims to recruit an equal number of participants leading a sedentary or non-active lifestyle.

Aim:

The study hopes to determine whether a correlation exists between diaphragm strength, diaphragm endurance and diaphragm thickness in young, healthy individuals. If a correlation is found to exist, it will provide researchers with a new non-invasive tool for the measurement of diaphragm strength in individuals who may not be able to actively participate in testing.

Procedures:

The researcher will explain the aims, objectives and study procedure to all potential study participants at each relevant university training facility. On the same day, all interested participants will be asked to complete a form of written informed consent, an information sheet enquiring participant age, gender, activity level (active/non-active) and type of activity as well as an additional form enquiring name, contact number and e-mail address. The height and weight of each participant will be measured and their overall BMI will be calculated. Participants will then be contacted via e-mail where another meeting will be scheduled so that measurements of diaphragm strength, thickness and endurance can be taken.

At the next scheduled meeting, participants will be measured individually at four different stations. At the first station the primary researcher will confirm the correct participant has arrived for testing and the participant will be issued with their data capture sheet that they will take with them to each station. On arrival, the breath rate per minute of each participant will be monitored and documented and the participant will be asked to rest for ten minutes prior to testing commencement. Participants will rest on a chair provided. At the second station, diaphragm thickness will be measured using ultrasound. At the third station, diaphragm strength and diaphragm endurance will be measured. At the last station, the researcher will work out a respiratory training program for each participant. The researcher will explain each step of the program to the participant to make sure they understand. The participant is then free to go

Why have you been invited to participate?

This study population needs to be a group of healthy male or female individuals of 18-24 years of age, who take part in sport at a university level or lead a non-active lifestyle

What will your responsibilities be?

All participants are expected to be punctual for their allocated time slot in which the measurements will take place. If the participant is unable to attend their allocated time slot, they are expected to contact the primary researcher via e-mail or cell phone. An email will be sent to all participants by the primary researcher prior to the second scheduled meeting explaining the location for testing, required dress code and requests regarding eating and exercise prior to testing, to ensure participant comfort during all testing procedures.

Will you benefit from taking part in this research?

Participants will benefit from this research by gaining information on individuals measurements of diaphragm strength, endurance and thickness. Participants will also help future researchers to further investigate this topic and benefit patients in the acute setting

Are there in risks involved in your taking part in this research?

This research involves no risks or adverse effects

Who will have access to your medical records?

The information collected during this study will remain confidential and protected at all times. Only the primary researcher, research assistant and statistician will have access to the data during the course of the study. Upon completion of the study, the researcher aims to publish the results of the study in an accredited peer reviewed journal. In addition, the results will be presented at an Academic Year-day and at one national conference. All participant information will remain anonymous.

Will you be paid to take part in this study and are there any costs involved?

You will not be paid to take part in the study, but there will be no costs involved for you, if you do take part.

Is there anything else that you should know or do?

- You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed

Declaration by participant

By signing below, I …………………………………..…………. agree to take part in a research study entitled: “Is there a correlation between diaphragm strength, diaphragm endurance and diaphragm thickness in young, healthy individuals??

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) ....…........……………. on (*date*) …………....……….. 2013.

……………………………………..   …………………………………..

Signature of participant   **Signature of witness**

Declaration by investigator

I *(name)* …………………………………………...……… declare that:

- I explained the information in this document to …………………………………..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) ....…......……………….. on (*date*) …………....……….. 2013.

……………………………………… ……………………………………..

Signature of investigator **Signature of witness**

ADDENDUM G

DATA CAPTURE SHEET

DEMOGRAPHICS

Participant Code	
Age	
Date of Birth	

GENDER	Male	Female	
ACTIVITY LEVEL	Active	Non-Active	
TYPE OF ACTIVITY	Rugby	Swimming	Cycling

MEASUREMENTS

BMI

Height	Weight	Calculated BMI

DIAPHRAGM THICKNESS

Measurement 1	Measurement 2	Measurement 3	Mean

DIAPHRAGM STRENGTH

Measurement 1	Measurement 2	Final measurement (Max); ($P_{initial}$)

DIAPHRAGM ENDURANCE

$P_{initial}$	P_{final}

60% $P_{initial}$:

FRI ($P_{final}/P_{initial}$) =

Time to task failure:

ADDENDUM H

Pilot Study

The pilot study was conducted prior to the commencement of the main study.

OBJECTIVES:

- To determine the intra-rater reliability of ultrasound measurements
- To determine the inter-rater reliability of ultrasound measurements
- To determine the strength of the diaphragm by measuring the PI_{max} of each participant
- To determine the endurance of the diaphragm by calculating the fatigue resistance index of each participant, after they inspired at 60% of their achieved PI_{max} to task failure
- To determine the time period required to complete measurements of diaphragm thickness, strength and endurance in each participant

METHODS

Study Setting: This pilot study was conducted at a private hospital in the Northern suburbs of Cape Town, South Africa.

Ethical Considerations: All participants provided informed consent

Sample: Sample of convenience; included six healthy individuals (18-24); three activity levels (sedentary; endurance-and strength related sporting activities; stratified for gender and BMI. See Table 1

Table 1: Demographic Profile of Participants

Participant code	Gender	Age	Activity level	Type of Activity	Height	Weight	BMI
1	Male	24	Active	Rugby	1.85	90	26.29657
2	Male	24	Active	Rugby	1.9	93	25.76177
3	Male	23	Non-active	None	1.78	80	25.24934
4	Female	22	Non-active	None	1.64	64	23.79536
5	Female	21	Non-active	None	1.68	63	22.32143
6	Female	22	Active	Cycling	1.56	46	18.90204

Measurements

Procedure: The PI scheduled three separate meetings with the group of six participants. At the first meeting, aims and objectives of the pilot study were explained and informed consent was obtained. At the second meeting, intra-rater reliability and inter-rater reliability of ultrasound measurements were established (Refer to Table 2). At the last meeting, all baseline measurements (height, weight, age, activity level) were recorded (Refer to Table 1) and diaphragm thickness, strength and endurance were measured by the PI (Refer to table). Measurements were completed in the same order by the primary investigator. The laboratory temperature and equipment set-up remained standardized and all equipment was calibrated daily prior to testing.

Intra-rater reliability: Ultrasounds measurements of diaphragm thickness (Refer to) were performed by the PI on the same day separated by a five minute interval, using the same ultrasound equipment with the participant in a relaxed, supine position. PI measurements were compared to establish intra-rater reliability

Inter-rater reliability: Ultrasound measurements of diaphragm thickness (Refer to) were performed by the expert ultra-sonographer (US) on the same day separated by a five minute interval, using the same ultrasound equipment with the participant in a relaxed, supine position. PI and US measurements of diaphragm thickness were compared to establish inter-rater reliability

Table 2: Intra-rater and inter-rater reliability of PI and US ultrasound measurements

US: Tdi 1	US: Tdi 2	US: Tdi 3	US: Tdi Mean	PI: Tdi 1	PI: Tdi 2	PI: Tdi 3	PI: Tdi Mean
1.3	1.3	1.3	1.3	1.3	1.2	1.3	1.3
1.7	1.7	1.7	1.7	1.7	1.7	1.6	1.7
1.2	1.2	1.2	1.2	1.2	1.3	1.2	1.2
1.0	1.0	1.0	1.0	1.0	1.1	1.0	1.0
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0

*US = Ultra-sonographer; Tdi = Diaphragm thickness; PI = Primary investigator

Diaphragm Thickness: Sonographic measurement of diaphragm thickness (Refer to section 3.2.5)

Diaphragm Strength: Mouth pressure manometer measurements for diaphragmatic strength (Refer to Section 3.2.5)

Diaphragm Endurance: Participants were instructed to breathe through a pressure threshold device at 60% of PI_{max} until task failure. The fatigue resistance index (FRI = PI_{max} final/PI_{max} initial) was calculated as a measure of endurance (Refer to Section 3.2.5)

Data Analysis: To determine the relationships between the variables, Pearson correlations were calculated. Due to the small sample size and possibility of outliers, Spearman correlations were also calculated and reported together with the Pearson correlations. A 5% significance level ($P<0.05$) was used as guideline for determining significant correlations. Trends were however still reported due to the small sample size and lack of power

RESULTS

We included six participants in this pilot study. The mean age of the sample was 22.67 (SD=1.21), with a mean BMI of 23.72 (SD=2.77) (Refer to table 1). Preliminary results indicate a positive correlation ($r=0.75$; $r^2=0.56$; $p=0.08$) between the mean thickness of the diaphragm and PI_{max} measurements (Figure 1), while a negative correlation ($r=-0.26$; $r^2=0.07$; $p=0.62$) was found between the mean thickness of the diaphragm and FRI (Figure 2). Inter-rater reliability (ICC agreement = 0.99) was established.

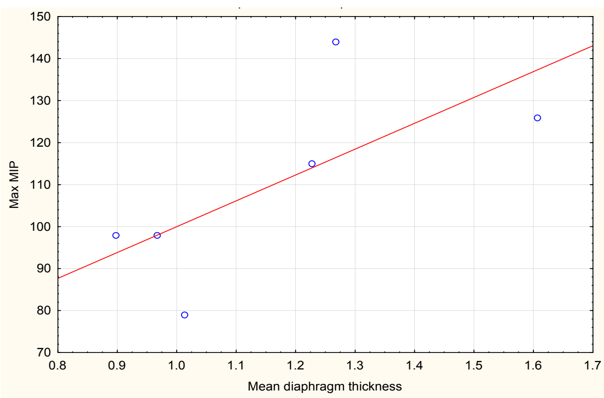


Figure 1: Mean PImax vs mean diaphragm thickness

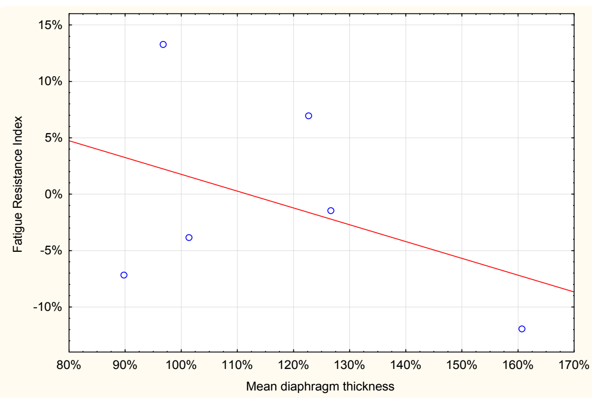


Figure 2: Mean FRI vs mean diaphragm thickness

CONCLUSION

This study will determine the correlation between diaphragm thickness; diaphragm strength and diaphragm endurance. This information could assist in the management of mechanically ventilated patients.

ADDENDUM I:

This poster was presented at the World Federation of Societies of Intensive and Critical Care Medicine (WFSICCM) in Durban this year

THE CORRELATION BETWEEN DIAPHRAGM STRENGTH, ENDURANCE AND THICKNESS

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BACKGROUND: Diaphragmatic function is an important determinant in the discontinuation of mechanical ventilation (MV). Measurements of diaphragm thickness using ultrasound imaging may be a suitable option for interpreting diaphragm function.[1] A correlation between diaphragm strength, diaphragm endurance and diaphragm thickness has not yet been established

OBJECTIVES: To determine the correlation between diaphragm strength, diaphragm endurance and diaphragm thickness

METHODS: Descriptive correlational study design. Sample of convenience; included healthy individuals (18-24 years); three activity-levels (sedentary; endurance- and strength related sporting activities); stratified for gender and BMI. Measurements included: Sonographic measurement of diaphragm thickness (Figure 1 and Figure 2); mouth pressure manometer measurements for diaphragmatic strength; and fatigue resistance index (FRI) as a measure of endurance. Participants were instructed to breathe through a pressure threshold device at 60% of PI_{max} until task failure. The FRI was calculated as $([PI_{max\ final}/PI_{max\ initial}] \times 100) - 100$. Intra-rater reliability established and testing procedures standardised a priori.[2]

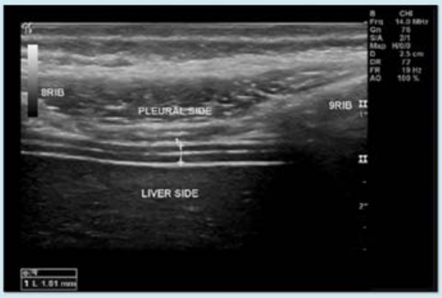

Figure 1: Example of sonographic imaging of the diaphragm

Figure 2: Example of sonographic imaging of the diaphragm in a 24 year old male with BMI = 25.76 and participating in a strength type sport

DATA ANALYSIS: To determine the relationships between the variables, Pearson correlations were calculated. Due to the small sample size and possibility of outliers, Spearman correlations were also calculated and reported together with the Pearson correlations. A 5% significance level ($P<0.05$) was used as guideline for determining significant correlations. Trends were however still reported due to the small sample size and lack of power

Table 1: Demographic profile of participants

Participant	Age	Activity Level	Type of Activity	BMI
001	24	Strength	Rugby	26.30
002	24	Strength	Rugby	25.76
003	23	Sedentary	None	25.25
004	22	Sedentary	None	23.80
005	21	Sedentary	None	22.32
006	22	Endurance	Cycling	18.90

RESULTS: We included six participants in this pilot study. The mean age of the sample was 22.67 (SD=1.21), with a mean BMI of 23.72 (SD=2.77) (Refer to table 1). Preliminary results indicate a positive correlation ($r=0.75$) between the mean thickness of the diaphragm and PI_{max} measurements (Figure 3), while a negative correlation ($r=-0.26$) was found between the mean thickness of the diaphragm and FRI (Figure 4)

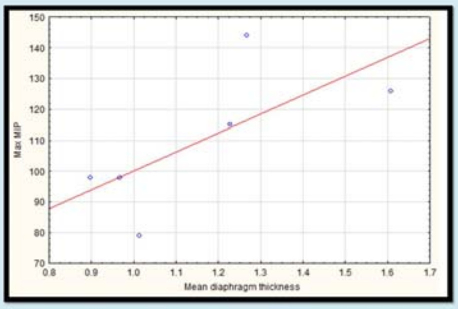

Figure 3: Mean diaphragm thickness vs PI_{max}

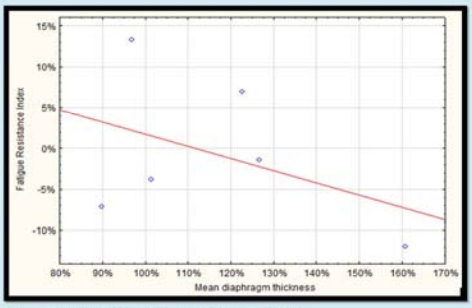

Figure 4: Mean diaphragm thickness vs FRI

CONCLUSION: This study will determine the correlation between diaphragm strength, endurance and thickness. This information could assist in the management of MV patients.

REFERENCES: (1) Grosu HB et al. Diaphragm Muscle Thinning in Mechanically Ventilated Patients. Chest 2012; 142(847): 1455-60
(2) Chang AT et al. Reduced inspiratory muscle endurance following successful weaning from prolonged mechanical ventilation. Chest 2005; 128(2): 553-9

ADDENDA RELATED TO RESULTS

ADDENDUM J

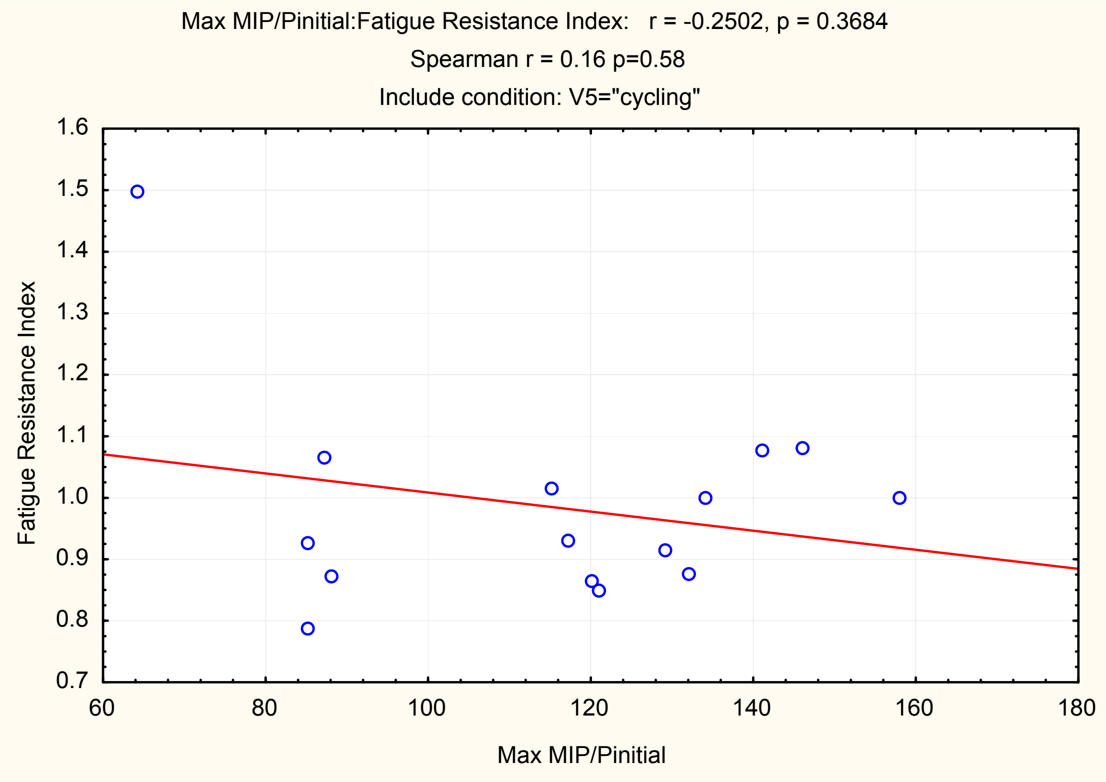

Figure A.1: Mean fatigue resistance index vs mean diaphragm strength in cyclists

ADDENDUM K

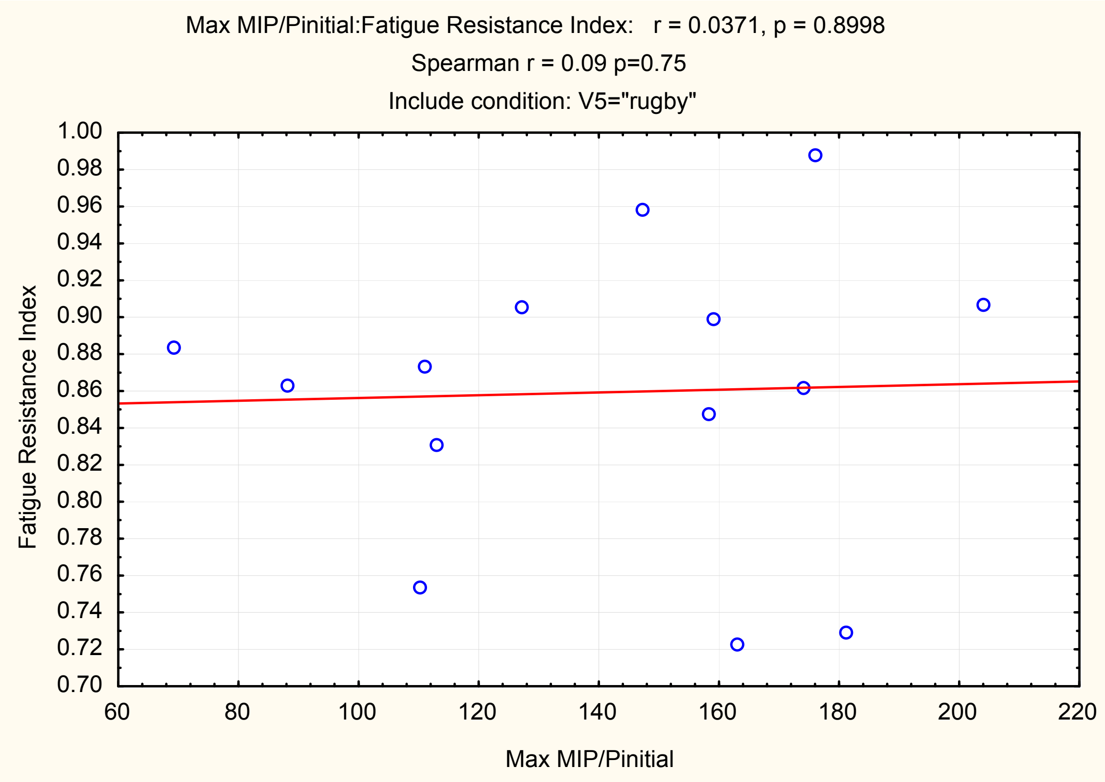

Figure A.2: Mean fatigue resistance index vs mean diaphragm strength in rugby players

ADDENDUM L

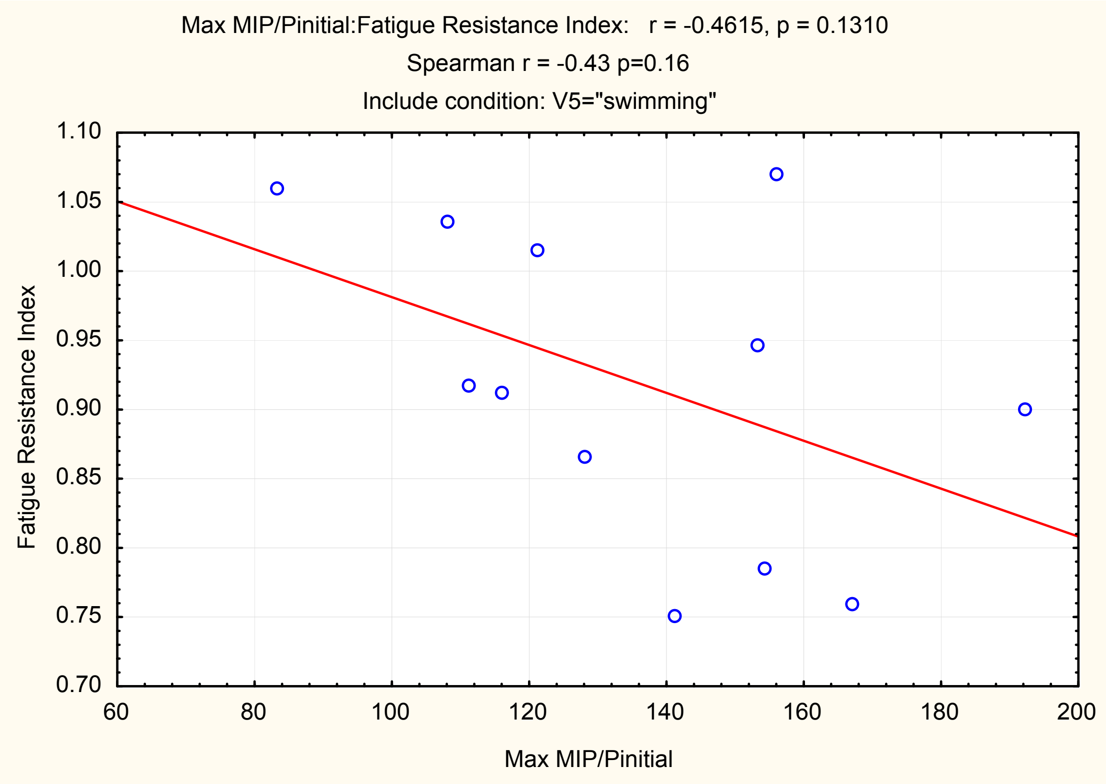

Figure A.3: Mean fatigue resistance index vs mean diaphragm strength in swimmers

ADDENDUM M

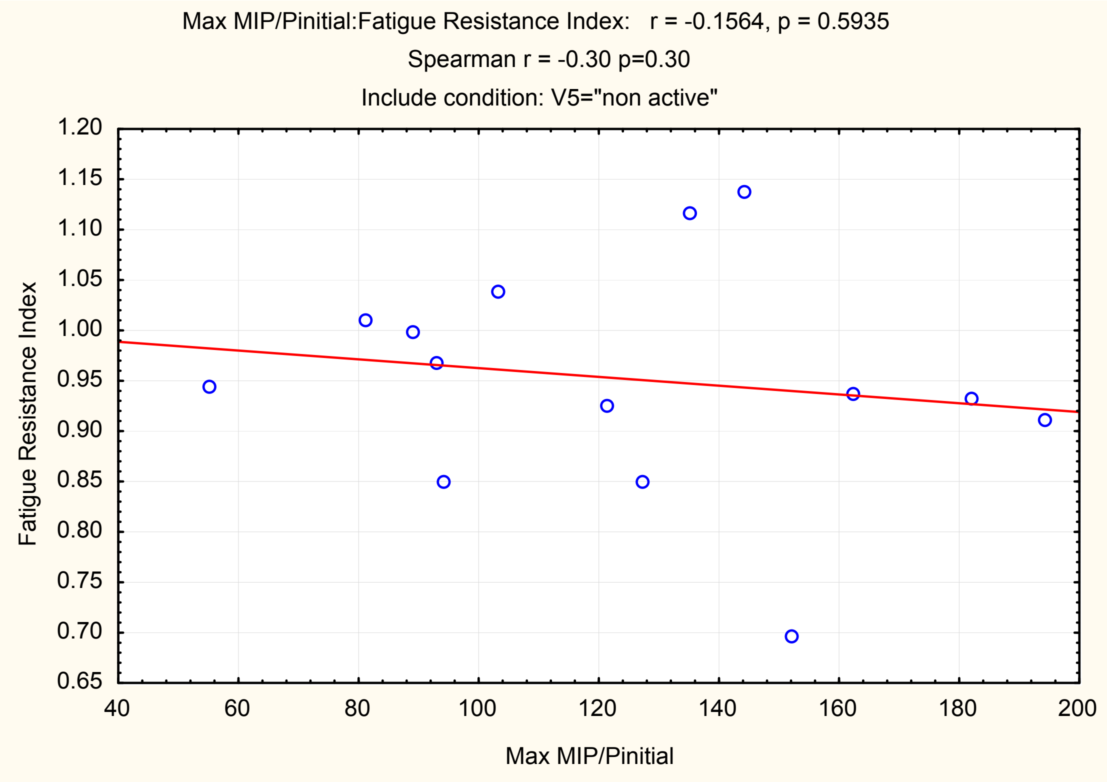

Figure A.4: Mean fatigue resistance index vs mean diaphragm strength in non-active subjects

ADDENDUM N

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Typography

- Please use double line spacing.
- Type the text unjustified, without hyphenating words at line breaks.
- Use hard returns only to end headings and paragraphs, not to rearrange lines.
- Capitalize only the first word, and proper nouns, in the title.
- All pages should be numbered.
- Use the *Critical Care* reference format.
- Footnotes are not allowed, but endnotes are permitted.
- Please do not format the text in multiple columns.
- Greek and other special characters may be included. If you are unable to reproduce a particular special character, please type out the name of the symbol in full. **Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF.**

Units

SI units should be used throughout (liter and molar are permitted, however).