Ultrasound imaging for the examination of diaphragm thickness in females with breathing pattern disorders

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Glossary

Note: references are added to those items that represent primary or secondary outcome measures used in the experimental study.

BMI: Body mass index, calculated by the formula of weight in kilograms divided by height in meters squared, and is expressed as kg/m^2 (Ministry of Health, 2017).

BMI+ group: participant group whose BMI is equal to or greater than $25kg/m^{2.}$, considered overweight in New Zealand (Ministry of Health, 2017).

BPD group: Participants with breathing pattern disorders.

Breathe hold time (BHT): assessment technique that involves a timed measurement of a breath hold with the nose-blocked on a tidal exhalation; the person holds their breath until they feel a moderate-strong urge to breathe (Grammatopoulou et al., 2014; Grammatopoulou et al., 2011).

CO_2: Carbon dioxide.

Dyspnoea: An unpleasant sensation of shortness of breath, difficulty breathing, or air-hunger.

Excursion: Movement of the of the diaphragm central tendon which is measured by ultrasound.

Healthy BMI group: participant group whose BMI falls within a healthy range of BMI 18.5-24.9 kg/m^2 (Ministry of Health, 2017).

Healthy group: healthy participants who do not have features of breathing pattern disorders.

Hi–Lo test : a manual and observational assessment completed in sitting, where the therapist asks the participant to place one hand on the abdomen, and one on the upper chest, and then observes if the patient has an abdominal or thoracic dominant pattern of breathing (Chaitow, 2014).

Hypercapnia: increased level of carbon dioxide in the blood.

Hypocapnia: a reduced level of carbon dioxide in the blood, that can be measured by capnography and can be described as end-tidal CO_2 < 35mmHg.

Hypoechogenic: a dark layer that represents reduced echo on ultrasound, that is seen in the diaphragm muscle, and may be bordered by brighter (hyperechogenic) layers.

Lung function tests (LFT): a set of tests that include spirometry, which measures the amount of air the lungs can hold, and how much air can be expelled in one second.

Manual assessment of respiratory motion (MARM): a manual therapy palpation technique that involves skilled palpation of the posterior rib cage to assess breathing pattern disorders (Courtney, Cohen, & Reece, 2009; Courtney & Dixhoorn, 2014).

Maximal inspiratory pressure (MIP): a measure of global inspiratory muscle strength and is measured from residual volume (Laveneziana et al., 2019).

Nijmegen Questionnaire (NQ): a 16-item outcome measure in which the patient rates the frequency of each BPD symptom on a 5-point scale from 0 (never) to 5 (very often), with a maximum score of 64 (Dixhoorn & Duivenvoorden, 1985).

Oxygen saturation (SpO_2): Oxygen saturation measured with a pulse oximeter device; normal levels are between 96-98% and a levels below 94% can indicate the presence of lung disease (Soguel Schenkel, Burdet, de Muralt, & Fitting, 1996).

TF: Thickening fraction of the diaphragm as calculated by the formula TF = (Tmax – Tvex) / Tvex (Laveneziana et al., 2019).

Tidal volume: The volume of air inspired and expired during normal quiet breathing.

Tmax: Thickness of the diaphragm at maximal inspiration.

Tmin: Thickness of the diaphragm at residual volume.

Total lung capacity (TLC): the volume of air present in the lung after a maximum inspiration.

Tvex: Thickness of the diaphragm at expiration to normal tidal volume or to expiratory reserve volume.

Tvin: Thickness of the diaphragm at inspiration to normal tidal volume.

USI: Ultrasound imaging, which uses high-frequency sound waves to evaluate muscle and soft tissue morphology inside the body (Teyhen, 2006).

Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Scott Peirce

22.5.2020

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Abstract

This research examined diaphragm thickness and diaphragm thickening fraction, using ultrasound imaging (USI), in females with breathing pattern disorders (BPD) and healthy female controls. The diaphragm is the key muscle of respiration, and people with BPD present with breathing that has increased accessory, chest and neck muscles use and decreased use of the diaphragm, and diaphragm wasting is hypothesised to occur over time. Establishing a clear diagnosis of BPD is difficult and USI has been proposed as a useful tool in respiratory physiotherapy for measurement of diaphragm morphology. The primary aim of this study was to answer the question: 'Does diaphragm thickness at tidal exhalation, as measured by USI, differ in females with BPD, when compared with healthy controls?'

A female BPD group (n=19) and female control group (n=18) with normal body mass index (BMI) were recruited in an outpatient setting. A second cohort of females (BPD group n=6, female control group n= 4) was recruited with an elevated BMI (BMI+). The inclusion criteria for the BPD group consisted of: elevated Nijmegen Questionnaire (NQ) score, elevated respiratory rate, dominant apical breathing pattern on the Hi-Lo test, and poor breath hold time. Diaphragm thickness was assessed by ultrasound imaging. Diaphragm thickness was measured at four points during the breathing cycle: tidal exhalation (Tvex), tidal inhalation (Tvin), maximum inhalation (Tmax), and exhalation to residual volume (Tmin). The diaphragm thickening fraction (TF) was then calculated by the formula TF = (Tmax – Tvex) / Tvex.

Results indicated significant differences in the primary measure of diaphragm thickness between the BPD and control groups at Tvex on the left side (BPD 1.583±0.341 mm, n=19; control 2.003±0.584, n=18; p=0.011) and on the right side (BPD 1.650±0.369, n=19; control 2.013±0.617, n=18; p=0.032). Other variables showed statistical significance for diaphragm thickness at Tvin, Tmax and TF on the left side, and at Tvin on the right side ($p<0.05$). Parametric statistical testing was unable to be performed due to inadequate sample size in the BMI+ groups. A trend of paradoxical diaphragm thickening was observed in both the BPD and BPD BMI+ groups from the Tvex to Tmin measurements on both the left and right sides.

These findings suggest that in females with a healthy BMI, diaphragm thickness is reduced in females with BPD compared with controls. USI is useful to quantify diaphragm atrophy in females with BPD, and future research in BPD should be directed towards replicating these results in a male BPD population, and in a BPD population with a >25kg/m^2 BMI sample. Based on these findings, patients with BPD should be assessed with USI to allow for identification of diaphragm atrophy, and treatment should focus on improving the morphology of the diaphragm with strengthening exercises.

Chapter 1. Introduction

1.1 Personal background to this study

I was first introduced to ultrasound imaging (USI) on a one day continuing professional development (CPD) course in 2007. It was exciting that a portable ultrasound machine could help access real-time visual data, which could be potentially useful to my patients in physiotherapy practice. I was also interested in how this information could be used as a reliable and valid objective measure and as a biofeedback treatment tool. I purchased an USI machine soon after that course. The following year, I attended a CPD course on breathing pattern disorders (BPD) taught by physiotherapists Dinah Bradley and Tania Clifton-Smith. As I had asthma, the course resonated with me personally. On a professional level, I had a new way of looking at the presenting problems of many of my patients. I was intrigued to see if I could combine USI and BPD and produce a measurement tool for the objective assessment of the diaphragm. I began trialling this technique first on myself and then on my patients. I observed positive results in my patients from the measurement process and the biofeedback treatment. As my USI skills evolved, I saw more patients with BPD who appeared to have atrophied diaphragm muscles and I speculated that there was a correlation between diaphragm atrophy and BPD. After reading more in the area of BPD, I noticed a gap in the literature, as no studies had been published on USI assessment of the diaphragm in a BPD population, therefore I decided to formally explore this question as part of a Masters of Health Science (Physiotherapy).

1.2 Statement of the problem

Hyperventilation, BPD or dysfunctional breathing are all terms used to describe a respiratory syndrome that has features of abnormal breathing and causes a variety of disjointed and confusing symptoms (Boulding, Stacey, Niven, & Fowler, 2016; Chaitow, Bradley, & Gilbert, 2014; Lum, 1975; Thomas, McKinley, Freeman, & Foy, 2001). However, the term BPD will be used for the purposes of consistency throughout this thesis. BPD are an umbrella term that is hypothesised to include many specific sub-patterns such as: hyperventilation, chronic mouth breathing, dominant apical

breathing, forced expiratory breathing, paradoxical breathing, erratic breathing, breath holding, and periodic sighing and yawning (Boulding et al., 2016; Chaitow, Bradley, & Gilbert, 2002; Lum, 1975; Rice, 1950).

Estimates of the prevalence of BPD range from 6% to 11% in healthy adult populations (M. Jones, Harvey, Marston, & O'Connell, 2013; Lum, 1975; Rice, 1950). Individuals with BPD may contribute significant cost and health burdens, as many of these patients present to emergency departments and require expensive screening to rule out other serious pathologies (Pfortmueller et al., 2015). One BPD case-study reported healthcare costs of $1,852.53 due to emergency department visits and health testing in New Zealand (Mooney & Candy, 2008).

Normal tidal respiration has a respiratory rate of 10–16 breaths per minute (in adults) (West, 2013), with approximately 400–500 ml per breath (Bowton & Scott, 2016). Tidal respiration primarily uses diaphragm muscle contraction to achieve adequate ventilation for the physiological needs of the body (Carrillo-Esper et al., 2016; De Troyer & Boriek, 2011). The diaphragm is comprised of slow twitch and fast twitch muscle fibres that are highly resistant to fatigue, which is necessary because of the near continuous nature of respiratory activity (Polla, D'Antona, Bottinelli, & Reggiani, 2004).

In extreme cases of BPD, individuals will have respiration that is in excess of metabolic requirements, which causes hypocapnia or lower levels of carbon dioxide (CO_2) in the blood, while oxygen (O_2) levels can be hyper-saturated (Chaitow et al., 2002, 2014; Lum, 1975). However, more commonly an individual with BPD presents with shortness of breath, apical dominant breathing, use of the accessory breathing muscles, and hyperinflation which alters the length-tension relationship and the function of the diaphragm (De Troyer & Boriek, 2011). These biomechanical changes can create further musculoskeletal pain including widespread muscular tension, chest pain, low back pain, neck pain (Chaitow, 2004; Chaitow et al., 2002, 2014) and psychological symptoms (e.g. anxiety and tension) (Gilbert, 1998, 2005, 2014). Many of the symptoms of BPD are listed in the Nijmegen Questionnaire (NQ) (Section 2.5.1; Appendix 1), and can be linked to the three domains of BPD: biomechanical,

biochemical, and psychological dysfunction (Courtney & Dixhoorn, 2014; Dixhoorn & Duivenvoorden, 1985; Dixhoorn & Folgering, 2015).

The exact pathological process of BPD continues to be a subject to debate, with multiple pathways existing that may create BPD (Clifton-Smith & Rowley, 2011; Gardner, 1996). Psychological stress has been implicated as a factor that may trigger the pathogenesis of BPD, as it activates voluntary ventilatory control pathways (Gardner, 1996; Jack et al., 2004; Jack, Rossiter, Warburton, & Whipp, 2003; Pfortmueller et al., 2015). Increased dominant apical breathing, and diaphragm muscle weakness and atrophy have also been hypothesised to exist in BPD, and may contribute to the aetiology of BPD (Chaitow et al., 2002, 2014; Clifton-Smith & Rowley, 2011). Other authors have suggested that various mechanical influences (e.g. enlarged tonsils or obstructed nasal airway) may lead to a BPD (Bartley, 2014). However, although individual triggers may exist to create BPD, it may be more likely that BPD have a multifactorial aetiology (Gardner, 1996). Triggers that are accepted to produced increased ventilatory drive and therefore are implicated in BPD pathogenesis include: excessive caffeine (Polla et al., 2004), acute and chronic pain (Jafari, Courtois, Van den Bergh, Vlaeyen, & Van Diest, 2017), sustained focus and stress (Grassmann, Vlemincx, von Leupoldt, Mittelstädt, & Van den Bergh, 2016), diaphragm muscle weakness (McCool & Tzelepis, 2012), anaemia (Chaitow et al., 2014; Clifton-Smith & Rowley, 2011), respiratory pathologies (e.g. asthma) (Thomas et al., 2001), and psychological conditions (e.g. anxiety and panic disorders) (Gilbert, 1998, 2014; M. Jones et al., 2013).

Treatment of BPD focuses on restoring normal breathing patterns, and improving diaphragm function, and this has been shown to improve quality of life in randomised controlled trials that have looked at BPD most commonly within asthma populations (Bruton et al., 2018; Grammatopoulou et al., 2011; Thomas et al., 2003). However, while treatment of BPD has been well studied, the diagnosis of a BPD is difficult, especially as no clear gold-standard diagnostic test is available (Courtney & Dixhoorn, 2014; Dixhoorn & Duivenvoorden, 1985; Dixhoorn & Folgering, 2015; Ogilvie & Kersten, 2015). This highlights the need to establish clear diagnostic criteria and screening tools for BPD (Pfortmueller et al., 2015). Therefore, further investigation into accurate and valid clinical assessment tools for BPD is warranted. Recent reviews

recommend a variety of measures for the assessment and diagnosis of BPD, which may include: the NQ, manual assessment of respiration motion (MARM), Hi-Lo test, measurement of breath-hold time (BHT), oxygen saturation, and respiratory rate (RR) (Chaitow et al., 2002, 2014; Courtney, 2017). However some debate exists as there are concerns about the validity and reliability of many of these measurement techniques, including the MARM (Ludwig, 2013) and NQ (Ogilvie, 2017; Ogilvie, Kayes, & Kersten, 2019; Ogilvie & Kersten, 2015). Furthermore, many of these assessment techniques are primarily subjective in nature, and none of the above measurements provides a valid or objective measurement of diaphragm muscle function, despite this having been hypothesised as weak and dysfunctional in BPD (Chaitow et al., 2002, 2014; Clifton-Smith & Rowley, 2011; Hruska, 1997). There is a need to research new tools in the field of BPD, and USI of the diaphragm may provide an objective, and valid and reliable tool for measuring diaphragm morphology.

Muscle morphology (i.e. muscle size and thickness) is closely correlated to muscle strength (i.e. function) (Bogdanis, 2012; Gao, Arfat, Wang, & Goswami, 2018), and diaphragm thickness correlates to inspiratory muscle strength and pulmonary function in healthy individuals (Cardenas, Santana, Caruso, Ribeiro de Carvalho, & Pereira de Albuquerque, 2018). Furthermore, USI of the diaphragm muscle is gaining popularity as a measurement tool that can be useful in the objective assessment of diaphragm morphology in populations with neurological conditions such as stroke (Cho, Lee, Kim, & Lee, 2018; Jung & Kim, 2014, 2015, 2017; Jung, Shim, Kwon, Kim, & Kim, 2014; Kim, Lee, Cho, & Lee, 2017), respiratory conditions such as chronic obstructive pulmonary disease (COPD) (Antenora et al., 2017; Baria et al., 2014; Crimi et al., 2018; Gorman, McKenzie, Pride, Tolman, & Gandevia, 2002), and musculoskeletal conditions such as low back pain (Calvo-Lobo et al., 2019; Finta, Nagy, & Bender, 2018). Furthermore, there is encouragement within contemporary literature for physiotherapists to use USI in clinical situations to provide safe, objective, valid and useful information to aid diagnosis and management of respiratory conditions (Hayward & Janssen, 2018; Le Neindre, Mongodi, Philippart, & Bouhemad, 2016; Leech, Bissett, Kot, & Ntoumenopoulos, 2015; Whittaker et al., 2019). USI measurement can provide information on the movement or excursion of the diaphragm, thickness of the diaphragm, thickening fraction (TF) of the diaphragm, as well as measurement and

diagnosis of pleural effusion and lung consolidation (Le Neindre et al., 2016). In BPD accessory muscles activity is increased (Boulding et al., 2016), and this is thought to lead to diaphragm unloading (Clifton-Smith, 2014), and this may cause further changes to the function, morphology, and thickness of the diaphragm muscle – therefore USI of the diaphragm thickness and TF may be able to assess diaphragm atrophy.

In lieu of the validity concerns of more traditional assessments of BPD (i.e. NQ and MARM), USI for diaphragm morphology and function may present a viable method to enhance the diagnosis of BPD. Measurement of diaphragm atrophy or thinning of the diaphragm is deemed clinically useful as it may provide a correlation between BPD and diaphragm atrophy, allowing clear identification of individuals with BPD. Results from this study may also help physiotherapists to identify a clear treatment target within BPD (e.g. strengthening of the diaphragm) and may help to inform and direct further research regarding the mechanisms behind the pathophysiology of BPD.

1.3 Purposes and aims

To date, no studies have examined diaphragm thickness in a population of people with BPD. The purpose of this study was to measure diaphragm thickness and TF at tidal exhalation (Tvex), tidal inhalation (Tvin), maximal inhalation (Tmax) and exhalation to residual volume (Tmin) in the left and right diaphragm in females with BPD using USI. The diaphragm thickening fraction (TF) was calculated by the formula TF = (Tmax – Tvex) / Tvex (Laveneziana et al., 2019). These measures were also compared with those for healthy females to explore any correlations between diaphragm thickness, TF, and BPD.

In order to minimise bias due to the confounding factors of gender and body mass index (BMI), the focus of this study was narrowed to USI of diaphragm thickness in females with BPD, stratified by two BMI groups: a healthy BMI group (BMI 18.5–24.9 kg/m^2) and a BMI+ group (BMI $\geq$25 kg/m^2).

Primary aim: To measure values of diaphragm thickness and TF using USI at Tvex, Tvin, Tmax and Tmin in females with BPD and a healthy BMI (18.5–24.9 kg/m^2) compared with healthy female controls with a healthy BMI (18.5–24.9 kg/m^2).

Secondary aim: To measure values of diaphragm thickness and TF using USI at Tvex, Tvin, Tmax and Tmin in females with BPD and a BMI $\geq$25 kg/m^2 compared with female controls with a BMI $\geq$25 kg/m^2.

Tertiary aim: To measure values of diaphragm thickness and TF using USI during Tvin and Tvex, and Tmin and Tmax in females with BPD and female controls with a healthy BMI (18.5–24.9 kg/m^2), compared with females with BPD and female controls with a BMI $\geq$25 kg/m^2.

1.4 Hypotheses

Research Hypothesis 1: There will be a significant difference in diaphragm thickness and TF between females with BPD and a healthy BMI (18.5–24.9 kg/m^2) compared with healthy control females in the matched group.

Research Hypothesis 2: There will be a significant difference in diaphragm thickness and TF between females with BPD and a BMI $\geq$25kg/m^2 (BMI+ group) compared with healthy control females in the matched group.

Research Hypothesis 3: There will be a significant difference in diaphragm thickness and TF between females with BPD and a healthy BMI (18.5–24.9 kg/m^2) and females with BPD and BMI $\geq$25 kg/m^2 (BMI+ group). There will also be a significant difference in diaphragm thickness and TF between control females with a healthy BMI (18.5–24.9 kg/m^2) and control females with a BMI $\geq$25 kg/m^2.

1.5 Significance of this study

To date, there is no research that has examined the use of USI of the diaphragm in the diagnosis of BPD. USI could provide a low-cost and repeatable analysis of the diaphragm in BPD that can be used in both clinical and research environments. Measurement of diaphragm atrophy or thinning of the diaphragm is potentially clinically useful as it may allow for identification of individuals with BPD and provide the first evidence of a correlation between BPD and diaphragm atrophy. These findings

may help to reduce the burden and cost of BPD in settings such as emergency departments and cardiac wards. The burden of BPD may be reduced as patients may spend less time waiting for expensive tests such as magnetic resonance imaging and cardiac ultrasound assessment (i.e. due to chest pain symptoms in BPD), and may progress to physiotherapy treatment in a more efficient manner. Cost savings to the health sector may occur as expensive testing may be reduced from a reported value of $1,852.53 (Mooney & Candy, 2008), to the lower cost of 6 sessions in either private physiotherapy or hospital outpatient physiotherapy.

If diaphragm atrophy is identified, it may also allow for a clear diagnosis and may inform a physiotherapy treatment strategy (e.g. strengthening of the diaphragm) in the treatment of BPD. These findings may be extrapolated to other areas of research involving pathologies in the respiratory system e.g. asthma and COPD (Grammatopoulou et al., 2014), psychological system e.g. anxiety and panic disorder (Vollmer, Strawn, & Sah, 2015), gastrointestinal system e.g. gastro oesophageal reflux disease (GORD) and supragastric belching (Eherer et al., 2012) and musculoskeletal system e.g. neck pain and low back pain (Chaitow, 2004; Dimitriadis, Kapreli, Strimpakos, & Oldham, 2013, 2014), where the diaphragm has been implicated.

Chapter 2. Background and literature review

This chapter reviews normal breathing, the dimensions of BPD, and discusses current methods for the assessment (both subjective and objective measures) and diagnosis, as well as the challenges surrounding these methods. The case for the use of USI to assess diaphragm thickness as an objective measure for diagnosis in BPD will also be discussed.

2.1 Mechanics of normal breathing

Normal breathing is primarily concerned with supplying the body with O_2, the removal of CO_2 and the stabilisation of the body's alkalinity and acidity (i.e. pH) (West, 2013). Normal tidal respiration i.e. relaxed breath inhalation/exhalation (Figure 1) has a respiratory rate of 10–14 breaths per minute (in adults) (West, 2013), and is approximately 400 mls in females and 500 mls in males per breath (Bowton & Scott, 2016). This creates a normal gas exchange per minute volume of 5–8 litres/minute (West, 2013). Normal tidal inhalation uses diaphragm contraction and caudal motion of the dome of the diaphragm to achieve ventilation of the lungs, along with subtle movements of the rib cage through the action of the intercostal muscles (Carrillo-Esper et al., 2016; De Troyer & Boriek, 2011; Lee, Chang, Coppieters, & Hodges, 2010). In contrast, resting exhalation is a passive process that is aided by elastic recoil of the lungs, ribs, intercostals and diaphragm (Bordoni & Zanier, 2013; De Troyer & Boriek, 2011). The inspiratory muscle action creates a negative pressure on the pleura and lungs, which draws air from the outside atmosphere through the nasal passage into the upper airways, and down within the lungs to the alveoli, where gas exchange of CO_2 and O_2 occurs (De Troyer & Boriek, 2011; West, 2013).

Figure 1. Lung volumes. Images Source: https://commons.wikimedia.org/wiki/File:LungVolume.jpg

The diaphragm is the most important inspiratory muscle (West & Luks, 2016), and contributes to approximately three-quarters of the inspiratory volume of vital capacity, whereas thoracic expansion contributes to one-quarter of this volume (Boussuges, Gole, & Blanc, 2009). The diaphragm is a dome shaped, parachute-like structure that separates the chest from the abdomen (Figure 2) (Bordoni & Zanier, 2013). The complex muscular structure of the diaphragm can be divided into a central tendinous portion and muscular portions with attachments to sternal, lumbar, costal, and crural fibres that surround the oesophageal opening. The sternal portion attaches to the xiphoid process (De Troyer, 1997; De Troyer & Boriek, 2011), and the costal section attaches bilaterally to the bottom six ribs; in this area, the diaphragm sits against the lower lateral rib cage (commonly ribs 7, 8, 9, and 10), with this region termed the zone of apposition (ZOA) (Figure 3) (McCool & Tzelepis, 2012). The ZOA is the area where the diaphragm muscle fibres contract, shorten and thicken to produce the descent of the dome of the diaphragm and inspiration (McKenzie, Gandevia, Gorman, & Southon, 1994). The ZOA has particular significance as its length is shortened with lung hyperinflation (De Troyer, 1997; De Troyer & Boriek, 2011) and in respiratory diseases such as COPD (Crimi et al., 2018), which reduces the efficiency of the diaphragm due to the alteration in length-tension relationship of its skeletal muscle fibres (De Troyer, 1997; De Troyer & Boriek, 2011). The lumbar section of the diaphragm attaches to the first, second and third lumbar vertebral bodies and corresponding lumbar discs (Bordoni & Zanier, 2013). The diaphragm has three openings for the ascending aorta, inferior vena cava and oesophagus, which allow the diaphragm to influence the cardiovascular and gastrointestinal system (De Troyer & Boriek, 2011).

Figure 2. Inferior view of the diaphragm showing openings for the aorta, oesophagus and vena cava, and the broad anatomical connections.
(Source: https://cnx.org/contents/FPtK1zmh@8.25:fEI3C8Ot@10/Preface)

The nerve supply of the diaphragm is through the right and left phrenic nerves, which create neurological drive to the right and left hemi-diaphragms (De Troyer, 1997; De Troyer & Boriek, 2011). Furthermore, the hemi-diaphragms are asymmetrical due to the position of the liver and heart, which produces differences in the motion of the diaphragm with deeper breathing. During deep breathing, the right diaphragm descends by 7.0±1.1 cm in males and 5.7±1.0 cm in females; the left diaphragm descends by 7.5±0.9 cm in males and 6.4±1.0 cm in females (Boussuges et al., 2009). Diaphragm motion is reduced and almost symmetrical during quiet breathing i.e. the right diaphragm descends by 1.8±0.4 cm in males and 1.6±0.3 cm in females, with similar motion of the left diaphragm (Boussuges et al., 2009). In comparison, the apical chest wall expands by a much smaller amount during tidal breathing, with expansion in an anterior posterior direction of 0.35±0.16 cm at the first thoracic vertebrae and 0.5±0.15 cm at the seventh thoracic vertebrae, and lateral expansion of 0.52 cm at the axilla level (Lee et al., 2010). Therefore, the motion of the diaphragm is much greater than chest motion during normal tidal breathing.

During inspiration to total lung capacity (Figure 1), greater muscular activity is seen in all inspiratory muscles, and the accessory inspiratory muscles also contract (Hudson, Gandevia, & Butler, 2007). With exhalation to residual volume, lung deflation is facilitated by the abdominal muscles contracting and producing an increased intra-abdominal pressure; the diaphragm thins and lengthens, and is displaced in a cranial direction into to the thoracic cavity (De Troyer & Boriek, 2011). Although all abdominal muscles are active during exhalation to residual volume, the transversus abdominis muscle produces the greatest expiratory force (De Troyer & Boriek, 2011; Misuri et al., 1997).

Figure 3. Picture of the lungs, diaphragm, and zone of apposition.
(Source: Umbrello & Formenti, 2016; http://rc.rcjournal.com/content/61/4/542/tab-figures-data)

For normal biomechanics of breathing to occur, the diaphragm must have adequate excursion to facilitate the indrawing of air into the lungs through pressure changes,

adequate muscular thickness and strength to create contractile force, and adequate length in the ZOA; as all of these variables create an optimal length-tension relationship for the muscle fibres of the diaphragm, which allows efficient ventilation of the lungs (De Troyer, 1997; De Troyer & Boriek, 2011).

The diaphragm can become dysfunctional (i.e. the diaphragm may begin to function in a manner that is no longer efficient) due to pathophysiological changes in lung tissue in some conditions such as COPD and severe asthma. For example, in some individuals with COPD, the lung becomes excessively hyperinflated and the ZOA is shortened, and this causes the diaphragm to become flattened (De Troyer, 1997; De Troyer & Boriek, 2011). When the muscular length of the diaphragm fibres is excessively shortened, the diaphragm may then function in a paradoxical manner, with diaphragm contraction causing exhalation, and diaphragm relaxation causing inhalation (De Troyer, 1997; De Troyer & Boriek, 2011). This paradoxical breathing pattern significantly increases the work of breathing, and creates accessory muscle fatigue and respiratory distress (De Troyer, 1997; De Troyer & Boriek, 2011).

There is some debate regarding whether the diaphragm can be considered as two distinct muscles. Because of its two independent (phrenic) nerve supplies and in conditions such as a phrenic neuropathy (Caleffi-Pereira et al., 2018), the diaphragm may function as right and left muscles (Boon et al., 2014; Gottesman & McCool, 1997; Massery, 2017; McCool & Tzelepis, 2012). Other anatomical perspectives suggest distinction between a respiratory and digestive or crural portion, which provide a sphincter action on the oesophagus and provide an anti-reflux barrier (De Troyer & Boriek, 2011; De Troyer, Sampson, Sigrist, & Macklem, 1981; Pickering & Jones, 2002). Despite these perspectives, the diaphragm's primary role is respiration, with many secondary roles that are controlled by precise neurological mechanisms in the central nervous system, such as musculoskeletal stability and postural control (Chaitow, 2004; Hodges & Gandevia, 2000a; Wallden, 2017), gastrointestinal roles (e.g. digestion and anti-reflux) (Eherer et al., 2012; Kessing, Bredenoord, & Smout, 2012), and fluid and lymphatic controls (e.g. lymphatic drainage of the abdominal cavity) (Abu-Hijleh, Habbal, & Moqattash, 1995).

2.2 Neurological control of breathing

The neurological basis of the rhythm of breathing is generated by a complex network neurons in the medullary region in the brainstem (the pre-botzinger complex and retrotrapezoid nucleus), with these rhythms further shaped by the brainstem and pons (Evans et al., 2009; Homma & Masaoka, 2008; Ikeda et al., 2017; Masaoka, Izumizaki, & Homma, 2014). Further respiratory pattern shaping is performed by premotor and respiratory motor neurons, and in the upper cervical spinal cord (Ikeda et al., 2017). Neural control of breathing is modulated by the many interactions that occur with respiration, such as swallowing (McFarland et al., 2016), vocalisation, coughing, postural stability challenges (Hodges & Gandevia, 2000a, 2000b; Hodges, Heijnen, & Gandevia, 2001), haemodynamic stability (blood flow, lymph flow) (Abu-Hijleh et al., 1995), digestion, maintenance of normal continence, and heat regulation (Tsuji, Hayashi, Kondo, & Nishiyasu, 2016). Due to the interactions of the above neural control networks, BPD may present with dysfunctions and substituted motor control strategies in many of these systems, such as breath holding, or inappropriate Valsalva-like manoeuvres, or altered postural stability with exercise (Clifton-Smith, 2014).

2.3 Breathing pattern disorders

2.3.1 Historical context of BPD

The diagnosis and treatment of BPD represent an emerging field of research (Chaitow et al., 2014). However, BPD symptoms were described as early as 1871 as an 'irritable heart' (De Costa, 1871). The original symptom description of irritable heart was almost exactly the same as the symptom cluster described in modern accounts of BPD: palpitations, digestive disturbances, pain in the chest, pins and needles, shortness of breath and dizziness (Chaitow et al., 2002, 2014; De Costa, 1871). In the 100 years following the work of De Costa (1871), various names were given to BPD, until the term hyperventilation syndrome (HVS) rose to prominence as a diagnosis in 1950–1970s (Lum, 1975; Rice, 1950). Doctors and physiotherapists made HVS diagnoses, and care was provided within private and public healthcare (Holloway & West, 2007; Lum, 1975; Rice, 1950).

The validity of the term ‘hyperventilation’ and the diagnosis of HVS were challenged in 1996, with a landmark paper by Hornsveld, Garssen, Dop, van Spiegel, and de Haes (1996) that showed both hypocapnia and hypercapnia produced symptoms associated with BPD. This resulted in a significant change in the understanding of the pathophysiology of BPD, and a change in terminology from HVS to dysfunctional breathing and BPD. Movement away from a BPD diagnosis based solely on hypocapnia has been steadily made (Boulding et al., 2016), and subsequent research evaluated prevalence, and showed treatment efficacy with randomised controlled trials in this field in the early 2000s (Holloway & West, 2007; Thomas et al., 2003; Thomas et al., 2001; Thomas et al., 2009), and the first textbook on BPD was published in 2002 (Chaitow et al., 2002).

2.3.2 Definition of BPD

> In BPD “there is no formal definition…and no gold standard diagnostic method” (Boulding et al., 2016, p. 287).

BPD is a syndrome, and acts as an umbrella term that describes a cluster of symptoms, and despite recognisable sub-patterns of BPD, there is still no formal definition for BPD (Boulding et al., 2016; Chaitow et al., 2014). This lack of a clear definition has led to confusion in the diagnosis of BPD (Barker & Everard, 2015).

There is also debate around the specific sub-patterns of BPD, with many patterns that have been described, including chronic mouth breathing (Courtney, 2009), hyperventilation, apical breathing, forced expiratory breathing, paradoxical breathing, periodic deep sighing and yawning (Boulding et al., 2016), erratic breathing, and breath holding (Gilbert, 1998, 2014). Although some patterns are commonly described such as hyperventilation, specifics in this pattern are still debated: with hyperventilation not considered to be linked with hypocapnia by some authors (Boulding et al., 2016), yet other authors consider this connection to be valid (Grammatopoulou et al., 2014; Jack et al., 2004; Jack et al., 2003; Pfortmueller et al., 2015). Therefore, there is an urgent need for a consensus-based approach to defining BPD and describing the patterns involved in BPD.

Overall, agreement exists in the literature that BPD are multifactorial in nature, and may be influenced by inter-related biochemical, psychological and biomechanical factors (Chaitow et al., 2002, 2014; Courtney, 2009, 2017). These factors are useful in understanding symptomology and pathophysiological processes and are helpful in guiding assessment and selecting treatment techniques.

A further issue concerning variation in the nomenclature, may be attributable to discipline bias, as research in BPD has originated from a variety of medical and complementary medical disciplines (e.g. cardiology, psychology, psychiatry, physiotherapy, respiratory medicine, exercise science and osteopathy). The largest evolution in terminology resulted from research by Hornsveld et al. (1996) that challenged the original biochemical concepts in BPD in a randomised placebo blind study. This research led to a decoupling of hyperventilation from hypocapnia (Boulding et al., 2016; Chaitow et al., 2014), with hypocapnia defined as reduced CO_2 (below 35 mmHg) (McLaughlin, 2009). The terminology now in use in current literature is dysfunctional breathing and BPD (Boulding et al., 2016; Chaitow et al., 2002, 2014; Gilbert, 1998; Thomas et al., 2001). There is a urgent need to gain a formal consensus on terminology to allow for a formal definition of BPD (Boulding et al., 2016; Bruton, Garrod, & Thomas, 2011; Gilbert, 1998, 2014), and this should be a goal for clinicians and researchers working in the field of BPD.

2.3.3 Prevalence of BPD

Estimates of the prevalence of BPD in general adult populations range from 6% to 11% (M. Jones et al., 2013; Lum, 1975; Rice, 1950). BPD have also been shown to be more prevalent in females (55%) than males (45%) (Pfortmueller et al., 2015). The prevalence of BPD in related respiratory conditions was reported as 29%–64% in asthmatic populations (Stanton, Vaughn, Carter, & Bucknall, 2008; Thomas et al., 2001), and 43% in people with COPD (De Vos et al., 2017). There is also a recognised overlap between BPD, anxiety and panic disorder, with an emergency department study reporting 39% of those who attended due to a BPD-related episode had a comorbid anxiety or panic disorder (Pfortmueller et al., 2015). There is also a known overlap between vestibular disorders and BPD, with 23% of patients attending a vestibular clinic reported to have BPD (Humphriss, Baguley, Andersson, & Wagstaff,

2004). Much of the research reporting the prevalence of BPD is from Europe and the United Kingdom, and relies on the use of the Nijmegen questionnaire for diagnosis of a BPD, (De Vos et al., 2017; Humphriss et al., 2004; Thomas et al., 2001). However there is debate around the validity of this questionnaire (refer to Section 2.5.1) (Ogilvie et al., 2019; Ogilvie & Kersten, 2015) and future prevalence research should use a variety of measures to diagnose BPD. Furthermore, New Zealand based research is needed to define the national prevalence of BPD.

2.3.4 Domains of BPD

Due to the confusing symptoms and pathophysiology of BPD, authors have organised BPD into three specific domains (i.e. aetiological areas) of: biomechanical, biochemical, and psychological (see Sections below) (Chaitow et al., 2002, 2014; Courtney, 2017). Furthermore, many of the symptoms that are seen in BPD and are listed in the NQ (see Section 2.5.1, and Appendix 1), can be connected with one or two specific domains (Courtney, 2017; Dixhoorn & Duivenvoorden, 1985; Dixhoorn & Folgering, 2015). BPD symptoms that may be more related to biomechanical mechanisms include: chest pain, feelings of tension, shortness of breath, bloated feeling in the stomach, inability to breathe deeply, tight feeling in the chest and faster or deeper breathing (Courtney, 2018; Courtney & Dixhoorn, 2014). Symptoms related to biochemical mechanisms may include: air hunger, shortness of breath, bloated feeling in the stomach, tingling fingers, stiff fingers and arms, tightness around the mouth, cold hands and feet, tight feelings in the chest and faster or deeper breathing (Courtney, 2018; Courtney & Dixhoorn, 2014). Finally, psychophysiological symptoms may include: feeling tense, blurred vision, pins and needles, dizzy spells, feeling confused, palpitations and feelings of anxiety or panic (Dixhoorn & Duivenvoorden, 1985; Dixhoorn & Folgering, 2015).

Biomechanical domain of BPD

Theories suggest that diaphragm muscle activity is reduced in BPD which may lead to its weakness (Chaitow, 2014; Clifton-Smith & Rowley, 2011; Hruska, 1997). This decreased diaphragm activity is compounded as accessory muscle overuse (e.g. pectoral, scalene, trapezius, sternocleidomastoid and upper intercostal muscles) is thought to be an important part of the pathophysiology of BPD. While these accessory

muscles attempt to maintain adequate ventilation in BPD, they lead to further mechanical unloading and weakness of the diaphragm (Clifton-Smith & Rowley, 2011; Gilbert, 2014; Hruska, 1997). A vicious cycle of dominant apical breathing with hypertonicity in the intercostal and chest wall muscles, hyperinflation, and unloading of the diaphragm is hypothesised to occur in BPD, and this may contribute to the unpleasant biomechanical symptoms of tension, chest pain and shortness of breath (Clifton-Smith & Rowley, 2011; Hruska, 1997). However, to date there is no been research to test these hypotheses in BPD or assess the validity of these observations.

Randomised controlled trials (RCTs) in BPD and asthma populations usually include breathing retraining exercises, and these exercises aim to improve the function of the diaphragm muscle (Bruton et al., 2018; Grammatopoulou et al., 2011; Thomas et al., 2003). One landmark BPD RCT study was strongly designed, had a large sample sizes (n=655), and adequate blinding, and showed that breathing retraining delivered by DVD was similar to face to face physiotherapy delivered over 3 sessions and both of these methods were superior to usual care with improvements seen in asthma quality of life questionnaire (Bruton et al., 2018). However, there were no significant changes in airway obstruction ($FEV_{1,}$ PEFR) or inflammation. These results are similar to earlier smaller and mid-sized studies that have shown positive treatment effects with improved asthma control, by utilising breathing retraining with diaphragm breathing (Holloway & West, 2007; Thomas et al., 2003; Thomas et al., 2009). Another smaller study (n=40) with less robust design (blind assessors, but no blinding of treatment) was able to demonstrate improvements in respiratory parameters (improved FEV_1 and End tidal CO_2) after 13 physiotherapy sessions (Grammatopoulou et al., 2011). Therefore, while these studies seem to link improvements in BPD populations, with treatment that focuses on restoration of normal diaphragm breathing, diaphragm morphology changes have not been assessed in these studies. However other possible mechanisms that may be responsible for improvements in BPD in the above studies may include neuroplasticity changes, or biochemical changes. Therefore, further research is required to identify if BPD populations have diaphragm atrophy or weakness and if breathing retraining improves the strength and function of the diaphragm.

Altered diaphragm muscle activity and hyperinflation, has also been further associated with abdominal muscle dysfunction (De Troyer & Boriek, 2011; Hruska, 1997), and

both weakness of the abdominal wall and hypertonicity of the abdominal wall have been described in BPD (Clifton-Smith & Rowley, 2011; Courtney, 2017; Whittaker, 2008). While abdominal muscle dysfunction has been described in healthy populations under hypercapnic conditions (Hodges et al., 2001), and in a unilateral low back pain population with BPD in one study (Whittaker, 2008), no research has so far assessed the correlation between hyperinflation, diaphragm atrophy and abdominal muscle dysfunction in BPD populations (refer to 2.5.3 for a discussion of clinical assessment techniques).

Biochemical domain of BPD

Some individuals with a BPD can present with normal oxygen saturations and hypocapnia (SpO_2 >98% and end-tidal CO_2 < 35mmHg), and this can lead to respiratory alkalosis (Gardner, 1996; Gilbert, 2005). Hypocapnia is defined as reduced CO_2 below 35 mmHg (McLaughlin, 2009), and the effects of hypocapnia and respiratory alkalosis are wide ranging, and have been linked to paraesthesia and muscle hypertonicity due to metabolic compensation, and the increased excretion of calcium and magnesium by the kidneys (Chaitow, 2004; Gardner, 1996). Hypocapnia also changes haemoglobin uptake of oxygen, creates coronary artery constriction (Laffey & Kavanagh, 2002) and reduced cerebral blood flow (Chang & Glover, 2009; Giardino, Friedman, & Dager, 2007). The contribution of biochemical dysfunction in BPD continues to be debated, with disparate results in several studies. Some studies show a correlation between hypocapnia and BPD (Grammatopoulou et al., 2014; Jack et al., 2003; Pfortmueller et al., 2015), and others studies show no relationship (Courtney, Greenwood, & Cohen, 2011; Hornsveld et al., 1996). Current expert opinion suggests that hypocapnia is thought to occur in some, but not all cases of BPD, and is therefore not an essential feature of BPD (Boulding et al., 2016; Clifton-Smith & Rowley, 2011; Courtney, 2017). Further research is needed to determine the proportion of BPD cases that have biochemical dysfunction, and the ideal method of assessing biochemical dysfunction (see Section 2.5.4).

Psychological domain

In situations of stress, prolonged concentration, or emotional difficulty, fluctuations can be observed in breathing volumes and respiration rate (Homma & Masaoka, 2008; Masaoka et al., 2014; Zelano et al., 2016). Furthermore, in anticipatory anxiety, repetitive sighing or a hyperventilation response is commonly observed (Gilbert, 1998, 2014). This is similar to the respiratory response seen during conditions of sustained mental focus and pain in experimental settings (Grassmann et al., 2016; Jafari et al., 2017). Therefore in healthy subjects, a variety of stressors creates increased demand on the respiratory system which may lead to BPD, if sustained (Gilbert, 2014).

BPD have been correlated to mental health conditions such as anxiety and panic disorder (Pfortmueller et al., 2015), and alterations in respiration of both hypocapnia and hypercapnia can produce both alkalinity and acidity in the brain, with both states implicated in panic attacks (Giardino et al., 2007; Vollmer et al., 2015; Wilhelm, Trabert, & Roth, 2001; Winter, Ahlbrand, Naik, & Sah, 2017). Respiratory therapy has been used to treat psychological conditions, with research showing improvements in mood and depression following breathing exercises (Ma et al., 2017; Qu, Olafsrud, Meza-Zepeda, & Saatcioglu, 2013), and respiratory desensitisation therapy has also been shown to reduce symptoms of panic attacks (Meuret, Ritz, Wilhelm, & Roth, 2005; Meuret, Ritz, Wilhelm, Roth, & Rosenfield, 2018).

Difficulties remain in reconciling the diverse biomechanical, biochemical, and psychological domains of BPD, and a clear synthesis of the pathophysiology of BPD is lacking.

2.4 Pathophysiology of BPD

Homeostasis was proposed as a concept by Claude Bernard over 100 years ago, with the assumption that organisms tend to maintain constant internal environments (Modell et al., 2015). A failure to achieve homeostasis has been implicated in the pathophysiology of BPD (Chaitow et al., 2002, 2014; Porges, 2009). BPD is a syndrome

or disorder, and does not represent a specific pathological-anatomical or psychological process (Chaitow et al., 2002, 2014; Wilhelm et al., 2001).

There are several steps to described to develop a BPD, with the first being a triggering stressor or condition. A maladaptive response due to a single or multiple triggers leads to inappropriate respiratory pattern (Chaitow et al., 2002, 2014). Common stressors include anxiety and asthma (Courtney, 2017; Gardner, 1996), but a wide variety of triggers have been described such as: emotional challenge (e.g. the death of a spouse), physiological challenges such as anaemia or altitude or biomechanical stressors such as nasal obstruction or neck pain (Chaitow et al., 2014; Clifton-Smith & Rowley, 2011). Furthermore many disease processes may also trigger the formation of a BPD including: lung disease such as asthma, COPD, bronchiectasis, diabetes, kidney or liver disease, neuromuscular disease, pregnancy, cardiovascular disease, anaemia, chronic low back pain, chronic neck pain, chronic thoracic pain (Boon et al., 2013; Chaitow et al., 2014; Clifton-Smith & Rowley, 2011; McCool, Benditt, et al., 1997; McCool & Tzelepis, 2012). The disease symptoms and BPD's symptoms may co-exist in individuals, and symptom overlap may further hinder diagnosis and treatment of the BPD (Lum, 1975).

After the triggering stressor the next steps in the development of a BPD are unclear and no clear synthesis is available, and various stress systems have been implicated including: the respiratory and biochemical system (Gardner, 1996; Lum, 1975), the autonomic nervous system (Clifton-Smith & Rowley, 2011; Porges, 2009), and the hypothalamic pituitary adrenal system output (Abelson, Khan, & Giardino, 2010). All of these systems can influence the neurological control of breathing (see Section 2.2), which in turn causes an alteration in respiratory patterns (Chaitow et al., 2014).

While these accessory muscles attempt to maintain adequate ventilation in BPD, they lead to further mechanical unloading and weakness of the diaphragm (Clifton-Smith & Rowley, 2011; Gilbert, 2014; Hruska, 1997). of dominant apical breathing with hypertonicity in the intercostal and chest wall muscles, hyperinflation, and unloading of the diaphragm is hypothesised to occur in BPD, and this may contribute to the unpleasant biomechanical symptoms of tension, chest pain and shortness of breath (Clifton-Smith & Rowley, 2011; Hruska, 1997).

One commonly described pattern is apical dominant breathing (Boulding et al., 2016), and this creates a vicious cycle of apical dominant breathing with hypertonicity in the intercostal and chest muscles, along with hyperinflation, and weakness and dysfunction of the diaphragm (refer to 2.3.4) (Clifton-Smith & Rowley, 2011; Hruska, 1997). Another common pattern is paradoxical breathing, and this has been described as a reversal of normal breathing patterns or a delay between rib cage and diaphragm contraction that results in breathing with poor efficiency (Boulding et al., 2016; Chaitow et al., 2014; Chapman, Hansen-Honeycutt, Nasypany, Baker, & May, 2016). The biomechanical consequences of paradoxical breathing are hypothesised to create inadequate respiratory tidal volume and hyperinflation, and over activation of the scalene, pectoral chest and other accessory breathing muscles (Chapman et al., 2016; Fregonezi et al., 2018; Hruska, 1997). However, there is no agreement to clarify the specific biomechanical pathophysiology within BPD, and no research has to date has measured the diaphragm muscle directly to confirm or refute these pathophysiological hypotheses.

Therefore, there is a gap in the literature regarding the relationship between BPD and diaphragm function and atrophy. Diaphragm atrophy is an important component of BPD to research, as it will allow for a more complete pathophysiological description of BPD and will support clearer diagnosis and treatment of BPD.

2.5 Clinical assessment and diagnosis of BPD

Current diagnosis of BPD is recommended through assessment using a variety of both subjective and objective measures, for example questionnaires such as the NQ and observation and measurements such as MARM, Hi-Lo test, measurement BHT and RR (Chaitow et al., 2002, 2014; Courtney, 2017; Courtney & Dixhoorn, 2014). Assessment of BPD is further discussed in this section.

2.5.1 The Nijmegen Questionnaire (NQ)

The NQ is the most common symptom-based assessment tool used in BPD research. The NQ is a self-administered 16-item outcome measure, with a maximum score of 64, in which the patient rates the frequency of each BPD symptom on a 5-point scale from

0 (never) to 5 (very often) (Dixhoorn & Duivenvoorden, 1985). Various NQ cut-off values for the diagnosis of a BPD have been described such as >22/64 (Dixhoorn & Duivenvoorden, 1985) and >19/64 (Dixhoorn & Folgering, 2015). Normative values have been described as 5±5/64 in a Chinese population (Han et al., 2004) and 11±7/64 in Australia (Courtney & Dixhoorn, 2014).

The NQ has also been widely used in research for asthma (Bruton et al., 2018; Grammatopoulou et al., 2014; Grammatopoulou et al., 2011; Holloway & West, 2007; Thomas et al., 2003; Thomas et al., 2001). The clinical validity of the NQ has been determined for people with asthma when compared to specialist assessment and objective examination (sensitivity 92.7%, specificity 91.6%) (Grammatopoulou et al., 2014). The NQ has also been established as valid in BPD with regards to content validity compared to interview data (94%)(n= 9), but demonstrated poor structural validity when assessed by Rasch analysis (n= 239 questionnaires analysed) (Ogilvie et al., 2019). Specifically, the symptoms of 'cold hands and feet', was recommended to be removed from the NQ to improve the structural validity of the NQ. However while this study appears to be strongly constructed, and have adequate sample size, diagnosis of BPD was by clinician diagnosis and this study did not include any detailed objective or subjective assessment methodology in the diagnosis of a BPD (Ogilvie et al., 2019). Furthermore these findings contrast with the views of Kiesel, Rhodes, Mueller, Waninger, and Butler (2017), who found the item "cold hands and feet' to be a statistically significant diagnostic feature of the NQ, in a convenience sample population (n=51). Limitations of this research were lack of observer blinding and the retrospective nature of these recommendations from a NQ item frequency count, but strengths of this study were the diagnosis of BPD by multidimensional measures including BHT, the MARM, Hi-Lo, capnography, and the NQ.

Concerns also exist regarding issues around cultural validation as the NQ was translated from the Dutch language to English and adaptation to be a culturally relevant tool requires a cultural validation process (Ogilvie, 2017; Ogilvie & Kersten, 2015). However, the NQ has been successfully validated in languages such as Greek and Farsi (Grammatopoulou et al., 2014; Ravanbakhsh, Nargesi, Raji, & Haddadzadeh Shoushtari, 2015) and as described above has content validity in the English language BPD (Ogilvie et al., 2019).

While controversies continue with regard to the NQ, consensus recommends that the NQ is used alongside other objective measures in the diagnosis of BPD (Chaitow et al., 2002, 2014; Courtney et al., 2009; Kiesel et al., 2017). Therefore, there is a need to utilise a robust and valid objective measurement to complement the assessment and diagnosis of BPD.

2.5.2 Other questionnaires

Two further questionnaires were developed for use in BPD: the Rowley Breathing Self-Efficacy Scale (ROBE) (Rowley & Nicholls, 2006) and the Self-Evaluation of Breathing Questionnaire (SEBQ) (Courtney & Dixhoorn, 2014; Courtney et al., 2011). The ROBE is a questionnaire developed in New Zealand which focused on the self-efficacy of the individual to control symptoms of BPD (Rowley & Nicholls, 2006). There is little research on this questionnaire, and the sample size was small (n=26), and underpowered and was unable to find a correlation to other questionnaires such as the NQ and the Hospital Anxiety and Depression (HAD) Scale.

The SEBQ was developed in Australia, and has 25 items that describe symptoms associated with lack of air and perception of inappropriate breathing (Courtney et al., 2011). The SEBQ has been shown to have good test-retest reliability for a heterogenous sample of patients in New Zealand (intraclass correlation coefficient [ICC]=0.88; 95% confidence interval [CI]: 0.84–0.91) (Mitchell, 2011). Furthermore, the SEBQ was also shown to have good correlation with the NQ in a heterogeneous healthy sample of individuals (ICC=0.75 P=0.0001) (Courtney et al., 2011). At present no specific cut-off scores have been established for a diagnosis of BPD with the SEBQ, and further testing using a Rasch analysis will help to identify if it has content and structural validity. The development of further questionnaires is a significant step in the field of BPD, and future research should aim to test the SEBQ and ROBE in a large sample of individuals with BPD to the NQ to further improve the ability to diagnose BPD.

2.5.3 Manual assessment in BPD

Various approaches are currently used to assess the biomechanical domain of BPD, and are based on observation and therapist manual palpation, these include the Hi-Lo

test, MARM, and manual palpation of the diaphragm. However, limitations exist for each of these techniques as they provide subjective measurements of respiration, diaphragm morphology and function.

Hi-Lo test

The Hi-Lo test is a manual and observational assessment completed in a sitting position (Chaitow, 2014). The therapist asks the patient to place one hand on their abdomen and the other on their upper chest, and then observes if the patient has an apical dominant pattern or abdominal/diaphragm breathing pattern. The Hi-Lo test is simple to administer and requires little practitioner skill. Importantly, it also provides information on the relative contribution of the abdominal wall -and thus the indirect motion of the diaphragm- to respiration and allows for comparison with the apical or upper chest wall. The assessment is scored either 'upper chest/apical dominant' breathing or 'abdominal/diaphragmatic dominant' breathing (Chaitow, 2004, 2014).

The Hi-Lo test has an emerging evidence base (Courtney, 2009; Kiesel et al., 2017; Roussel, Nijs, Truijen, Smeuninx, & Stassijns, 2007) with inter-tester reliability of 88% in a healthy population (kappa 0.75; n=43) (Kiesel et al., 2017). The study by Kiesel et al. (2017) aimed to develop a screening process for BPD, and measured the agreement of an examination by three blinded assessors, to other BPD measures such as the NQ, BHT, and capnography. The limitations of this study were the use of a convenience sample of healthy participants, the lack of a sample size or power calculation, and the possible inclusion of smokers or people with asthma, may have confounded the outcome of the study by including participants with lung disease.

The Hi-Lo test has been shown to accurately distinguish apical breathing (96% of the time) and diaphragm breathing (97% of the time) in simulated breathing experiments in a healthy population (n=56 osteopaths and osteopathic students) (Courtney et al., 2009). However, this study had significant weaknesses due to the alternation of the roles of both the participant and the assessors, so that participant or observer blinding was not possible. Furthermore, data numbering errors meant three result scoring sheets were lost to analysis, which casts some doubt on the accuracy of these results.

The Hi-Lo test has also been used in research in low back pain (Roussel et al., 2007), and other heterogeneous healthy populations (H. Bradley & Esformes, 2014; Courtney et al., 2009; Kiesel et al., 2017), but not specifically in BPD populations. Therefore, while the Hi-Lo may have good inter-tester reliability, two major weakness of the Hi-Lo technique are the paucity of research in specific BPD populations, and a lack of comparison to assess validity against other gold-standard measurements such as respiratory inductance plethysmography, functional MRI or ultrasound.

Manual assessment of respiratory motion (MARM)

MARM is a manual therapy palpation technique that involves palpation of the posterior rib cage (Courtney et al., 2009; Courtney & Dixhoorn, 2014). The therapist sits behind the patient and palpates the lower posterior abdomen and posterior and lateral costal ribs expansion (Courtney et al., 2009; Courtney & Dixhoorn, 2014). In one study the MARM was found to have good inter-rater reliability in healthy populations (ICC=0.85, p=0.0001, 95% CI: 0.78–0.89) (Courtney, van Dixhoorn, & Cohen, 2008), and the technique was able to distinguish apical breathing from lower costal breathing when compared to respiratory inductance plethysmography (as tested through the use of the worn vest biofeedback device). However, this study has weaknesses due to a small sample size (n=12) and has confounding factors of the wearing a vest while measuring the MARM, which may have altered the breathing pattern due to increased restriction to breathing. A further study using MARM (Ludwig, 2013) showed poor inter- and intra-rater reliability (inter-rater: ICC range −0.18 to 0.36; intra-rater: ICC range = −0.51 to 0.92). Therefore, the reliability of the MARM is unclear, and there is no specific evidence in BPD populations, and while it may have applications for manual therapy, at present it may not be suitable for widespread multidisciplinary clinical use in respiratory practise.

Palpation of the diaphragm

Manual palpation of the diaphragm as an assessment technique, is a more recent approach (Bordoni, 2019; Bordoni, Marelli, Morabito, & Sacconi, 2016; Bordoni & Zanier, 2013), and involves palpating the underside of the seventh to tenth, anterior costal cartilages to assess diaphragm tension, and motion (Rocha et al., 2015).

However, to date there has been no research that has been conducted on palpation of the diaphragm in BPD populations. Diaphragm palpation techniques have been used in conjunction with myofascial diaphragm treatment release techniques in COPD (Nair et al., 2019; Rocha et al., 2015), and healthy populations (Mancini et al., 2019), but not in BPD. One strength of this technique is that improvement of palpated diaphragm motion has been correlated to changes on diaphragm motion on ultrasound, after treatment by myofascial release in COPD and healthy populations (Mancini et al., 2019; Nair et al., 2019; Rocha et al., 2015). So, although this technique shows promise, further research is needed adapt this technique to BPD populations.

In summary, of the manual approaches to assessment of BPD, the Hi-Lo has the largest evidence base, and while it has not been specifically validated in BPD populations it remains the current best measure of the biomechanical domain when compared to the MARM, and palpation of the diaphragm. As manual palpation techniques require an element of subjective analysis, and therefore by association, may lack inter-therapist reliability, there is a need to develop new robust and valid objective measures to complement manual assessment in BPD.

2.5.4 Other objective measures in BPD

Respiratory rate

Measurement of an individual's RR is a simple objective measurement that can be achieved by observation of the total number of respirations in one minute with a stop watch, with 12–16 breaths/minute considered normal (Main & Denehy, 2016). A RR at rest above 16 breaths per minute is considered to indicate a BDP (Chaitow et al., 2014), and in one asthma and BPD study (n=164) a RR of 18 was associated with a diagnosis of BPD in asthma, when compared to the NQ, and capnography (Grammatopoulou et al., 2014).

Oxygen saturation

Oxygen saturation (SpO_2) is measured with a pulse oximeter. SpO_2 is a cheap and fast assessment technique that is useful for screening for lung pathologies such as COPD, and levels below 94% can indicate the presence of lung disease (Chaitow et al., 2002,

2014; Soguel Schenkel et al., 1996). Therefore, pulse oximetry is a useful diagnostic tool to rule in or out the possibility of BPD.

Breath hold time (BHT)

BHT commonly involves a timed measurement of a breath hold with the nose-pinched on a tidal exhalation whilst the person holds their breath until they feel a moderate-strong urge to breathe (Grammatopoulou et al., 2014; Grammatopoulou et al., 2011). BHT is thought to be an indirect measurement of CO_2, however, there is debate in the literature around BHT and the diagnosis of BPD. One problem is the lack of agreement regarding a cut-point for the BHT that indicates BPD. For example, in one observer-blinded study examining a large cohort (n=162) of people with asthma, an exhale-BHT below 30 seconds was correlated with BPD, hypocapnia, and an elevated NQ (Grammatopoulou et al., 2014). A further study showed that people with BPD (n=39) could complete an inhale-BHT hold time of 21±12 seconds and this correlated to hypocapnia (arterial PCO_2 of 28mm)(Jack et al., 2004). Another study, this time in a heterogeneous population (n=51) and without observer blinding, showed that a BHT below 25 seconds indicated the presence of a BPD (Kiesel et al., 2017). Limitations of this study were the lack of control group, and the diagnosis of BPD being broadly defined as dysfunction in any one of the measures of BPD (positive NQ score, or positive Hi-lo or positive capnography or positive SEBQ), instead of a combination of positive BPD measures. Conversely, Courtney et al. (2011) showed BHT was negatively correlated with end tidal CO_2. This research used a heterogenous population and had poor blinding procedures, and as such may lack generalisability to BPD populations. Collectively, this body of research suggests that while BHT may be useful in the diagnosis of a BPD, it is not indicative of precise changes of CO_2 in the body.

Blood gas analysis, cardiopulmonary exercise testing and capnography

Arterial blood gas analysis is an assessment method that involves removal of a small arterial blood sample. Whilst this is generally considered as the gold-standard measurement of blood-gas, and is able to identify hypocapnia and respiratory alkalosis definitively, its use is limited primarily to emergency departments, and requires

medical training, that is outside the scope of physiotherapy (Gardner, 1996; Pfortmueller et al., 2015).

Cardiopulmonary exercise testing has been described as a method of diagnosis in patients where BPD is suspected during exercise, and the diagnosis is focused on alterations in technical gas exchange measures (i.e. transcutaneous pCO_2 below 35 mmHg, or supra-lactate threshold ratio of volume of air inhaled in one minute (VE) / volume of CO_2 (VCO_2) ratios) (Brat et al., 2019; Dickinson, McConnell, Ross, Brown, & Hull, 2015; Stanton et al., 2008; Ugonna, Barker, Kirkby, & Thevasagayam, 2019). One study by (Stanton et al., 2008) completed cardiopulmonary exercise testing on a small number of (n= 17) participants with BPD. The tested participants were recruited from a larger sample of 65 participants who had a positive NQ – and there were a large amount of exclusions due to non-attendance (n=20), comorbidities (n=21), wheeze (n=4) or locomotor problems (n=3). There were no control subjects in this study. This poor recruitment and inability to test a large portion of the study population was not adequately addressed in the results. The results of the cardiopulmonary exercise testing were that positive transcutaneous pCO_2 gas exchange below 35mmHg were present in 59% of the BPD patients, suggesting that hypocapnia is not the sole diagnostic feature of BPD. Another study that aimed to study cardiopulmonary exercise testing on patients diagnosed with BPD had adequate sample size (n=58) and power, but had poor observer blinding (Brat et al., 2019). During peak exercise gas exchange of End Tidal CO_2 was reduced to 27mmHg from 35mmHg in the BPD group and VE/ VCO_2 was also higher during exercise (38 units vs 31 units) in the BPD group during exercise. There were minimal changes in the healthy group. The changes in of End Tidal CO_2 and VE/ VCO_2 were found to be specific for participants with a BPD (83% and 93%). Although cardiopulmonary exercise testing has validity in the assessment of gas changes (i.e. it measures and analyses all CO_2 and O_2 during exercise), it appears poor specificity in the diagnosis of BPD, as not all subjects with a BPD have hypocapnia. Furthermore, there is the need for an expensive technical laboratory set up, and this assessment is not a routine physiotherapy assessment tool. Together these results suggest that there are benefits and limitations to the application of cardiopulmonary exercise testing, with many patients being unable to complete testing if they have comorbidities and may only aid in the diagnosis of BPD patients with hypocapnia.

A further CO_2 assessment tool is capnography, which is used to screen for low end-tidal CO_2 below 35mmHg (Courtney et al., 2011; McLaughlin, 2009). End tidal CO_2 assessment requires wearing nasal prongs and measurement of expired tidal CO_2; this measurement is thought to closely reflect blood-based CO_2 measurement (Courtney et al., 2011; McLaughlin, 2009). An advantage of capnography is that it can give a waveform readout that can be utilised to assess the respiratory pattern (e.g. forced exhale pattern or sighing), and can be useful as a biofeedback tool for patients, and it is simple and cost-effective (McLaughlin, 2009). The disadvantages of this technique are similar to those of cardiopulmonary exercise testing, in that it may capnography may only aid in the diagnosis of BPD patients with hypocapnia. Therefore, its routine use in the diagnosis of BPD is indicated when there is suspicion of hypocapnia (Boulding et al., 2016; Courtney, 2017).

Other tests of respiratory muscle function

Other measures have been described in the assessment of respiratory muscle function, including: fluoroscopy, USI, magnetic resonance imaging, maximal inspiratory pressure, sniff nasal inspiratory pressure, electromyography, transcranial magnetic stimulation and invasive measures such as gastric or oesophageal pressures (Laveneziana et al., 2019). None of these above measurement techniques have to date been used in the field of BPD, and research is needed to assess the ability of these techniques to assess and aid the diagnosis of BPD.

2.5.5 Summary of clinical assessments for BPD

As described above, although many measures have been evaluated within BPD, there is a specific need to develop cost-effective, robust, and valid objective measures that have the ability to accurately measure the biomechanics of BPD. Furthermore, a technique that assesses the function and morphology of the diaphragm in BPD is needed to support the diagnosis of BPD. Therefore, further investigation into accurate and valid clinical assessment tools for BPD is warranted.

2.6 USI for assessment of the diaphragm

In the last decade, USI has been growing in use to assess diaphragm thickness (Figures 4 and 5) and diaphragm excursion, both in clinical practice and research (Laveneziana et al., 2019; Le Neindre et al., 2016). The advantages of USI as a technique in point of care assessment are numerous, and include simple and rapid assessment, device portability, cost-effectiveness and enhanced patient comfort as it is non-invasive (Laveneziana et al., 2019; Le Neindre et al., 2016; Teyhen, 2006; Whittaker et al., 2007).

Using USI, the diaphragm is easily identified below the ribs and intercostal muscles, and consists of a hypoechogenic layer bordered by hyperechogenic peritoneum and pleural lines (Boon et al., 2013) (Figure 5). There are several components of the diaphragm that can be assessed using USI including:

a) excursion or motion of the diaphragm using B-mode and M-mode (Boussuges et al., 2009; Epelman, Navarro, Daneman, & Miller, 2005; Houston, Angus, Cowan, McMillan, & Thomson, 1994) (see 2.6.1).

b) static measurement of the thickness of the diaphragm at one or many different lung volumes (most commonly at tidal exhalation or Tvex and total lung capacity or Tmax) (Boon et al., 2013; Cardenas et al., 2018; Scarlata, Mancini, Laudisio, & Raffaele, 2019; Seok, Kim, Walker, Kwak, & Kwon, 2017) (see 2.6.2).

c) thickening ratio (TR) which is calculated by the formula: inspiratory diaphragm thickness/expiratory diaphragm thickness (Boon et al., 2013; Scarlata et al., 2019) (see 2.6.3).

d) thickening fraction (TF) which is calculated by the formula: (inspiratory diaphragm thickness – expiratory diaphragm thickness) / expiratory diaphragm thickness (Cardenas et al., 2018; Seok et al., 2017) (see 2.6.3).

There is no consensus regarding which of these above measures is the most relevant descriptor of normal diaphragm function or dysfunction (Laveneziana et al., 2019). However, diaphragm thickness can provide data that indicates either hypertrophy or

atrophy of the diaphragm, and diaphragm thickness can be used to generate the TR or TF, which indicates the contractility and relative motion of the diaphragm.

2.6.1 Assessment of diaphragm excursion

Measurement of excursion of the diaphragm would be useful in BPD as it is able to assess a reduction in the use of the diaphragm during tidal and deeper breathing. The use of USI in assessing BPD has not been examined to date, but patterns of breathing have been examined by Blaney, English, and Sawyer (1999) in a healthy population. Twelve participants were coached in three different styles of breathing (apical breathing, diaphragm breathing and thoracic lateral expansion) while breathing through a respirometer at a stable volume. Findings indicated reduced diaphragm motion during apical breathing (2.2 cm; ICC=0.86) and thoracic lateral expansion breathing (2.4 cm; ICC=0.75), and more motion during diaphragm breathing (3.1 cm; ICC=0.79). In another study by Jones et al. (2017) the effect of taught diaphragmatic breathing control exercises was assessed by USI in 20 healthy students (n=10 control group, and n=10 breathing control group). Both groups had similar diaphragm motion at rest (diaphragm breathing 1.79 ± 0.57 cm vs. control 1.69 ± 0.26 cm), but the diaphragmatic breathing group had significantly greater diaphragm motion after an exercise step test (diaphragm breathing 3.57 ± 0.70 cm vs. control 2.59 ± 0.54 cm; p=0.02). This effect was significant and continued at one, two, and three minutes post exercise. This suggested that USI was able to detect changes in breathing pattern with exercise. While both these studies had no control groups, had small heterogeneous healthy sample, and utilised poor participant blinding procedures, the results suggested that USI was effective in measuring changes in diaphragm motion during varied breathing patterns and has good intra-observer reliability.

Therefore, it appears that USI of the diaphragm is sensitive enough to observe changes both at rest and during exercise in manipulated breathing patterns, and therefore may be sensitive enough to observe diaphragm changes in populations such as BPD.

Figure 4. Schematic picture of the USI position used to measure of diaphragm thickness, the zone of apposition and approximate position of the ultrasound probe
(Source: https://radiologykey.com/diaphragm-ultrasound-in-the-intensive-care-unit/)

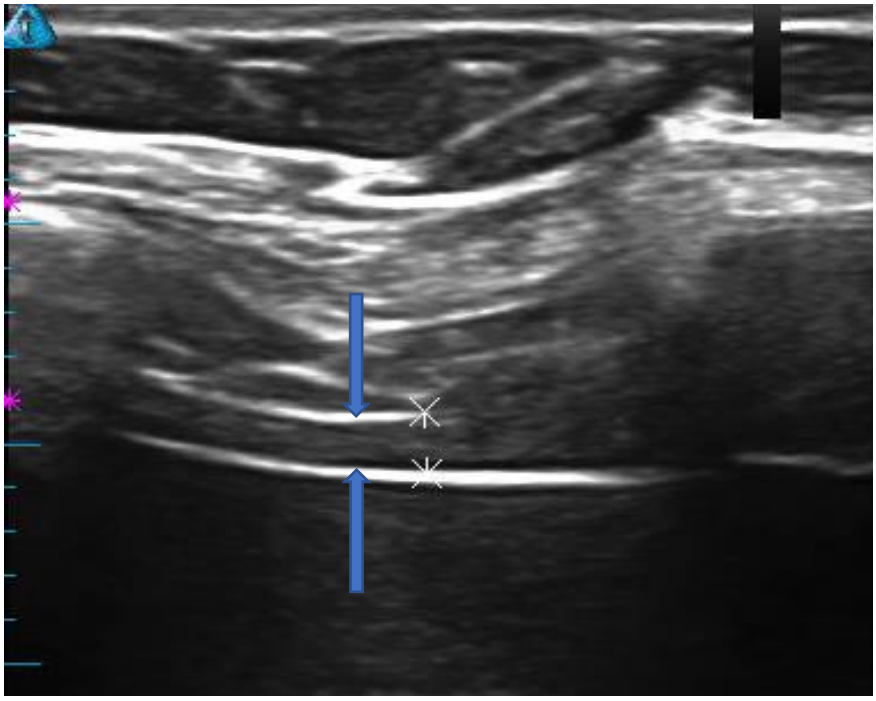

Figure 5. Ultrasound image of the diaphragm thickness in the zone of apposition
The diaphragm thickness is the measure as the distance between the two blue arrows (source: personal images of author)

2.6.2 Diaphragm thickness

Like many skeletal muscles, there is a strong relationship between diaphragm strength (function) and diaphragm thickness (morphology) (Suetta et al., 2008). Multiple studies have examined diaphragm thickness using USI in healthy subjects and have reported high levels of intra- and inter-observer reliability (Baldwin, Paratz, & Bersten, 2011; Boon et al., 2013; Cohn, Benditt, Eveloff, & McCool, 1997; Enright, Chatham, Ionescu, Unnithan, & Shale, 2007). Diaphragm thickness has not been specifically assessed in BPD. However, changes in diaphragm thickness have been correlated to simulated changes in breathing pattern in one study by Kaneko, Otsuka, Kawashima,

and Sato (2010). The authors measured diaphragm thickness in 24 healthy male participants and participants underwent cycles of relaxed breathing (control condition), and then periods of apical chest restriction (experimental condition). Apically restricted breathing was achieved by inflating a sphygmomanometer cuff to restrict the expansion of the upper chest during breathing. The change of thickness of the diaphragm during inhalation increased from 1.89 mm to 2.02 mm ($P<0.05$) with chest restriction, and then returned to 1.80 mm ($P<0.05$) with removal of chest restriction. Other significant changes were observed in the diaphragm Thickening Fraction (TF) ($p<0.05$). This case control study also showed good intra-rater reliability (0.925 ICC). The main limitation of this study is the lack of a true control group, and participant blinding was not feasible, but nevertheless this study suggests that USI is sensitive to changes in diaphragm thickness when breathing is externally manipulated or altered.

Considerable debate exists as to the normal values for diaphragm thickness at Tvex (refer to Table 1) with mean values of resting diaphragm thickness, in females at Tvex, ranging from 0.14±0.03 cm (95% CI: 0.13–0.15 cm) (right diaphragm, n=54) (Carrillo-Esper et al., 2016) to 0.31±0.19 cm (95% CI: 0.27–0.35 cm) (left diaphragm, n=77) (Boon et al., 2013). A major limitation of the normative study by Boon et al. (2013) is that it included a large proportion of smokers (39%) and a population that had a high BMI (27.9 kg/m^2 males; 26.6 kg/m^2 females), which may have impacted on diaphragm thickness as smoking is associated with lung damage or COPD, and possible lung hyperinflation which may create changes in thickness of the diaphragm fibres (Antenora et al., 2017). Furthermore, increased BMI and obesity has been correlated with increased thickness of the diaphragm (McCool, Benditt, et al., 1997; McCool & Tzelepis, 2012), thus reducing the generalisability of these results. Therefore, it is likely that the study by Boon et al. (2013) over-estimates diaphragm thickness in normal populations with a healthy BMI and non-smokers.

There is debate as to the lower limit of normal diaphragm thickness (as calculated by the fifth percentile) at Tvex, which has been reported to be 0.15 cm in healthy subjects (Boon et al., 2013) and 0.15 cm (left) to 0.16 cm (right) in patients with COPD (Baria et al., 2014). The lower limit of normal figure of 0.15cm seems to concur with other published normative studies (see table 1), as this figure is below the mean of many of

these studies. However, in one single study by Carrillo-Esper et al. (2016) the mean of diaphragm thickness is below this level (0.14±0.03 cm; 95% CI: 0.13–0.15 cm). Two weaknesses of this study are poor observer blinding, and the presence of five outliers above the standard distribution of the female cohort (n=54). It is also unclear if these

Table 1. Normative populations studies of diaphragm thickness

	Diaphragm thickness tidal exhale (Tvex)		Diaphragm thickness tidal inhale (Tvin)		Diaphragm thickness at TLC (Tmax)		Thickening fraction or thickening ratio	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baldwin et al; 2011								
Male and Female	0.178							
Boon et al; 2013							TR	
Male	0.38	0.15					1.8	0.5
Female	0.27	0.1					1.8	0.5
Cardenas et al; 2018							TF	
Males	0.187	0.03			0.559	0.09	204.2	61.9
Females	0.179	0.03			0.481	0.09	169.7	44.7
Carrillo-Esper et al; 2016								
Males	0.19	0.04						
Female	0.14	0.03						
Scarlata et al; 2019							TR	
Males	0.187	0.04			0.278	0.05	1.57	0.04
Females	0.167	0.04			0.239	0.05	1.47	0.04
Seok et al; 2017							TF	
Male	0.22	0.05			0.47	0.12	118	56
Female	0.17	0.03			0.32	0.08	92	41
Ueki et al; 1995								
Male and Female	0.17	0.02			0.45	0.09		
Vishwanath et al; 2016								
Male and Female	0.189	0.05	0.261	0.06				

All results are shown for the right-side diaphragm only in cm. TR= thickening ratio; TF= thickening fraction; TLC= total lung capacity

outliers were removed, or included from the calculation of the mean which hinders interpretation of these results (Carrillo-Esper et al., 2016). Furthermore, current review and expert opinion are uncertain as to whether a Tvex value below the threshold of 0.15 cm can be used to diagnose diaphragm atrophy (Laveneziana et al., 2019). As diaphragm function is comprised of both excursion and thickness, it may be optimal to measure both of these variables, to diagnose diaphragm atrophy and changes in function (Laveneziana et al., 2019).

Diaphragm thickness does not seem to change with age, with the exception of age groups less than 18 years or greater than 70 years (Boon et al., 2013; Thimmaiah, Geetha, & Jain, 2016). However, diaphragm thickness may be influenced by ethnic morphological features, with differences in diaphragm thickness noted between American (Boon et al., 2013), Italian (Scarlata et al., 2019), Indian (Thimmaiah et al., 2016) and Mexican healthy populations (Carrillo-Esper et al., 2016). It would be of benefit to measure a healthy population in New Zealand to establish if there are unique ethnic morphological differences in diaphragm thickness.

Other important relationships that influences diaphragm thickness at Tvex include: smoking (Boon et al., 2013), gender (Boon et al., 2013; Carrillo-Esper et al., 2016), position (e.g. supine vs. prone vs. sitting) (C. Brown, Tseng, Mitchell, & Roddey, 2018; Hellyer et al., 2017), physical activity (P. Brown et al., 2013; Scarlata et al., 2019), and BMI or body composition (Boon et al., 2013; Enright et al., 2007; McCool, Benditt, et al., 1997). Female gender has been correlated to reduced diaphragm thickness, when compared to males (Boon et al., 2013; Carrillo-Esper et al., 2016), and supine postures has been correlated to reduced diaphragm thickness when compared to sitting postures (C. Brown et al., 2018; Hellyer et al., 2017). Reduced physical activity has been correlated to reduced diaphragm thickness when compared to increased physical activity, but no meaningful cut-offs have been described for physical activity (P. Brown et al., 2013; Scarlata et al., 2019). Lower BMI values have been correlated to reduced diaphragm thickness, when compared to higher BMI values (Boon et al., 2013; Enright et al., 2007; McCool, Benditt, et al., 1997). Therefore, prospective research needs to

control for the above confounding variables of BMI, gender, positioning, activity level and smoking.

Clinical evidence of diaphragm atrophy

As mentioned earlier, skeletal muscle thickness (the diaphragm) is closely correlated to strength. For example, when the diaphragm is unloaded or its nerve supply is injured, the diaphragm can begin to thin and atrophy, and its mechanical strength decreases (McCool & Tzelepis, 2012).

Diaphragm atrophy has been consistently shown in people with phrenic neuropathy or phrenic nerve injury (the nerve that controls diaphragm contraction) due to impaired nerve conduction and therefore muscle disuse (Boon et al., 2014; Caleffi-Pereira et al., 2018). Furthermore, diaphragm atrophy has also been consistently described in patients with stroke, when the affected side (hemiplegic side) is compared with the less affected side, at measures of Tmax and thickening fraction (TF) (Jung & Kim, 2017; Kim et al., 2017).

Diaphragm wasting was described in a study involving athletes with low back pain (n=20) compared with controls (n=20) (Calvo-Lobo et al., 2019). This study utilised a strong methodological design, had adequate power, and had adequate observer blinding procedures. The study found diaphragm thickness at Tvex was significantly reduced ($p<0.015$) in the LBP group (right (1.6±0.7 mm) and left (1.3±1.2 mm) diaphragm thickness) compared to controls (right (2.3±0.6 mm) and left (2.0±1.0 mm) diaphragm thickness) (Calvo-Lobo et al., 2019).

There is conflicting evidence in ICU and mechanical ventilation based populations, with some studies showing a rapid rate of diaphragm wasting or atrophy (Dubé et al., 2017; Goligher et al., 2018; Grosu et al., 2012; Martin, Smith, & Gabrielli, 2013; Turton, Alaidarous, & Welters, 2019). Despite the majority of studies showing that diaphragm wasting occurred during mechanical ventilation, expert opinion suggests that diaphragm thickness cannot currently offer a useful measure for predicting failure to wean or successful ventilator weaning (Dubé et al., 2017; Laveneziana et al., 2019). Due to ethical considerations, all ventilator-based research utilises heterogenous

populations, with varying levels of sedation and convenience samples, and this hinder generalisation and interpretation of ICU studies to other populations.

Diaphragm thickness has also been examined with USI in respiratory populations including: chronic asthma (de Bruin, Ueki, Watson, & Pride, 1997), and COPD (Antenora et al., 2017; Baria et al., 2014; Hafez & Abo-Elkheir, 2017), but it is difficult to characterise the relationship of diaphragm thickness to these disease processes in a straightforward manner. For example, de Bruin et al. (1997) measured diaphragm thickness in a small population with moderate asthma (n=9, males n=4,) (diaphragm thickness at Tvex 2.2±0.4 mm) and compared this with a healthy population (n=9, males n=3) (diaphragm thickness at Tvex 1.7±0.3 mm), and showed no diaphragm atrophy in asthma. These results lacked generalisability due to the heterogenous asthma population that included smokers, the small sample size and lack of measurement blinding. These results are similar to a well-designed study that studied cases of mild or moderate COPD (as classified using the Global Initiative for Chronic Obstructive Lung Disease or GOLD classification), and found that diaphragm thickness was not reduced compared to healthy patients, but diaphragm thickness and thickening fraction (TF) was reduced in a small subgroup of severe COPD patients (Hafez & Abo-Elkheir, 2017). This study utilised a large sample size (n= 113 COPD males, n= 114 control males), showed good intra-observer agreement with repeated measures, but did not utilise observer blinding. A subset of COPD participants (n=13) was further defined and analysed that had both diaphragm atrophy (Tvex <1.8mm) and severe COPD. However, within the severe COPD group, diaphragm atrophy was not seen 78% of cases. These results have been corroborated by other well designed studies in COPD (Antenora et al., 2017; Baria et al., 2014). Therefore, diaphragm atrophy and dysfunction are only present in a small proportion of people with severe COPD, and generally diaphragm thickness is preserved in mild and moderate COPD and asthma.

Clinical evidence of diaphragm hypertrophy

The diaphragm has been shown to have increased thickening (hypertrophy) on USI after mechanical loading via inspiratory muscle training (IMT), which is designed to strengthen the inspiratory muscles via resistance exercise loading (Kaneko et al.,

2009). In addition, repetitive loading of the diaphragm over time, either through direct inspiratory loading using IMT (Enright, Unnithan, Heward, Withnall, & Davies, 2006) or indirect exercise loading (e.g. sit ups and bicep curls) can lead to diaphragm hypertrophy (DePalo, Parker, Al-Bilbeisi, & McCool, 2004). For example, in patients with stroke the diaphragm thickness at Tmax and asymmetry between the affected and less affected side is improved with IMT (Cho et al., 2018; Jung & Kim, 2014, 2015; Jung et al., 2014). Thickening ratio (TR) in stroke was significantly improved (thickening ratio pre 1.65±026; post 2.25±0.48; $P<0.05$) on the affected side after IMT (Jung & Kim, 2015). This effect was also seen in another study (Jung et al., 2014) that used a combination of IMT and abdominal electric stimulation for exhalation training (thickening ratio pre 1.74±0.27 ; post 2.16±0.29; $P<0.05$). These studies had adequate sample sizes, and reported outcomes in a clear manner, but had poor observer blinding, and no true control group, and thus caution is needed interpreting these results. However, studies in stroke populations may serve as a model of diaphragm atrophy and weakness, and reversal of diaphragm atrophy and weakness can be observed with strength-based training using IMT.

Hypertrophy or thickening and strengthening of the diaphragm has been consistently demonstrated in people who lift weights or exercise more compared with matched healthy populations (P. Brown et al., 2013; DePalo et al., 2004; McCool, Benditt, et al., 1997; McCool, Conomos, et al., 1997; Summerhill, Angov, Garber, & McCool, 2007). The diaphragm has been shown to consistently respond to IMT, with increasing maximal inspiratory pressure (MIP) and diaphragm thickness on USI across a variety of age groups in healthy populations (Enright et al., 2006; Mills, Johnson, Barnett, Smith, & Sharpe, 2015; Souza et al., 2014; Summerhill et al., 2007). Furthermore, increased diaphragm thickness has also been consistently positively correlated with increased general physical activity levels and increased MIP (P. Brown et al., 2013; Chaitow et al., 2002, 2014; Courtney & Dixhoorn, 2014; McCool, Benditt, et al., 1997; McCool, Conomos, et al., 1997; Summerhill et al., 2007).

2.6.3 Diaphragm thickening fraction (TF) and thickening ratio (TR)

TF and TR can provide an indication of diaphragm thickening during inspiration and measurement of TR and TF are reproducible, and these measures can express the

change in thickness and thus provide information that pertains to the function of the diaphragm fibres (Goligher et al., 2018; Harper et al., 2013). A normal tidal TR of 20% has been described in normal population (the formula in that study was diaphragm thickness at Tvin/ diaphragm thickness at Tvex) (Harper et al., 2013), and a lower limit of normal TF has been described as <20% in healthy subjects and people with COPD (Baria et al., 2014; Boon et al., 2013; Boon et al., 2014). One study found that a low TF correlated with diaphragm dysfunction in COPD exacerbations, and was an accurate indicator that non-invasive ventilation would fail (Antenora et al., 2017). Another study suggested that TF was unable to predict acute respiratory exacerbation in people with COPD (Eryuksel, Cimsit, Bekir, Cimsit, & Karakurt, 2017). General consensus suggests that a TR or TF < 20% may represent almost full paresis of the diaphragm, as the mean values for TR in healthy subjects are usually greater than 100% (Boon et al., 2013; Boon et al., 2014; Harper et al., 2013; Laveneziana et al., 2019).

TF and TR have been shown in some studies to have a strong correlations with other measures of global respiratory muscle function such as MIP (r^2=−0.82; P<0.01) (Cardenas et al., 2018; Ueki, De Bruin, & Pride, 1995), but other reviews suggest that the correlation of TR and TF with inspiratory effort is poor (Laveneziana et al., 2019). There is no current consensus as to benefits of the TF versus TR, with some authors favouring TF calculations (Cardenas et al., 2018; Seok et al., 2017) and others TR calculations (Boon et al., 2013; Harper et al., 2013; Scarlata et al., 2019), and therefore choice of the specific measurement calculation does not appear to be important.

In summary, diaphragm thickness and both TR or TF can provide morphological and functional information, that may guide treatment in stroke, aid in the diagnosis of phrenic neuropathy, and may be useful in low back pain, ICU and specific lung disease populations (i.e. severe COPD).

2.7 USI of diaphragm thickness in the assessment and diagnosis of BPD

As described earlier in Section 2.5, difficulties exist in the diagnosis of BPD, based on the use of the NQ, the Hi-Lo test, RR and BHT (Chaitow et al., 2002, 2014; Courtney et al., 2009; Courtney & Dixhoorn, 2014; Dixhoorn & Duivenvoorden, 1985; Dixhoorn &

Folgering, 2015; Kiesel et al., 2017; Ogilvie, 2017; Ogilvie & Kersten, 2015). Diaphragm dysfunction has been implicated in the pathophysiology of BPD, and while current best practice is to diagnose BPD with a variety of measures, many of these measures lack the ability to objectively assess the diaphragm.

USI has been used widely in other populations, and can provide valid, safe and useful clinical information to aid in respiratory assessment and diagnosis (Le Neindre et al., 2016; Ntoumenopoulos, Parry, & Le Neindre, 2018). USI is able to directly assess diaphragm thickness and contractility with TF or TR, and has excellent levels of sensitivity and specificity when compared with fluoroscopy and needle EMG studies (Boon et al., 2014; Houston, Fleet, Cowan, & McMillan, 1995). USI is sufficiently sensitive to detect differences in: normal and manipulated styles of breathing (Blaney et al., 1999; A. Jones et al., 2017; Kaneko et al., 2010), movement and exercise (A. Jones et al., 2017), hypertrophy (P. Brown et al., 2013; McCool, Benditt, et al., 1997; McCool, Conomos, et al., 1997) and atrophy of the diaphragm (Dubé et al., 2017; Goligher et al., 2018; Martin et al., 2013; Turton et al., 2019). USI investigation of diaphragm thickness has not been assessed in BPD to date.

The potential clinical value of this approach will be the ability to accurately identify diaphragm atrophy, and alterations of diaphragm thickness and TF, in individuals with BPD. As diaphragm thickness and TF can be measured with USI and re-measured in future sessions, USI may provide a valid and practical way to objectively measure progress across treatment sessions (Blaney et al., 1999). USI of the diaphragm may become a reliable tool to diagnose BPD or diaphragm atrophy and may in turn represent a method for examining treatment efficacy for those strategies employed to improve diaphragm morphology and function.

2.8 Summary

BPD are a significant health problem in New Zealand. While the pathophysiology of BPD is unclear, it appears that the diaphragm muscle is implicated in BPD. Furthermore, successful treatment of BPD involves breathing exercises that aim to improve the function of the diaphragm. However, while treatment of BPD has been

well studied, the diagnosis of BPD is difficult, as no gold-standard diagnostic test is available. There is a need to establish clear diagnostic tools for BPD. USI of diaphragm thickness and TF has been shown to provide useful information in many related pathological conditions and is sensitive enough to measure artificially manipulated breathing patterns in healthy populations. Therefore, an observational cross-sectional study will compare the use of diaphragm USI between females with BPD and healthy females.

Chapter 3. Methods

3.1 Study Design

To answer the research questions, an observational cross-sectional quantitative study was used. Study participants were stratified into two BMI groups based on BMI measurements to evaluate our hypothesis. Along with relevant demographic and clinical variables, diaphragm thickness was measured, and TF calculated at one point in time for females with a BPD and matched healthy females. Female gender has clear correlation with reduced diaphragm thickness compared to males (Boon et al., 2013; Carrillo-Esper et al., 2016), and because of the higher rate of BPD in females (Pfortmueller et al., 2015), this research focused on the female gender. Furthermore increased BMI is clearly correlated with increased diaphragm thickness (Boon et al., 2013; Enright et al., 2007; McCool, Benditt, et al., 1997). Therefore this design was chosen to avoid the effect of the strongest confounding variables of BMI and gender which is described in detail in Section 2.6.2 (Lederer et al., 2018).

3.2 Participants and recruitment

Participants were recruited from 29 March 2018 to 30 June 2019 throughout Auckland, New Zealand. Due to the difficulties in recruiting a random sample, for the BPD group, a convenience sample of participants was recruited through referrals to a private physiotherapy clinic and other public referral sources (e.g. hospitals and university respiratory outpatient clinics) using flyers, emails, social media, posters and promotional talks. A convenience sample of healthy female participants was recruited for the control group through gyms, Auckland University of Technology (AUT) campuses, yoga studios and other health and physiotherapy centres.

3.3 Eligibility criteria

3.3.1 Inclusion criteria

Specific screening measures were used to assess participants' eligibility, specifically: the NQ, RR, BHT, and the Hi-Lo test (for descriptions and relevance of these measures see Section 2.5). The inclusion criteria for eligible females for the BPD group were:

aged 18–65 years, a NQ score ≥23, SpO_2 of >96%, RR >16 breaths per minute, a BHT ≤30 seconds and presence of an apical dominant breathing pattern on Hi-Lo test.

Inclusion criteria for healthy control females: aged 18–65 years, a NQ score <16, SpO_2 of >96%, a RR of 8–15 breaths per minute, a BHT >30 seconds and presence of an abdominal dominant breathing pattern on Hi-Lo assessment.

Participants from both the BPD and healthy control groups were divided into two further BMI groups – healthy BMI group (BMI 18.5–24.9 kg/m^2) and BMI+ group (BMI ≥25kg/m^2) (Ministry of Health, 2017).

3.3.2 Exclusion criteria

Participants were excluded if they had: a SpO_2 of >94%, lung disease (e.g. asthma, COPD, bronchiectasis), current or previous smoking history, diabetes, kidney or liver disease, neuromuscular disease, pregnancy, cardiovascular disease, anaemia, chronic low back pain, chronic neck pain, chronic thoracic pain, recent upper respiratory tract infection, steroid use or recent surgery. All of these factors may influence either the respiratory or musculoskeletal system, and therefore lead to the sampling of a heterogeneous population which may reduce to external validity of the current study Participants in either the BPD or control groups who did not meet all the inclusion criteria were excluded. For example, if a participant had an apical dominant breathing pattern, but they had a normal RR and normal NQ score they were excluded.

3.4 Ethical considerations and participant recruitment

Ethical approval was obtained from the Auckland University of Technology Ethics Committee (AUTEC; reference: 17/419) (Appendix 2). The major risk to participants in this study was the potential coercion of patients referred to *Breathing Works* who were patients of Scott Peirce (primary researcher). *Breathing Works* is a private respiratory clinic that focuses on the treatment of BPD and is owned by the primary researcher. For this reason, all affiliates or patients of Scott Peirce were excluded from participation to negate any conflict of interest. In addition, to negate the risk for

coercion, all potential participants were contacted by the clinic receptionist by phone to discuss the study overview and participation. Potential participants were screened against the study eligibility criteria during this initial phone call.

Following this initial screening, interested participants were provided with a participant information sheet (Appendix 3), consent form, and study advertisement (via by email or post), and participants were asked to reply if they wished to be included in this study. All interested participants were provided with consent documentation to complete, and a time was scheduled for data collection. Participants had opportunities to ask questions or voice any concerns at any time, including before providing consent and throughout the study.

Participants' confidentially and privacy was maintained throughout the study by ensuring all participants results were anonymised to others, except the primary researcher. All collected data were kept secure in a password-protected file and stored in keeping with AUTEC privacy and legal recommendations. Participants were asked to attend data collection sessions at AUT or other study locations; given the burden of travelling to these locations, participants received a thank you card and $20 supermarket voucher as koha.

The primary investigator conducted all measurements to ensure consistency and protocol standardisation. Participants were assigned a randomly generated unique identifying code to aid in blinding of the study USI measures to the primary researcher, and then completed the measures described below.

3.5 Screening and eligibility measures

A group of measures was used in the diagnosis of a BPD and to determine inclusion in the control and BPD groups. These measures included: the NQ, BHT, RR, pulse oximetry and the Hi-Lo test (Chaitow et al., 2002, 2014; Courtney et al., 2009; Kiesel et al., 2017). Methods for these measures are detailed in 2.5.

The NQ

The NQ is described in detail in Section 2.5.1 (see Appendix 1). This questionnaire is commonly used for the diagnosis of BPD, and remains the most commonly used questionnaire to assess BPD in research (Courtney & Dixhoorn, 2014; Dixhoorn & Duivenvoorden, 1985; Dixhoorn & Folgering, 2015). As various NQ values have been described for the diagnosis of a BPD (Dixhoorn & Duivenvoorden, 1985; Dixhoorn & Folgering, 2015), a conservative cut-off point of ≥23 out of 64 was used as the inclusion criterion for the BPD group. In addition, various NQ values have been described for a healthy normal population (Courtney & Dixhoorn, 2014; Dixhoorn & Folgering, 2015; Han et al., 2004); therefore, a conservative value of ≤16 out of 64 was chosen as the inclusion criterion for the healthy control

Hi-Lo test

The Hi-Lo test (described in Section 2.5.3) is an observational assessment frequently in clinical situations and research (H. Bradley & Esformes, 2014; Courtney et al., 2009; Kiesel et al., 2017; Roussel et al., 2007), and is completed in a sitting position. The inclusion criterion for the BPD group was the presence of a upper chest/apical dominant pattern of breathing, whereas for the healthy control group it was the presence of an abdominal/diaphragmatic dominant breathing (D. Bradley, 1998; Chaitow, 2014).

Oxygen saturation

SpO_2 was measured with a pulse oximeter (described in Section 2.5.4). Levels below 94% were used to exclude individuals from participation in this study as this may indicate the presence of a lung disease (Chaitow et al., 2002, 2014; Soguel Schenkel et al., 1996).

Breath hold time (BHT)

BHT measurement occurs after a period of normal breathing and is measured during a pause at tidal exhale (described in Section 2.5.4). The person pinches their nose and holds their breath until they feel a moderate-strong urge to breathe (Grammatopoulou et al., 2014; Grammatopoulou et al., 2011). Due to variability in published cut-off

values for BHT (Grammatopoulou et al., 2014; Jack et al., 2004; Kiesel et al., 2017), this study used a conservative cut-off of a BHT time of >30 seconds to represent normal breath hold on tidal exhale. Participants were excluded from the healthy female control group if they could not reach this threshold. Participants in the BPD group were included if their BHT was ≤30 seconds.

Respiratory Rate (RR)

Measurement of the RR is assessed by observation of the total number of respirations in one minute (described in Section 2.5.4)(Main & Denehy, 2016). A respiratory rate of ≥16 breaths per minute was used as the cut-off for inclusion in the BDP group, and a rate of 8-15 was used for inclusion in the healthy control group (Chaitow et al., 2014; Grammatopoulou et al., 2014).

3.6 Demographic variables

Demographic information was collected regarding participants' general health, smoking history, age, gender, and approximate exercise hours per week. This information was used to confirm the absence of a smoking history which was a confounding factor and exclusion criterion. Exercise levels influence diaphragm thickness and so were collected for analysis in the results (P. Brown et al., 2013; Scarlata et al., 2019).

A stadiometer was used to measure participants' height, and calibrated electronic scales were used to measure their weight. BMI was calculated by the formula: BMI = weight in kilograms / height in meters^2 (Ministry of Health, 2017). From this data, female participants were recruited prospectively into four groups as described below.

1. Healthy BMI: Participants with a BMI in a healthy range (18.5–24.9 kg/m^2) who either had a BPD (BPD group) or control participants (without a BPD/control group).
2. BMI+: Participants with a BMI ≥25kg/m^2 who either had a BPD (BPD BMI+ group) or control participants (control BMI+ group).

3.7 Primary outcome measure: diaphragm thickness

The primary outcome measure in this study was diaphragm thickness. This was assessed with USI using an 8300 Chison ultrasound machine with a 10 MHz linear probe (Chison Ultrasound, China, see Figure 6). Diaphragm thickness was measured on the left and right sides of the thorax, as described in previous studies (Boon et al., 2013; Boon et al., 2014) (Figures 5 and 9).

Figure 6. Picture of the 8300 Chison ultrasound machine
(Image source: www.ebay.com/itm/CHISON-8300-Portable-Ultrasound-w-1-probe-choice-of-linear-or-convex-probe-/161497880782)

Measurements of diaphragm thickness was recorded by placing the USI probe in a longitudinal orientation at the level of the ZOA (rib space 8–9, or 9–10) (Figure 7). The primary investigator, who has 10 years of experience in USI assessment of the diaphragm, collected all USI data and was blinded to the diaphragm thickness measurement. Blinding was achieved by obscuring the part of the USI screen that displayed the diaphragm thickness measurement values. The primary investigator then analysed the USI data later; and this analysis was blinded to participants' identity by the use of unique identification numbers. Accurate calibration of equipment was ensured by an independent technician.

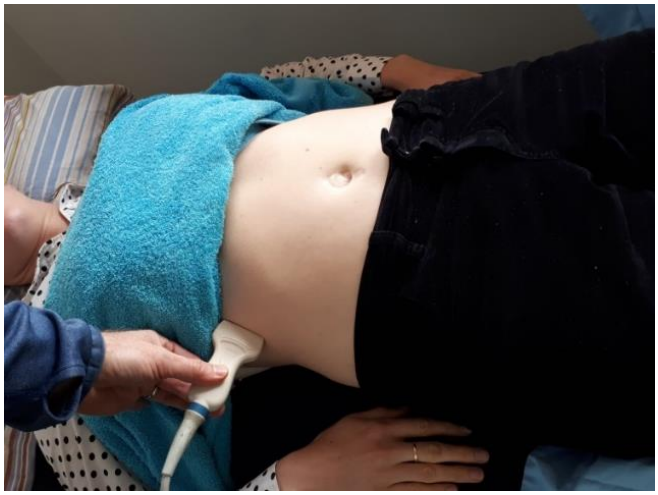

Figure 7. Participant position
Picture of the position of the ultrasound probe to assess diaphragm thickness (Left picture). Picture of participant positioning with pillows behind the participants head and under participants knees (right picture). Source: authors images.

Diaphragm thickness was assessed during a momentary pause in specific respiratory conditions: Tvin, Tvex, Tmax and Tmin (Figure 8). These diaphragm thickness values were used to calculate the TF (refer to Section 3.8.1: Secondary outcome measures).

Figure 8. Lung volumes and diaphragm thickness labels as measured by USI. (Image source: https://commons.wikimedia.org/wiki/File:Lungvolumes_Updated.png). Key: Tmax = thickness of diaphragm at total lung capacity; Tvin = thickness of diaphragm at tidal inhalation; Tvex = thickness of diaphragm at tidal exhale; Tmin = thickness of diaphragm at residual volume.

3.8 Secondary outcome measures

3.8.1 Thickening fraction (TF)

TF can be used to measure changes in diaphragm thickness, and has been shown to have strong correlations with other measures of diaphragm muscle function such as MIP (r^2=−0.82; P<0.01) (Cardenas et al., 2018; Ueki et al., 1995). The TF was calculated using the formula: TF = (diaphragm thickness at Tmax − diaphragm thickness at Tvex) / diaphragm thickness at Tvex (Cardenas et al., 2018; Ueki et al., 1995).

3.8.2 Descriptive analysis for dysfunctional breathing patterns

Any specific dysfunctional breathing patterns seen during data collection were described descriptively. For example, it is expected that the diaphragm will become thicker as a person progressively moves from Tmin to Tvex to Tvin and finally to Tmax (Ueki et al., 1995). If it were the case that the diaphragm became thinner between each of these progressive measures, this would be consistent with paradoxical thinning of the diaphragm (or the reverse if paradoxical thickening were to occur).

3.9 Participant positioning and instruction

The participant was first briefed on the research set up and positioning required for the ultrasound scan and made comfortable. Participants were positioned supine on a plinth, with a pillow behind their head and pillow support under their knees to relax the abdomen and assist the thoracic and lumbar spine to achieve a neutral posture (Figure 7)(Boon et al., 2013; Calvo-Lobo et al., 2019). No clothing was removed during the scan, but participants' tee-shirt/shirt was repositioned to reveal the lower five ribs on both the left and right sides to allow for the lateral ultrasound probe position. Participants were given paper towels to protect their clothing from ultrasound gel. Towels were also provided to assist participant comfort and privacy.

3.10 USI machine and measurement specifics

The unique identification code for each participant was entered and saved to the USB drive attached to USI machine. A small amount of gel was used as a coupling medium for placement of the ultrasound probe. The ultrasound probe was placed on the participant's lateral trunk in line with the anterior axillary line and the anterior superior iliac spine. The ribs were palpated to find rib 11 laterally, and then the ribs counted superiorly until ribs 10–9–8 were identified. The ultrasound probe was then placed spanning ribs 10–9 or 9–8 to allow visualisation of the diaphragm (Boon et al., 2013) (Figure 7). The diaphragm appears as a three-layered structure below the ribs and intercostals, and consists of a hypoechogenic layer bordered by hyperechogenic peritoneum and pleural lines (Boon et al., 2013) (Figure 9). Optimisation of the

ultrasound image with respect to frequency, gain and focus was completed, along with scrolling and fanning of the probe to ensure optimal visualisation (Whittaker, 2007). The participant was then asked to take tidal volume breaths, followed by a deep breath to maximal inhalation and exhalation to assess the general position of the probe and image quality, and allow images to be captured at Tvin, Tvex, Tmax and Tmin.

Each participant was then asked to breathe using specific verbal cues during measurement (Appendix 4) to provide a consistent cue to the size of breath (e.g. 'gently do a relaxed breath in' was the verbal cue for Tvin) (Figure 8). The image of the diaphragm was then frozen, and a calliper measurement was taken on the visual portion of the diaphragm. The image was visually inspected for clarity before it was saved to a flash drive and labelled with a short description (e.g. R Tvin).

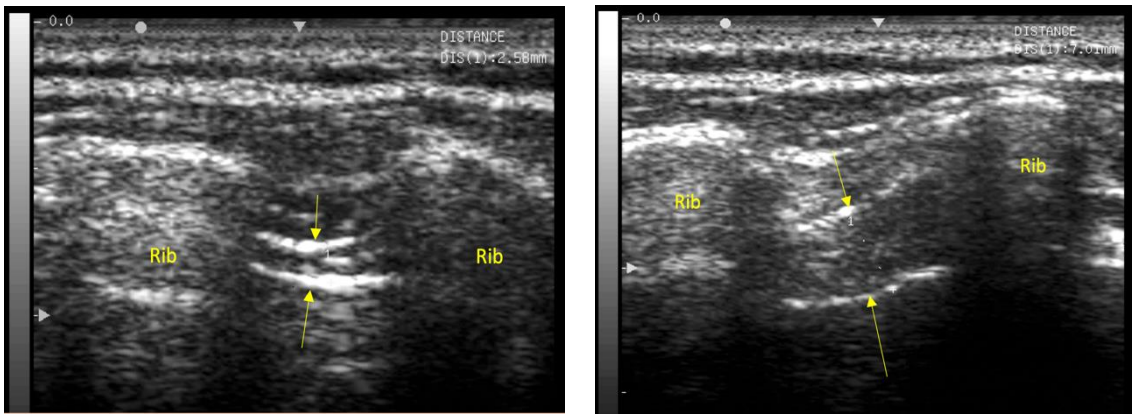

Figure 9. Ultrasound of the diaphragm thickness measurement at Tvex or tidal exhale (left image) and at Tmax or maximum inhale (right image). Key: Yellow arrows depict the top and bottom boundaries of the diaphragm muscle. Source: authors own images.

Previous studies have shown that large variations may exist between the right and left diaphragm in healthy people (Boon et al., 2013; Harper et al., 2013); therefore, bilateral thickness measures of the diaphragm were assessed in each participant. After all measurements were completed, participants in the BPD group were offered usual

care for their condition as per the study flow chart (Appendix 5). A measurement protocol or run sheet was followed to reduce USI measurement errors (Appendix 6).

3.11 Statistical analysis

SPSS version 25.0.0.0 (IBM Corporation, New York, USA) was used to analyse the data. Appropriate descriptive statistics were reported for all recorded measures. Statistical analysis was separately carried out for both groups, described below.

1: Healthy BMI: Participants with a healthy BMI (18.5–24.9 kg/m^2) in the BPD group or the control group.

2: BMI+: Participants with a BMI ≥25kg/m^2 in the BPD BMI+ group or the control BMI+ group.

3.11.1 Sample size calculation

A review of the primary investigators' clinic notes over a 3-month period in 2017 was performed to facilitate the sample size calculation. This audit (n=20) showed that people presenting with a BPD with a healthy BMI (18.5–24.9 kg/m^2) had a mean diaphragm thickness of 1.44±0.31 mm, and those with a BMI ≥25kg/m^2 had a thickness of 1.75±0.34 mm. Data from a well-designed large-scale (n=150, female n=77) normative study by Boon et al. (2013) showed the mean diaphragm thickness was 3.1±1.9 mm. The hypothesis was tested at a 5% level of significance. Using the diaphragm thickness data collected from the clinical audit and retrieved from Boon et al. (2013), G*Power software version 3.1.9.4 (University Dusseldorf) was used to calculate a sample size of 20 cases in the healthy BMI group (18.5–24.9 kg/m^2) and 28 cases in the BMI+ group (≥25 kg/m^2). Another 14 participants were added to allow for the confounding variable of age (Aday & Cornelius, 2011). This provided a total sample size of 76 participants: 17 each in BPD and control groups for healthy BMI, and 21 each in BPD and control groups for BMI+.

3.11.2 Means comparisons

Measurable data were reported as mean and standard deviation (SD) with an accompanying histogram. Outcome measures were then assessed for normality using

a Kolmogorov-Smirnov or Shapiro-Wilk test. A Levene test was used for equality of variances. If the result of the Kolmogorov-Smirnov test was significant ($P<0.05$), the data were transformed to a logarithm scale, and reassessment of normality was performed.

For the healthy BMI groups (BPD group and control group) the sample size and power were adequate, and primary analysis using independent t-tests was performed to investigate the differences between the means of the BPD and control groups for diaphragm thickness and TR for each BMI group. For the BMI+ groups where the sample size and power was not adequate (BPD BMI+ group and the control BMI+ group) means and 95% confidence intervals were presented for each BMI group, but parametric testing was not conducted as recommended by current statistical guidelines (Lederer et al., 2018). This study also provided descriptive details of left and right diaphragm thickness in the BPD and control groups, and any trends were discussed descriptively.

3.11.3 Inclusion of outlier values

The BPD groups (BMI+ and healthy BMI) included participants with a variety of specific BPD (refer to 3.8.2), and some participants were considered likely to have paradoxical thickening of the diaphragm when the diaphragm was expected to be thinning. Therefore, outlier diaphragm thickness values were included in the analysis. Paradoxical diaphragm thickening and thinning were reported separately using descriptive statistics.

3.12 Funding

Funding for this study was obtained from the Cardiothoracic Special Interest Group Scholarship Fund (a special interest group of Physiotherapy New Zealand). AUT also provided funding through a master's research stipend. *Breathing Works Ltd* provided further assistance with the supply of consumables such as stationery, and printing supplies.

Chapter 4. Results

This focus of this chapter is to present the study results, ranging from participant recruitment to statistical analysis, and aims to answer the research questions: 1) does diaphragm thickness differ in females with a BPD compared with healthy female controls? and 2) does the diaphragm TF differ in females with a BPD compared with healthy female controls? Descriptive data are then presented for understanding the population characteristics. A separate section follows that describes the participant characteristics the BMI+ groups, with these results reported using descriptive statistics due to a small sample size.

For clarity, the four groups are described below.

Healthy BMI (BMI in a healthy range (18.5–24.9 kg/m^2)) (refer to 4.2):

a) Participants with BPD (BPD group)
b) Control participants without a BPD (control group).

BMI+ (BMI $\geq$25kg/m^2) (refer to 4.3):

c) Participants with a BPD (BPD BMI+ group)
d) Control participants (control BMI+ group).

4.1 Participants

Recruitment took place between 29 March 2018 and 30 June 2019 (Figure 10). In total, 84 females initially responded to the invitation to participate by email and phone. Of these participants, 37 were did not meet the inclusion criteria and were excluded (Table 2). The remaining 47 participants met the inclusion criteria (Figure 10) and progressed as study participants.

In total, 47 participants met the inclusion criteria, 37 of them in the healthy BMI group and the rest in the BMI+ group. 19 participants were in the BPD group and 18 in the control group. However only six and four participants were found for BPD BMI+ group and the control BMI+ group, respectively. There was difficultly in recruiting subjects into the BMI+ group, (see Section 5.8) and five participants of healthy BMI were excluded as they volunteered after the healthy BMI group had been finalised.

Most of the excluded volunteers were done so by phone or email after the initial enquiry about the study, and reasons included: spinal fusion, pregnancy, low back pain chronic thoracic pain, bronchiectasis, asthma, chest infection, anaemia, under-age, and smoking history. The remaining were excluded by not meeting the screening and eligibility measures (see Table 2, and Table 3).

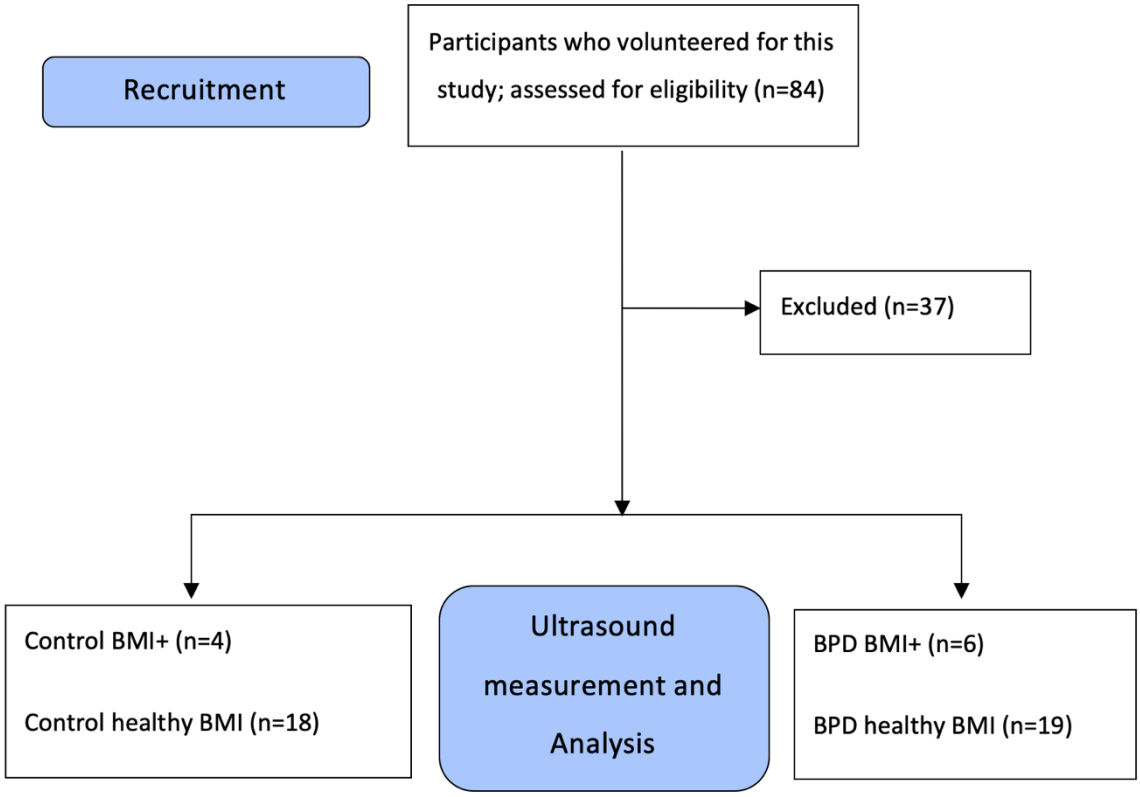

Figure 10. Modified CONSORT flow diagram detailing flow of participants
From 'CONSORT 2010 Flow Diagram', by Altman, Moher and Schulz, The CONSORT group (2010). Retrieved from: http://www.consort-statement.org/consort- statement/flow-diagram

Table 2. Excluded control participants for both BMI groups

Exclusion categories	Numbers excluded
Breath-hold time <30 sec	1
Chest breathing pattern	1
Breath hold time <30 sec, NQ >16	1
Breath hold time <30 sec, RR >16	1
Chest breathing pattern, breath-hold time <30 sec	7
Chest breathing pattern, breath-hold time <30 sec, RR >16	5
Chest breathing pattern, breath-hold time <30 sec, RR >16, NQ >16	2
BMI exclusion	5
Chronic thoracic pain	1
Low back pain	1
Pregnant	2
Spinal fusion	1
Total	28

Note: RR = respiratory rate; NQ = Nijmegen Questionnaire; BMI (body mass index) exclusion = participants who were outside the desired BMI parameters for the BMI+ group.

Table 3. Excluded BPD participants for both BMI groups

Reasons for exclusion from the BPD and BPD BMI+ groups	Number excluded
NQ <23	1
NQ <23, abdominal breathing pattern	1
Ex-smoker	2
Bronchiectasis	1
Chest infection	1
Under-age	1
Anaemia	1
Asthma	1
Total	9

Notes: NQ = Nijmegen Questionnaire.

4.2 Results for the healthy BMI groups

4.2.1 Demographic and respiratory characteristics of all participants with a healthy BMI

Descriptive statistics for the demographic characteristics are presented in Table 4. The mean (standard deviation; SD) age was 36 (12) years in the BPD group and 29 (11) years in the control group. The mean BMI was 22.09 kg/m^2 in the BPD group and 22.01 kg/m^2 in the control group.

Table 4. Demographic and respiratory characteristics in participants with a healthy BMI

	Group	N	Mean	Standard deviation	P-value
Age (years)	BPD	19	36.32	11.56	
	Control	18	29.61	11.27	0.083
Height (m)	BPD	19	1.65	0.07	
	Control	18	1.67	0.06	0.547
Weight (kg)	BPD	19	60.84	6.72	
	Control	18	62.35	8.12	0.542
BMI (kg/m^2)	BPD	19	22.09	1.94	
	Control	18	22.21	1.76	0.851
Pulse oximetry (%)	BPD	19	98	0.96	
	Control	18	98	0.49	0.059
Respiratory rate (breaths/min)	BPD	19	19.68	4.26	
	Control	18	13.94	1.11	0.000
Nijmegen Questionnaire	BPD	19	35.16	8.59	
(score out of 64)	Control	18	6.61	3.60	0.000
Breath hold time (seconds)	BPD	19	17.47	4.98	
	Control	18	32.67	3.40	0.000
Exercise hours/week *	BPD	19	3.47	2.36	
	Control	18	6.75	4.22	0.008

Notes: BPD = breathing pattern disorder; BMI = body mass index;

There was no difference in all demographic variables between groups, and likewise for SpO_2. For the remaining clinical variables (RR, BHT, and NQ score) statistically significant differences were observed between the BPD and control groups ($P<0.05$).

These findings were expected, as these variables were determinants used for the inclusion and exclusion criteria for the BPD and control groups. Exercise hours per week were also significantly different (P=0.008) between the BPD and control groups.

4.2.2 Diaphragm thickness at Tvex

The mean with standard deviation for the left and the right sides of diaphragm thickness at Tvex are presented in original scale (mm) in Table 5. To allow for parametric test (t-test), data was transferred to log scale in order to satisfy the normality assumption.

Table 5. Diaphragm thickness at Tvex on the right and left in participants with a healthy BMI

	Group	N	Mean	Standard deviation	Statistic (t)	Mean difference (95% CI)	P-value
R_Tvex (mm)	BPD	19	1.650	0.369		0.363 (0.026, 0.700)	
	Control	18	2.013	0.617			
R_Tvex_Log	BPD	19	0.208	0.089	−2.227	−0.078 (-0.150, -0.007)	0.032
	Control	18	0.287	0.123			
L_Tvex (mm)	BPD	19	1.583	0.341		0.420 (0.095, 0.745)	
	Control	18	2.003	0.584			
L_Tvex_Log	BPD	19	0.190	0.089	−2.674	−0.094 (-0.166, -0.167)	0.011
	Control	18	0.285	0.123			

BPD = breathing pattern disorder; CI = confidence interval; Tvex = tidal exhale; R_Tvex = right Tvex; R_Tvex_Log = right Tvex measurement converted to logarithm; L_Tvex = left Tvex; L_Tvex_Log = left Tvex measurement converted to logarithm.

For both the left and right sides, the diaphragm thickness at Tvex in the control group is significantly greater than the BPD group (Table 5 and Figure 11).

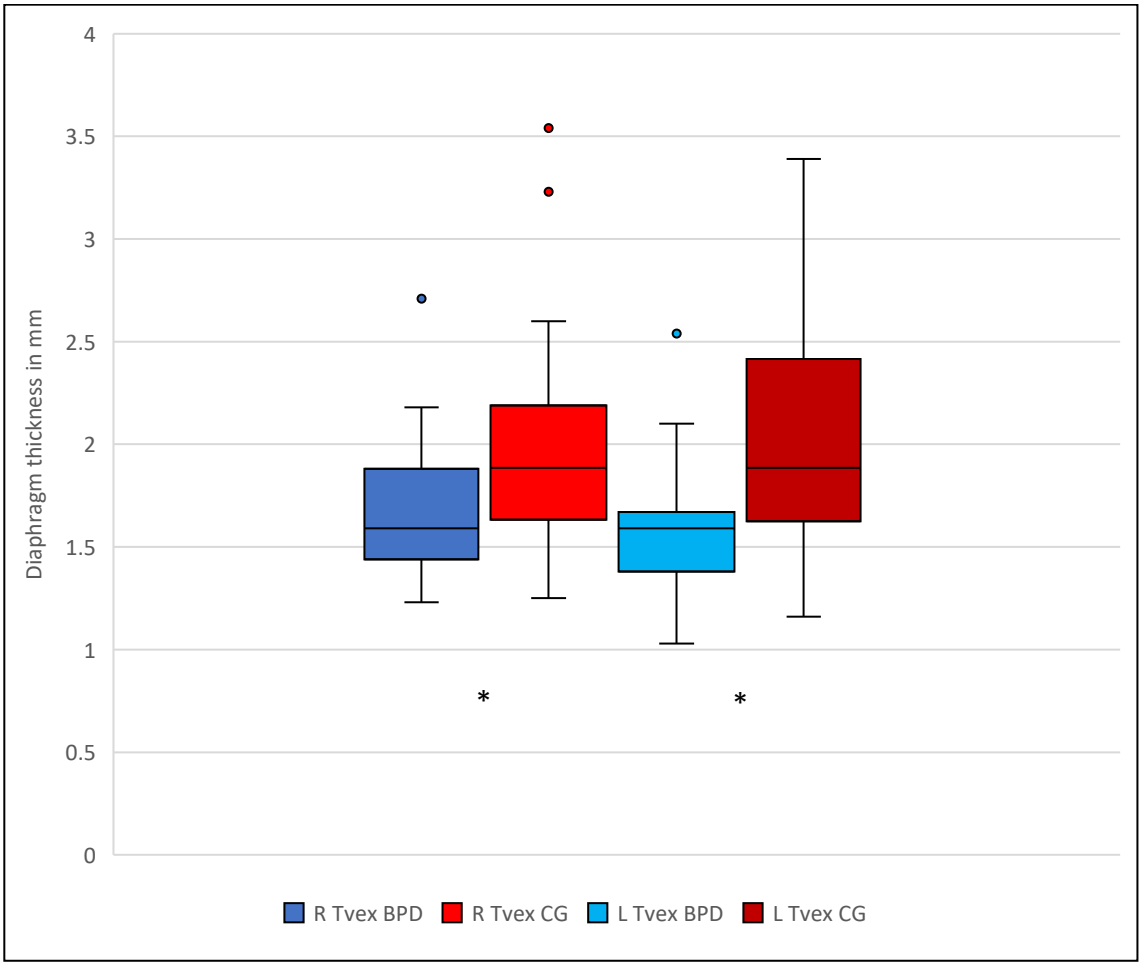

Figure 11. Box and whisker plot of diaphragm thickness at Tvex on the right and left in participants with a healthy BMI.
Note. The boxes represent the 25th and 75th percentiles, the centre line the median and the whiskers 1.5 of the median. * indicates significant difference between groups. Outliers are indicated by the small circles. Tvex = tidal exhale; BPD = breathing pattern disorder; R Tvex BPD = right Tvex in BPD group; L Tvex BPD = left Tvex in BPD group; R Tvex CG= right Tvex in control group; L Tvex CG = left Tvex in control group.

4.2.3 Diaphragm thickness at Tvin

Diaphragm thickness at Tvin on the right and left sides is presented in Table 6. Right Tvin data are presented in millimetres (mm), and in logarithm scale to allow use of parametric testing (t-test). Left Tvin data did not require transformation as a normal distribution was observed.

The right and left side diaphragm thickness at Tvin showed a significant difference between the BPD and control groups ($P<0.005$), with the diaphragm thinner in the BPD group when compared to the control group (Figure 12).

Table 6. Diaphragm thickness at Tvin on the right and left in healthy BMI.

	Group	N	Mean	Standard deviation	Statistic (t)	Mean difference (95% CI)	P-value
R_Tvin (mm)	BPD	19	1.861	0.358		0.615 (0.202, 1.028)	
	Control	18	2.476	0.772			
R_Tvin_Log	BPD	19	0.262	0.085	−3.038	−0.112 (−0.187, −0.037)	0.004
	Control	18	0.374	0.135			
L_Tvin (mm)	BPD	19	1.747	0.397	−4.001	−0.691 (−1.042, −0.341)	0.000
	Control	18	2.438	0.634			

BPD = breathing pattern disorder; CI = confidence interval; Tvin = tidal inhale; R_Tvin = right Tvin; R_Tvin_Log = right Tvin measurement converted to logarithm; L_Tvin = left Tvin; *denotes significant differences between groups.

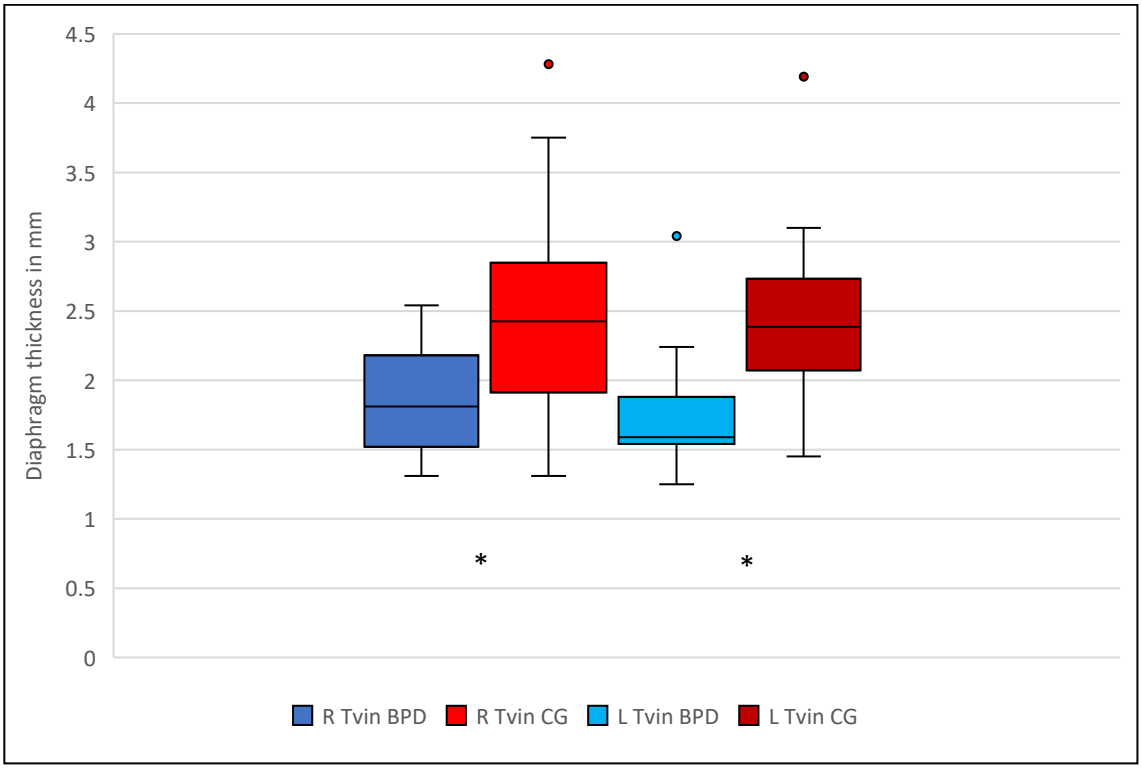

Figure 12. Box and whisker plot of diaphragm thickness at Tvin on the right and left in participants with a healthy BMI.
* indicates significant difference between groups. Outliers are indicated by the small circles. BPD = breathing pattern disorder; Tvin = tidal inhale; R Tvin BPD = right Tvin in BPD; L Tvin BPD = left Tvin in BPD; R Tvin CG = right Tvin in control group; L Tvin CG = left Tvin in control group.

4.2.4 Diaphragm thickness at Tmax

The thickness of the diaphragm at Tmax was measured using ultrasound on the right and left sides (Table 7). Right Tmax data are presented in mm and logarithm scale. Left Tmax data was normally distributed. Parametric t-test were used.

The left side diaphragm thickness at Tmax is significantly greater in the control group compared to the BPD group (P=0.001, Table 7 and Figure 13). However, the right side at Tmax (logarithm) was marginally unable to detect a difference between the BPD group and the control group (P=0.073, Table 7 and Figure 13).

Table 7. Diaphragm thickness at Tmax on the right and left in participants with a healthy BMI

	Group	N	Mean	Standard deviation	Statistic (t)	Mean difference (95% CI)	P-value
R_Tmax (mm)	BPD	19	3.603	1.344		0.891 (−0.167, 1.950)	
	Control	18	4.494	1.806			
R_TMax_log	BPD	19	0.529	0.160	−1.846	−0.096 (−0.201, 0.010)	0.073
	Control	18	0.624	0.157			
L_Tmax (mm)	BPD	19	3.147	1.418	−3.479	−1.683 (−2.66, −0.701)	0.001
	Control	18	4.830	1.523			

BPD = breathing pattern disorder; CI = confidence interval; Tmax = maximum inhale; R_Tmax = right Tmax; R_Tmax_Log = right Tmax measurement converted to logarithm; L_Tmax = left Tmax; *denotes significant differences between groups.

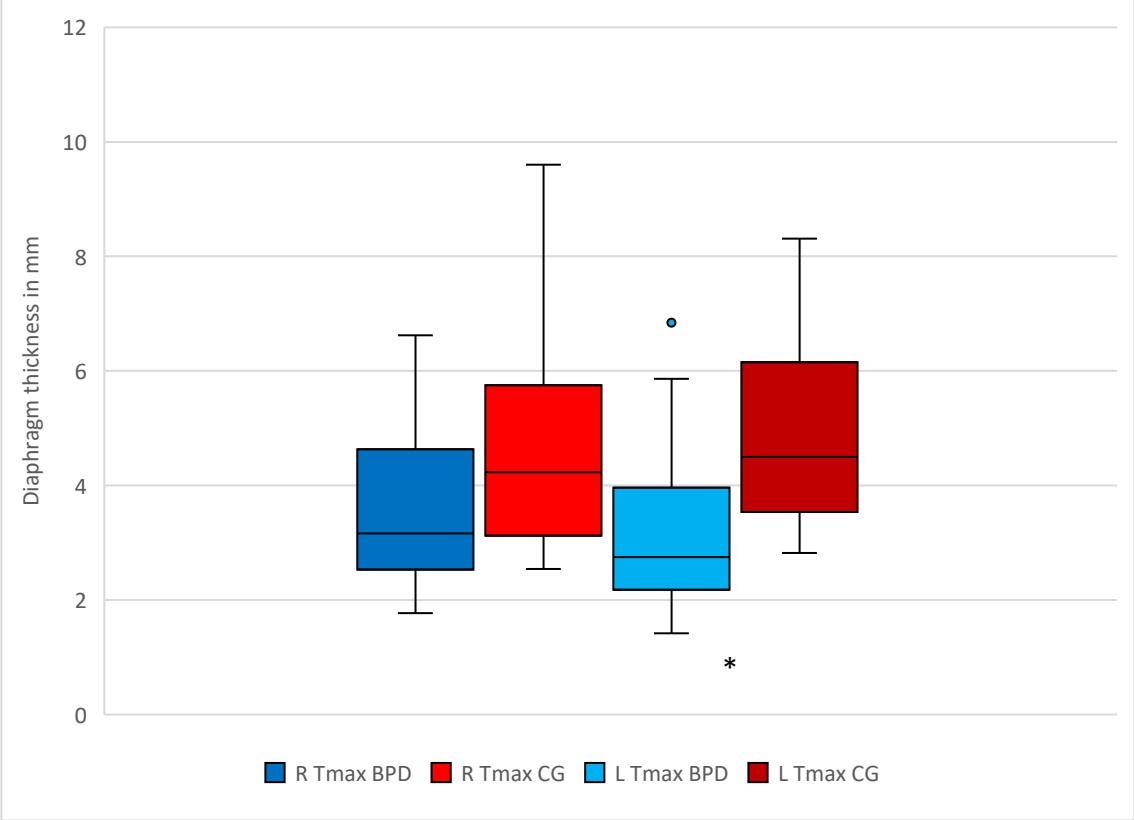

Figure 13. Box and whisker plot of diaphragm thickness at Tmax on the right and left in participants with a healthy BMI.
Note: Outliers are indicated by the small circles. BPD = breathing pattern disorder; Tmax = maximum inhale; R Tmax BPD = right Tmax in BPD; L Tmax BPD = left Tmax in BPD; R Tmax CG = right Tmax in control group; L Tmax CG = left Tmax in control group; * indicates significant difference between groups.

4.2.5 Diaphragm thickness at Tmin

The thickness of the diaphragm at Tmin the right and left sides are presented in Table 8. The right side at Tmin (logarithm) was marginally unable to detect a difference between the BPD group having a thicker diaphragm than the control group (P=0.063, Table 8, Figure 14). The left side Tmin logarithm (P=0.916, Table 8, Figure 14) showed no significant difference between the BPD and control groups.

Table 8. Table of diaphragm thickness at Tmin on the right and left in healthy BMI

	Group	N	Mean	Standard deviation	Statistic (t)	Mean difference (95% CI)	P-value
R_Tmin (mm)	BPD	19	2.145	0.519		−0.284 (−0.621;0.052)	
	Control	18	1.861	0.487			
R_Tmin_log	BPD	19	0.321	0.095	1.918	0.064 (−0.004; 0.132)	0.063
	Control	18	0.256	0.109			
L_Tmin (mm)	BPD	19	1.938	0.391		0.069 (−0.292; 0.430)	
	Control	18	2.008	0.663			
L_Tmin_log	BPD	19	0.28	0.084	−0.106	−0.0038 (−0.076; 0.069)	0.916
	Control	18	0.283	0.130			

Note: BPD = breathing pattern disorder; CI = confidence interval; Tmin = maximum exhale; R_Tmin = right Tmin, R_Tmin_Log = right Tmin converted to logarithm. L_Tmin = left Tmin, L_Tmin_Log = left Tmin converted to logarithm; *denotes significant differences between groups.

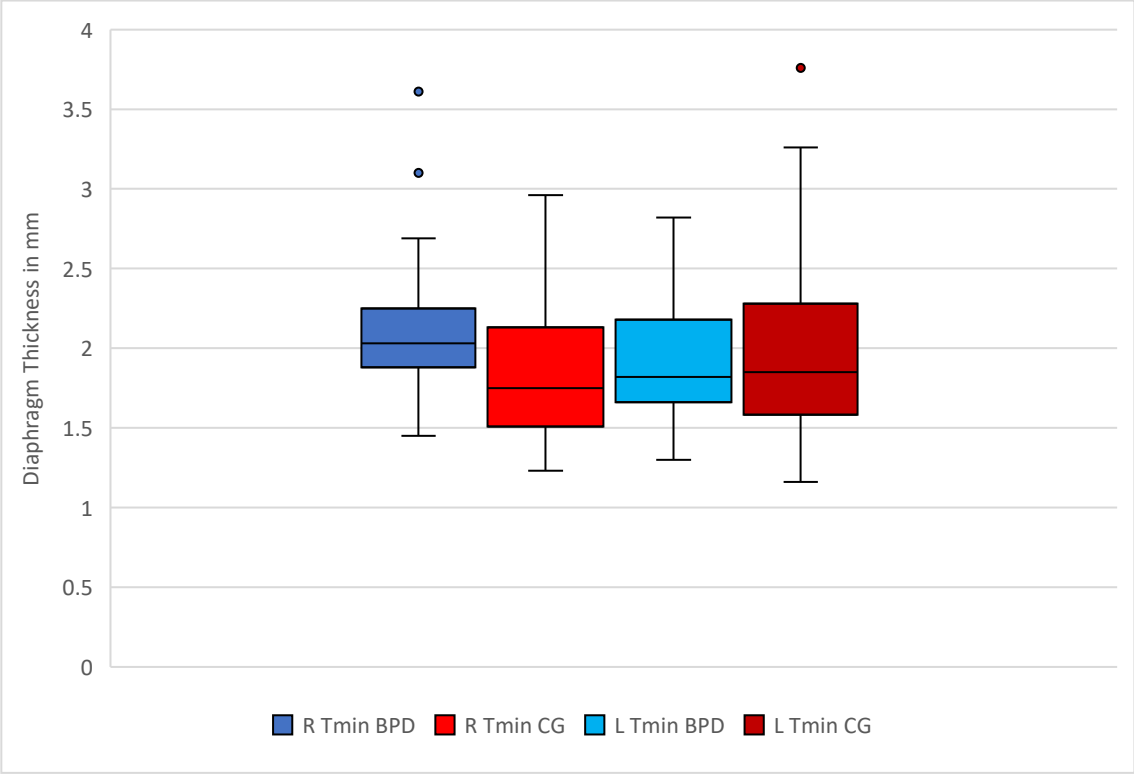

Figure 14. Box and whisker plot of diaphragm thickness at Tmin on the right and left in participants with a healthy BMI.

Note: Outliers are indicated by the small circles. BPD = breathing patter disorder; Tmin = maximum exhale; R Tmin BPD = right Tmin in BPD; L Tmin BPD = left Tmin in BPD; R Tmin CG= right Tmin in control group; L Tmin CG = left Tmin in control group.

4.2.6 Diaphragm thickening fraction (TF)

The left side diaphragm TF showed a significant difference between the BPD and control group, with a lower TF in the BPD group than the control group (P=0.039, Table 9 and Figure 15). The right-side diaphragm TF did not show a significant difference between the BPD group and the control group (P=0.943, Table 9, Figure 15).

Table 9. Diaphragm TF on the right and left in participants with a healthy BMI

	Group	N	Mean	Standard deviation	Statistic (t)	Mean difference (95% CI)	P-value
R_TF	BPD	19	1.243	0.859	0.072	0.016 (−0.446, 0.479)	0.943
	Control	18	1.226	0.467			
L_TF	BPD	19	0.995	0.762	−2.163	−0.441 (−0.861, −0.024)	0.039
	Control	18	1.436	0.447			

BPD = breathing pattern disorder; CI = confidence interval; TF = thickening fraction; R_ TF= right TF; L_TF= left TF; *denotes significant differences between groups.

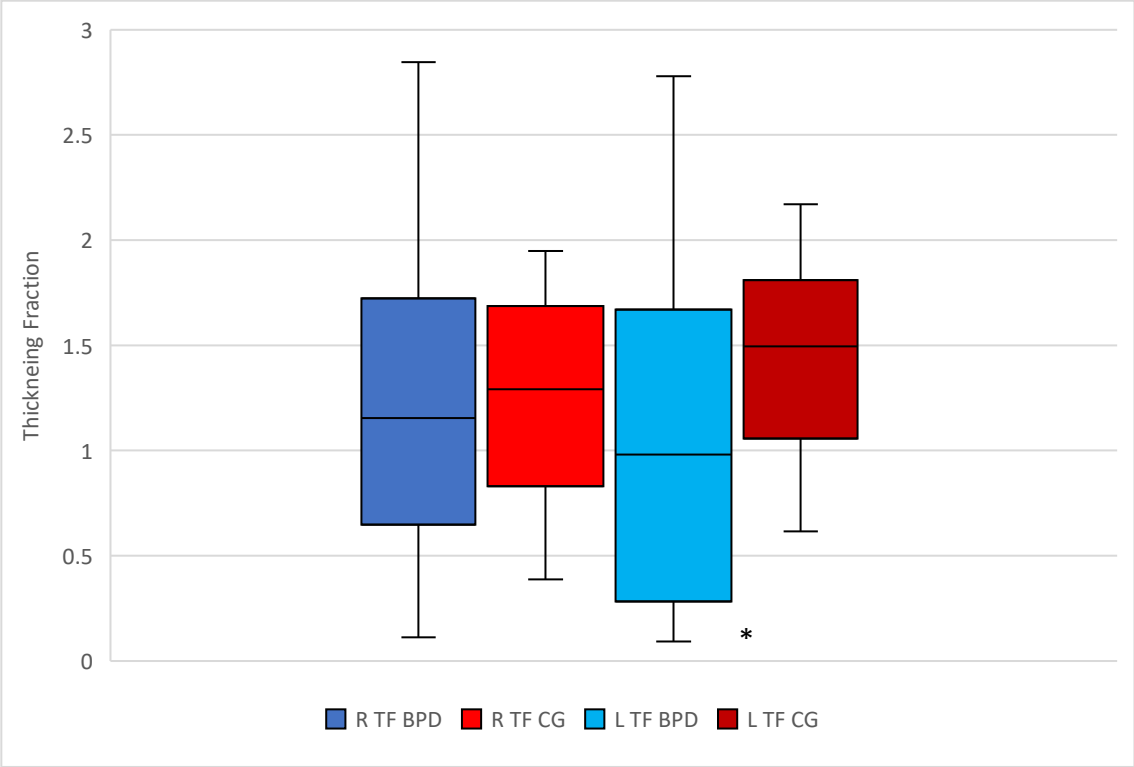

Figure 15. Box and whisker plot of diaphragm TF on right and left in participants with a healthy BMI.

Note. BPD = breathing pattern disorder; TF = thickening fraction; R TF BPD = right TF in BPD; L TF BPD = left TF in BPD; R TF CG= right TF in control group; L TF CG = left TF in control group; * indicates significant differences between groups.

4.2.7 Changes in diaphragm thickness at progressive measurement point

The diaphragm is expected to increase in thickness in a sequential fashion from Tmin, to Tvex, to Tvin and finally to Tmax. The progression of diaphragm thickness was described sequentially through each of the breathing measures on the right and left sides (Figures 16 and 17).

The right diaphragm in the control group increased in thickness progressively from Tmin (1.861 mm) through each breathing measure to Tmax (4.494 mm) in an expected fashion. The right diaphragm in the BPD group was thicker at Tmin (2.145 mm) than at both Tvex (1.649 mm) and Tvin (1.862 mm) and reached maximal thickness at Tmax (3.603 mm, Figure 16). This was an unexpected change of diaphragm thickness and suggests the increase in thickness from Tvex to Tmin in the BPD group may represent paradoxical diaphragm thickening.

The left diaphragm in the control group did not change in thickness from Tmin (2.008 mm) to Tvex (2.003 mm) and increased in thickness progressively from Tvex to Tvin (2.438 mm) and Tmax (4.830 mm). In the BPD group, the diaphragm was thicker at Tmin (1.938 mm) than at both Tvex (1.583 mm) and Tvin (1.747 mm) and reached maximal thickness at Tmax (3.147 mm) (Figure 17). As per the right diaphragm, the left diaphragm showed an unexpected change of diaphragm thickness, with the increased thickness from Tvex to Tmin in the BPD group being suggestive of paradoxical diaphragm thickening.

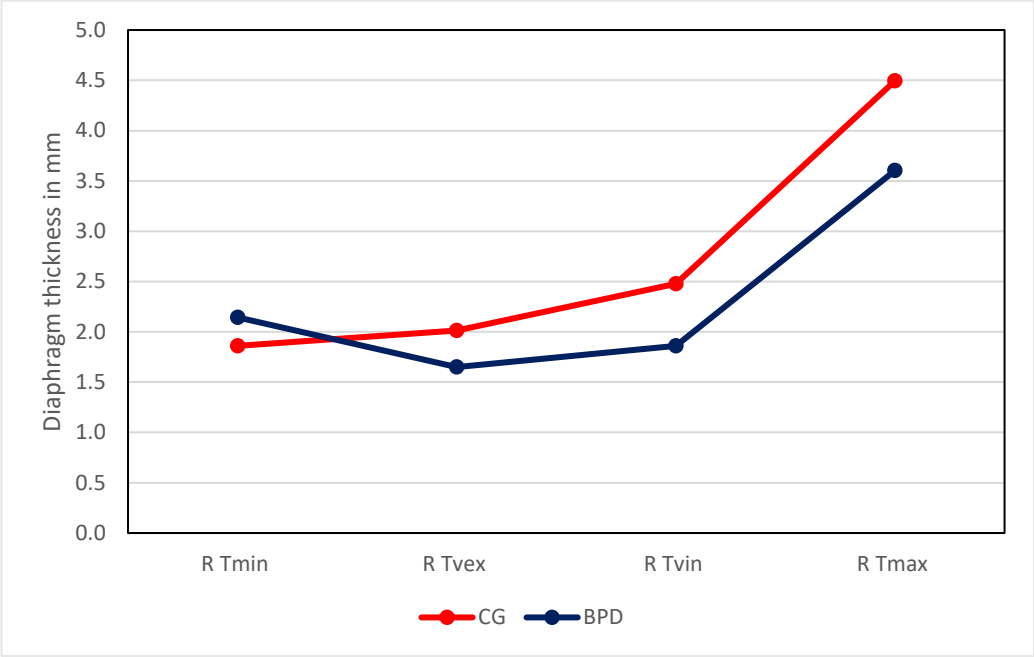

Figure 16. Line graph of mean diaphragm thickness across diaphragm measures on the right side in participants with a healthy BMI
Note: CG = control group; BPD = breathing pattern disorder group.

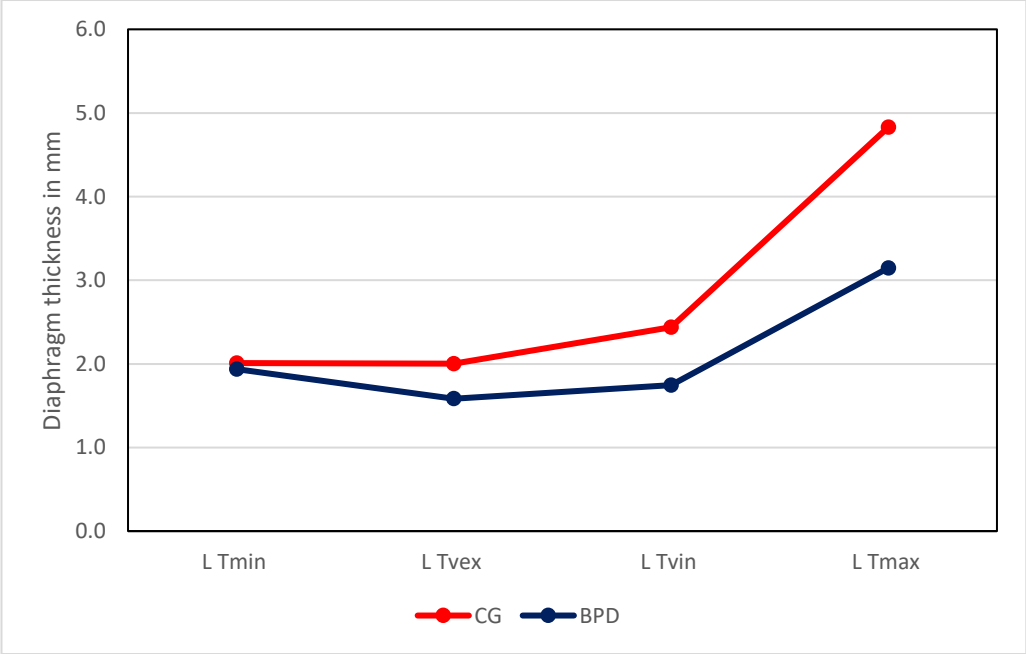

Figure 17. Line graph of mean diaphragm thickness across diaphragm measures on the left side in participants with a healthy BMI
CG = control group; BPD = breathing pattern disorder group.

4.2.8 Paradoxical changes in diaphragm thickness in participants with a healthy BMI

In three individual cases in the BPD group, paradoxical diaphragm thickening was observed. In these cases, the diaphragm was thicker at Tmin than at Tmax (Table 10 and Appendix 7). One participant had paradoxical thickening of the diaphragm at Tvex that was greater than at Tvin. These observations of paradoxical diaphragm activity are not unusual for people with BPD (refer to 3.11.3), and therefore were not considered as outliers in the statistical analysis.

Table 10. Paradoxical changes in diaphragm thickness in individuals with a healthy BMI in the BPD group

	R Tmin (mm)	R Tvex (mm)	R Tvin (mm)	R Tmax (mm)	L Tmin (mm)	L Tvex (mm)	L Tvin (mm)	L Tmax (mm)
Control (n=18)	1.86	2.01	2.48	4.49	2.01	2.00	2.44	4.83
BPD (n=19)	2.15	1.65	1.86	3.60	1.94	1.58	1.75	3.15
Participant A	**2.69**	2.18	2.54	**2.46**	1.88	**1.88**	**1.83**	2.18
Participant B	2.03	1.59	2.03	2.65	**1.95**	1.30	1.38	**1.42**
Participant C	**2.03**	1.31	1.31	**1.77**	1.66	1.52	1.70	1.95

Note: BPD = breathing pattern disorder; R = right side; L = left side; Tmin = maximum exhale; Tvex = tidal exhale; Tvin = tidal inhale; Tmax = maximum inhale. Values in bold indicate paradoxical changes in diaphragm thickness in comparison to mean values in BPD group and control group.

4.3 Results for the BMI+ groups

This section describes the demographic and respiratory characteristics of participants in the BPD BMI+ and control BMI+ groups. The sample size calculation suggested recruitment of 21 BPD BMI+ participants and 21 control BMI+ participants (total n=42). Unfortunately, it was not possible to recruit the desired number of participants for the BMI+ group despite multiple strategies (refer to Section 5.8 and Appendix 8). Due to the difficulty of recruiting people from BMI+ population, 10 participants who satisfied our inclusion criteria were recruited for the study. Descriptive statistics and confidence intervals were reported as recommended by current statistical guidelines (Lederer et al., 2018).

4.3.1 Demographic and respiratory characteristics of all participants in the BMI+ groups

Descriptive statistics for the demographic characteristics are presented in Table 11. Participants' mean (SD) age was 46.33 (15.81) years in the BPD group and 35.50 (9.68) years in the control group. The mean BMI was 30.27 kg/m^2 in the BPD group and 26.45 kg/m^2 in the control group. Due to the small sample size, parametric testing for baseline comparisons was not performed (Lederer et al., 2018)

Table 11. Demographic and respiratory characteristics for participants in the BMI+ group

	Group	N	Mean	Standard deviation
Age (years)	BPD	6	46.33	15.81
	Control	4	35.50	9.68
Height (m)	BPD	6	1.65	0.05
	Control	4	1.72	0.08
Weight (kg)	BPD	6	82.72	11.46
	Control	4	78.00	10.30
BMI (kg/m^2)	BPD	6	30.27	3.00
	Control	4	26.45	2.19
Pulse oximetry (%)	BPD	6	0.98	0.01
	Control	4	0.98	0.01
Respiratory rate (breaths/min)	BPD	6	18.00	1.27
	Control	4	13.50	1.00
Nijmegen Questionnaire (out of 64)	BPD	6	36.83	10.57
	Control	4	9.50	0.58
Breath-Hold Time (seconds)	BPD	6	15.33	5.75
	Control	4	33.25	1.26
Exercise hours/week	BPD	6	3.00	2.37
	Control	4	6.50	2.89

Note: BMI = body mass index; BPD = breathing pattern disorder; * exercise hours per week included aerobic and resistance exercise.

4.3.2 Diaphragm thickness at Tvex

Diaphragm thickness for both the right side and left side at Tvex showed no difference between the BPD and control groups (Table 12 & Figure 18). The right diaphragm showed a reversal of the trend of the control group being thicker than the BPD group.

Table 12. Diaphragm thickness at Tvex on the right and left in the BMI+ group

	Group	N	Mean	Standard deviation	Mean difference (95% CI)
R_Tvex (mm)	BPD	6	2.108	0.427	−0.196 (−0.740; 0.346)
	Control	4	1.912	0.222	
L_Tvex (mm)	BPD	6	2.002	0.096	−0.156 (−0.775; 0.463)
	Control	4	2.158	0.667	

BPD = breathing pattern disorder; Tvex = tidal exhale.

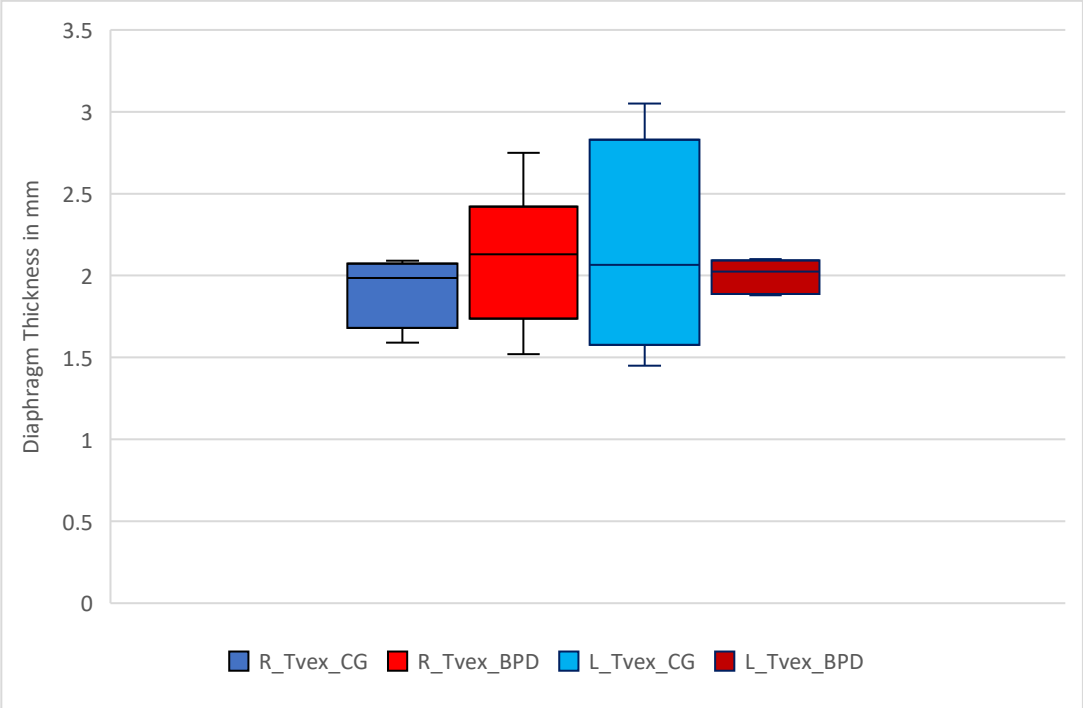

Figure 18. Box and whisker plot of diaphragm thickness at Tvex on the right and left sides in the BMI+ groups

Note: BPD = breathing pattern disorder; Tvex = tidal exhale; R Tvex BPD = right Tvex in BPD BMI+; L Tvex BPD = left Tvex in BPD BMI+; R Tvex CG= right Tvex in control BMI+; L Tvex NP = left Tvex in control BMI+.

4.3.3 Diaphragm thickness at Tmin, Tvin and Tmax

The thickness of the diaphragm at Tmin, Tvin and Tmax in the BMI+ groups is displayed in Table 13. Mean difference and confidence intervals are reported.

Table 13. Diaphragm thickness at Tmax, Tmin and Tvin on the right and left in participants with BMI+

	Group	N	Mean	Standard deviation	Mean difference (95% CI)
R_Tmin (mm)	BPD	6	2.243	0.364	−0.463 (−0.919; 1.28)
	Control	4	1.780	0.173	
L_Tmin (mm)	BPD	6	2.342	0.250	−0.204 (−0.977; 0.569)
	Control	4	2.138	0.785	
R_Tvin (mm)	BPD	6	2.562	0.444	0.171 (−0.945; 1.287)
	Control	4	2.733	1.082	
L_Tvin (mm)	BPD	6	2.425	0.537	0.088 (−0.741; 0.916)
	Control	4	2.513	0.588	
R_Tmax (mm)	BPD	6	4.127	1.208	2.238 (−0.692; 5.169)
	Control	4	6.365	2.812	
L_Tmax (mm)	BPD	6	4.202	1.523	0.618 (−1.920; 3.156)
	Control	4	4.820	1.972	

Note: BPD = breathing pattern disorder; Tmin = maximum exhale; Tvin = tidal inhale; Tmax = maximum inhale. R_ Tmax = right Tmax, R_Tmin= right Tmin, R_Tvin= right Tvin; L_ Tmax = left Tmax, L_Tmin = left Tmin, L_Tvin = left Tvin; BPD_OW = BPD >25 kg/m^{2}BMI group; NP_OW= Healthy female group BMI ≥25 kg/m^2; *denotes significant differences between groups.

The right diaphragm at Tmin was thicker in the BPD group than in the control group which was not an expected result (BPD group 2.243 mm; control group 1.780 mm) (Table 13 & Figure 19). In scale, the right diaphragm at Tmax was thicker in the control group compared with the BPD group (BPD group 4.127 mm; control group 6.365 mm) (Table 13 & Figure 20). The left diaphragm Tmin mean was thicker in the BPD group compared with the control group (BPD group 2.342 mm; control group 2.138mm), however this difference is not noticeable due to the distribution of the data (Table 13 & Figure 19). The left diaphragm Tmax mean was thicker in the control group compared with the BPD group (BPD group 4.202 mm; control group 4.820mm), however again this difference is not noticeable due to the distribution of the data (Table 13 & Figure 20). The right side Tvin and the left side Tvin were similar in the BPD and control groups.

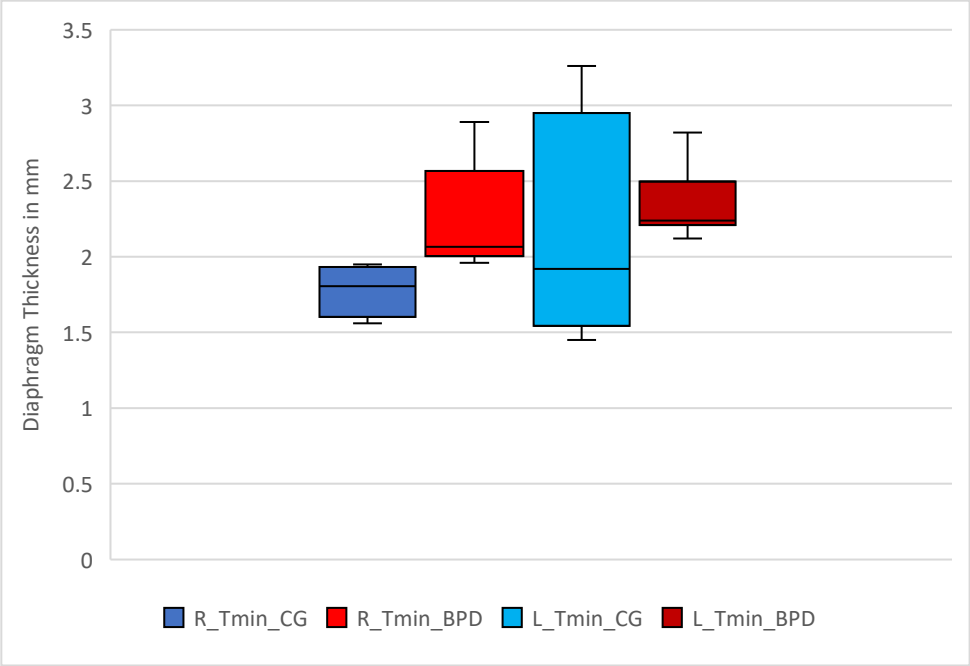

Figure 19. Box and whisker plot of diaphragm thickness at Tmin on the right and left in the BMI+ groups.

Note. BPD = breathing pattern disorder; BMI = body mass index; Tmin = maximum exhale; R_Tmin BPD = right Tmin in BPD BMI+; L_Tmin BPD = Left Tmin in BPD BMI+; R_Tmin CG = right Tmin in control BMI+; L_Tmin CG = Left Tmin in control BMI+.

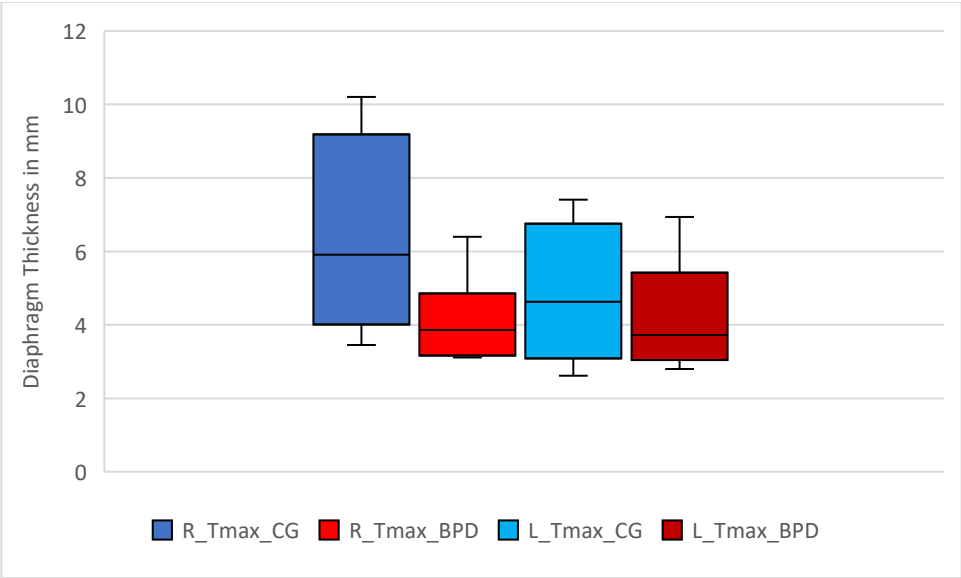

Figure 20. Box and whisker plot of diaphragm thickness at Tmax on the right and left in the BMI+ groups

Note: BPD = breathing pattern disorder; BMI = body mass index; Tmax = maximum inhale; R_Tmax BPD = right Tmax in BPD BMI+ group; L_Tmax BPD = left Tmax in BPD BMI+ group; R_Tmax CG = right Tmax in control BMI+ group; L_Tmax CG = left Tmax in control BMI+ group.

4.3.4 Diaphragm thickening fraction (TF)

The TF in the BMI+ groups are displayed in Table 14. Mean difference and confidence intervals are reported.

Table 14. Diaphragm TF on the right and left in participants with BMI+

	Group	N	Mean	Standard deviation	Mean difference (95% CI)
L_TF	BPD	6	1.094	0.732	−0.0864 (−0.982; 0.809)
	Control	4	1.180	0.266	
R_TF	BPD	6	1.030	0.722	−1.236 (−2.651; 0.178)
	Control	4	2.266	1.241	

Note: BPD = breathing pattern disorder; TF = thickening fraction; R_TF = right TF; L_TF = left TF.

In size, the right thickening fraction measurement showed a greater TF in the control group compared with the BPD group (BPD group 1.030, control group 2.266, Table 14 & Figure 21). The left side TF measurement was similar between the groups (BPD group 1.094; control group 1.180, Figure 21), and difference is not noticeable due to the distribution of the data.

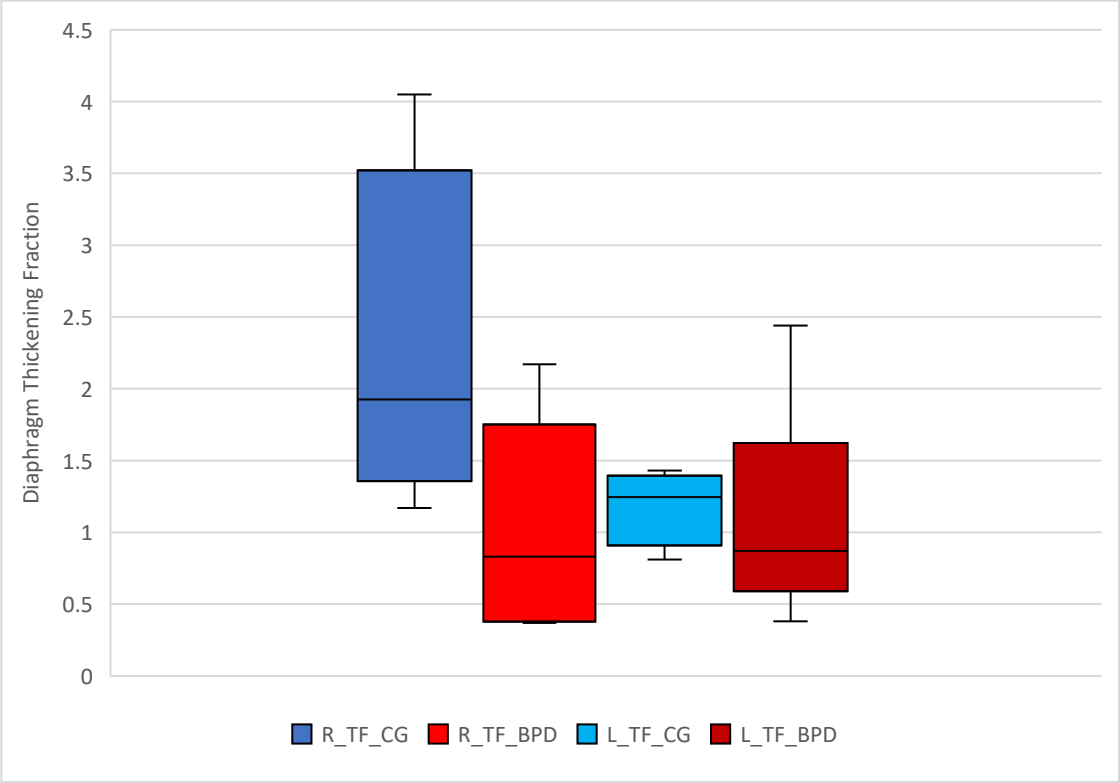

Figure 21. Box and whisker plot of diaphragm TF on the right and left in the BMI+ groups
Note: BPD = breathing pattern disorder; BMI = body mass index; TF = thickening fraction; R_TF_BPD = right thickening fraction in BPD BMI+; L_TF_BPD = left thickening fraction in BPD BMI+; R_TF_CG = right thickening fraction in control BMI+; L_TF_CG = left thickening fraction in control BMI+.

4.3.5 Changes in diaphragm thickness at progressive measurement points

The diaphragm is expected to increase in thickness in a sequential fashion from Tmin, to Tvex, to Tvin and finally to Tmax. The progression of diaphragm thickness is shown sequentially through each measure on the right and left sides (Figures 22 and 23). The right diaphragm in the control group increased in thickness progressively from Tmin (1.780 mm) through to Tmax (6.365 mm), which was an expected progression. In the BPD group, the right diaphragm was thicker at Tmin (2.243 mm) than at Tvex (2.108 mm), before progressively thickening through Tvin (2.562 mm) and reaching maximal thickness at Tmax (4.127 mm). This was an unexpected change of diaphragm thickness and is similar to the results of the healthy BMI group. This suggests the increase in thickness from right Tvex to Tmin in the BPD BMI+ group is likely to represent paradoxical diaphragm thickening.

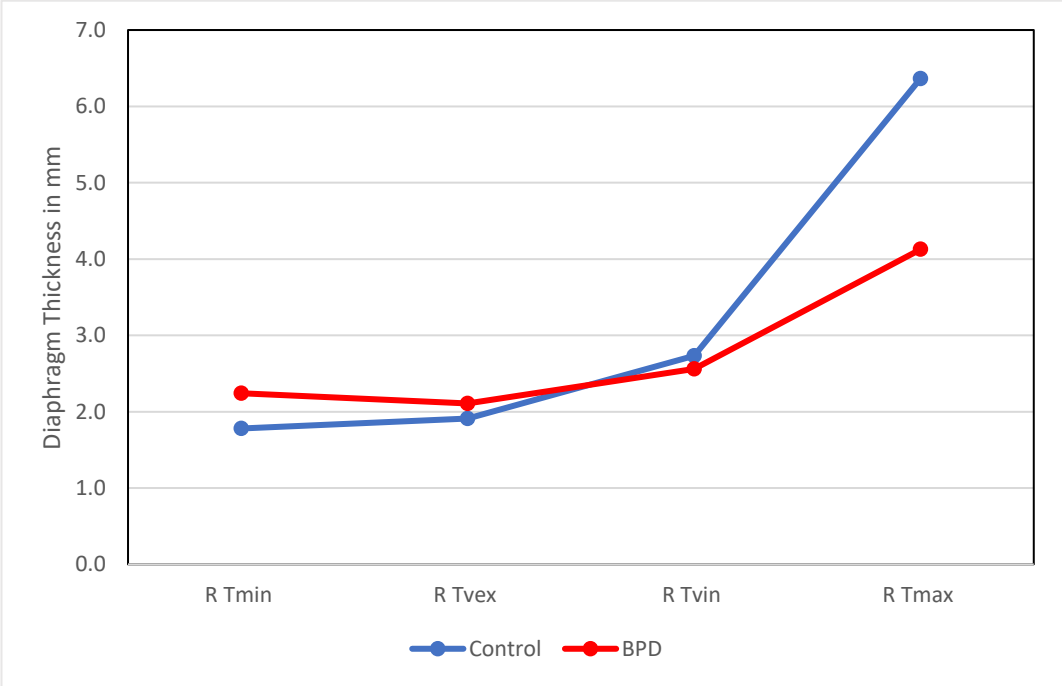

Figure 22. Line graph of mean diaphragm thickness across measures on the right side in the BMI+ group

BPD = breathing pattern disorder; Tmin = maximum exhale; Tvex = tidal exhale; Tvin = tidal inhale; Tmax = maximum inhale.

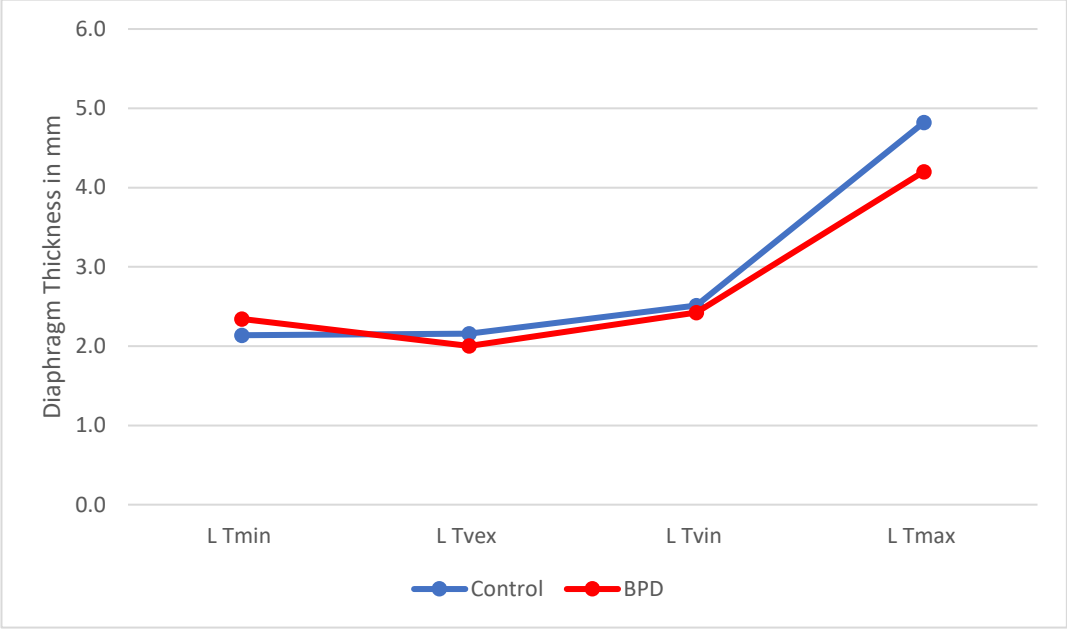

Figure 23. Line graph of mean diaphragm thickness across measures on the left side in the BMI+ groups

BPD = breathing pattern disorder; Tmin = maximum exhale; Tvex = tidal exhale; Tvin = tidal inhale; Tmax = maximum inhale.

The left diaphragm in the control group thickened progressively from Tmin (2.138 mm) through all measures to Tmax (4.820 mm). In the BPD group, the diaphragm was thicker at Tmin (2.342 mm) than at Tvex (2.002 mm), before progressively thickening

at Tvin (2.425 mm) and reaching a maximal thickness at Tmax (4.202 mm) (Figure 23). As per the right diaphragm, the left diaphragm showed an unexpected change of diaphragm thickness, with the increase in thickness from Tvex to Tmin in the BPD group likely to represent paradoxical diaphragm thickening.

4.3.6 Paradoxical diaphragm thickening in the BPD BMI+ group

Analysis of individual participant data in the BPD BMI+ group showed no individual cases of paradoxical thickening. This differed from the healthy BMI BPD group (refer to Section 4.2.8), where three individual cases of significant paradoxical thickening were found.

4.4 Descriptive differences of changes in diaphragm thickness between the healthy BMI and BMI+ groups.

Combined data of diaphragm thickness between healthy BMI groups and BMI+ groups were reported with descriptive statistics because of large differences in sample sizes. These results are displayed in Tables 15 and 16, and Figures 24 and 25.

For the right diaphragm, the BPD group with healthy BMI had the lowest values at Tvex, Tvin and Tmax when referenced to all other groups. For the right diaphragm both BMI+ groups had increased Tvin and Tmax thickness when compared to the heathy BMI groups. The control BMI+ group had the highest value of diaphragm thickness at Tmax which was expected. Therefore, the BMI+ groups (BPD and Control) have increased diaphragm thickness when referenced to healthy BMI groups at measures of right Tvin and right Tmax.

From Tvex to Tmin, both the control group and control BMI+ group showed thinning of the diaphragm, which was as expected. In contrast, both BPD groups (healthy BMI and BMI+) showed paradoxical thickening of the diaphragm from Tvex to Tmin, and this was an unexpected change of diaphragm thickness between these two measures.

Table 15. Mean right diaphragm thickness at Tmin, Tvex, Tvin and Tmax in all groups

	N	R Tmin (mm)	R Tvex (mm)	R Tvin (mm)	R Tmax (mm)
BPD	19	2.145	1.649	1.862	3.603
BPD BMI+	6	2.243	2.108	2.562	4.127
Control	18	1.861	2.013	2.477	4.494
Control BMI+	4	1.780	1.913	2.733	6.365

BPD = breathing pattern disorder; BMI = body mass index; Tmin = maximum exhale; Tvex = tidal exhale; Tvin = tidal inhale; Tmax = maximum inhale.

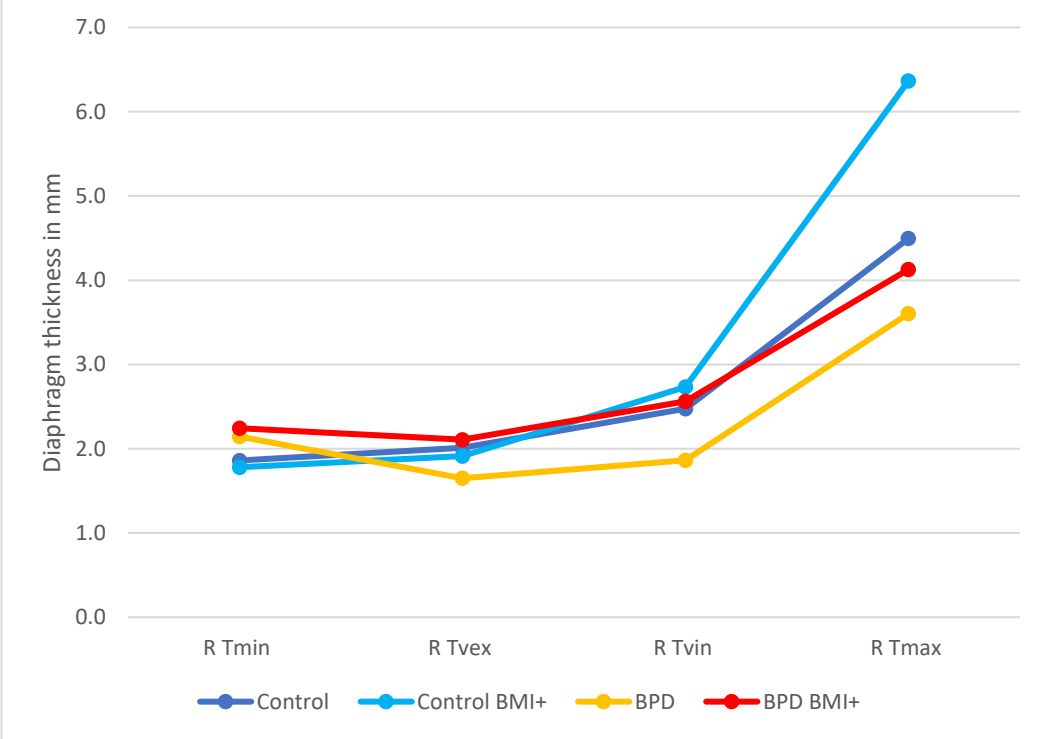

Figure 24. Line graph of right diaphragm thickness across each measure in all groups
BPD = breathing pattern disorder; BMI = body mass index; Tmin = maximum exhale; Tvex = tidal exhale; Tvin = tidal inhale; Tmax = maximum inhale.

On the left side, the BPD (healthy BMI) group had the lowest values at Tvex Tvin and Tmax, but not at Tmin when referenced with all other groups (Table 16, Figure 25). The BMI+ groups (control and BPD) had thicker diaphragm values at Tvex and Tvin when compared to the healthy BMI (control and BPD) groups. The control group, control BMI+ group and BPD BMI+ group had a similar range of thickness at Tmax; however, the BPD group had a lower value. Therefore, the BMI+ groups (BPD and control) have increased diaphragm thickness when referenced to healthy BMI groups at measures of left Tvex, and left Tvin.

Table 16. Mean left diaphragm thickness values at Tmin, Tvex, Tvin and Tmax in all groups

	N	L Tmin (mm)	L Tvex (mm)	L Tvin (mm)	L Tmax (mm)
BPD	19	1.938	1.583	1.747	3.147
BPD BMI+	6	2.342	2.002	2.425	4.202
Control	18	2.008	2.003	2.438	4.83
Control BMI+	4	2.138	2.158	2.513	4.82

Note: BPD = breathing pattern disorder; BMI = body mass index; Tmin = maximum exhale; Tvex = tidal exhale; Tvin = tidal inhale; Tmax = maximum inhale.

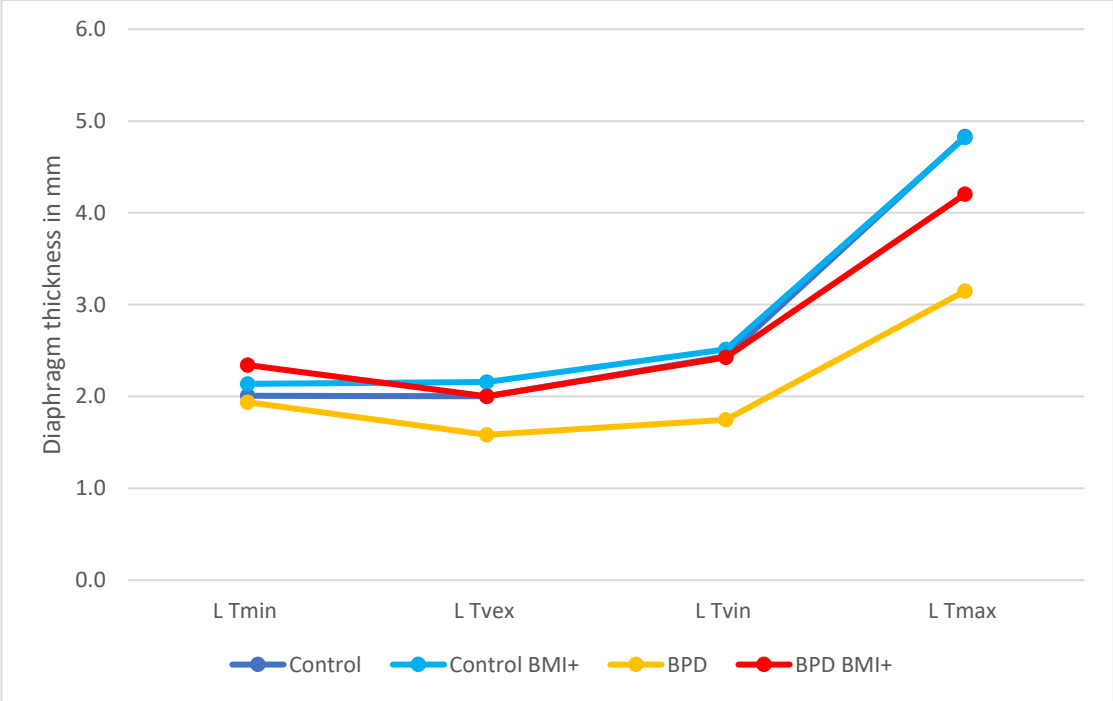

Figure 25. Line graph of diaphragm thickness on the left side across each measure in all groups
BPD = breathing pattern disorder; BMI = body mass index; Tmin = maximum exhale; Tvex = tidal exhale; Tvin = tidal inhale; Tmax = maximum inhale.

From Tvex to Tmin, both the control group and control BMI+ group showed slight or no thinning of the diaphragm, which was as expected for the left diaphragm. In contrast, both BPD groups (healthy BMI and BMI+) showed paradoxical thickening of the left diaphragm from Tvex to Tmin, and this was an unexpected change of diaphragm thickness between these two measures that is also seen on the right diaphragm .

4.5 Summary of results sections

In the healthy BMI group (n=19 BPD; n=18 control), a significant difference was seen in the measures of right diaphragm thickness at Tvex and Tvin, and left Tvex, Tvin and Tmax, as well as the left TF, with the diaphragm consistently thinner in the BPD participants compared to controls. Paradoxical diaphragm thickening was present in the BPD group, from Tvex to Tmin on both the left and right sides, which was not seen in the control group. Three individual cases of paradoxical changes in diaphragm thickness were observed in the BPD group.

In the BMI+ group (n=6 BPD; n=4 control), due to a small sample size, descriptive statistics along with mean differences and the confidence intervals were presented. In scale the difference between the right diaphragm thickness in the BPD and control group at Tmax and TF was observed, but these findings need to be interpreted in the context of a small sample size. Once again paradoxical diaphragm thickening was observed from Tvex to Tmin, in the BPD BMI+ group on both the left and right sides and this was not seen in the control BMI+ group.

Due to a mixed sample size, descriptive statistics were used to describe the impact of BMI on diaphragm thickness. The BMI+ groups (BPD and control) had increased diaphragm thickness when referenced to comparable healthy BMI groups (BPD and controls) at measures of left Tvex, left Tvin and right Tvin and right Tmax.

Chapter 5. Discussion

This is the first study to examine diaphragm thickness using USI in females with a BPD, and the first study to measure healthy diaphragm thickness in a New Zealand female population. The primary hypothesis of this study was that there would be a significant difference in diaphragm thickness at Tvex between the BPD group and control group for participants with a healthy BMI (healthy BMI group). This hypothesis was proven true at Tvex for both the left and right diaphragm with the control group having significant greater diaphragm thickness when compared to the BPD group. Significant differences were seen in diaphragm thickness at Tvin and Tmax for the left diaphragm, and Tvin for the right diaphragm with the control group having significant greater diaphragm thickness when compared to the BPD group. Furthermore, the hypothesis was proven true with the control group having a significantly larger TF compared to the BPD group.

The above significant results confirm that USI is likely to be a useful clinical tool in the diagnosis of diaphragm morphology and atrophy in BPD and may also provide useful feedback in the treatment and management of BPD. Furthermore, these results suggest that as there is an association between diaphragm atrophy and BPD, which due to these findings is now a likely part of the biomechanical pathophysiological process in BPD. Therefore, the use of USI is now likely to be a useful diagnostic aid in BPD and may also be useful to help guide the evaluation of treatments in BPD.

For the second hypothesis, in the BMI+ group, poor recruitment and a small sample size (n=10) meant that descriptive results have been reported, without the capacity for statistical means comparisons. Therefore, it was not appropriate to formally test the second hypothesis that there would be a significant difference in diaphragm thickness for participants in the BPD BMI+ group compared with the BMI+ control group. Descriptive analysis of diaphragm thickness for participants in the control BMI+ group showed increased diaphragm thickness at Tmax and increased TF on the right side, when compared to the BPD BMI+ group. The mean diaphragm thickness findings on the left side showed increased diaphragm thickness at Tmax and TF in the control group, but when the means were referenced to the distribution of the data the groups showed no differences. And interaction between reduced exercise levels, symptoms

of BPD, and increased BMI seen in the BPD BMI+ group may be responsible for these differences in Tmax and TF, but caution is needed when interpreting these results due to the small sample size and lack of parametric testing.

For the third hypothesis, in the heathy BMI and BMI + groups, combined data were not parametrically tested due to the large differences in the sample sizes. Therefore, again it was not appropriate to formally test the third hypothesis that there would be a significant difference between the BMI and BMI+ groups. Descriptive analysis showed that the BMI+ groups (BPD and control) had increased diaphragm thickness when referenced to healthy BMI groups at measures of Tvin and Tmax on the right side, and Tvex and Tvin on the left side. These results need to be interpreted with caution due to the differences in sample size and lack of parametric testing. However these results that suggest increased BMI is associated with increased diaphragm thickness, are similar to observations reported in other studies (Boon et al., 2013; Enright et al., 2007; McCool, Benditt, et al., 1997).

Unexpectedly, for the right and left diaphragm, both BPD groups (healthy BMI and BMI+) showed consistent paradoxical thickening of the diaphragm from Tvex to Tmin. Paradoxical thickening of the diaphragm may represent a specific dysfunctional breathing pattern that is used during maximal exhalation to Tmin in BPD populations. This chapter interprets and discusses all the above findings in relation to previous research in healthy and BPD populations.

5.1 Diaphragm thickness in BPD

The primary hypothesis of this study was that there would be a significant difference in diaphragm thickness between the BPD group and control group for participants a BMI between 18.5–24.9 kg/m^2 (healthy BMI group). This was proven true at Tvex, Tvin, Tmax, and TF for the left diaphragm, and at Tvex and Tvin for the right diaphragm, where the diaphragm was thinner in participants with BPD compared to controls. These findings were consistent with previous literature that suggested reduced diaphragm function or diaphragm atrophy is a component of BPD (D. Bradley, 1998; Chaitow, 2004; Chaitow et al., 2002; Clifton-Smith, 2014; Clifton-Smith & Rowley, 2011; Gilbert, 2014; Hruska, 1997). The mechanisms for diaphragm atrophy in BPD are likely due to BPD creating increased hypertonicity in the intercostal and chest wall

muscles, along with hyperinflation, leading to mechanical unloading of the diaphragm, and atrophy of the diaphragm (Clifton-Smith & Rowley, 2011; Hruska, 1997). A vicious cycle of weakening of the diaphragm may create further reliance on the accessory muscles to create the forces requires for breathing (Clifton-Smith & Rowley, 2011; Gilbert, 2014; Hruska, 1997).

The significant difference in levels of exercise per week between the BPD group (3.47 hours/week) and control groups (6.75 hours/week) seen in the current study may provide an insight into the differences seen in diaphragm function and thickness between the groups. For example, increased exercises levels have been correlated to diaphragm hypertrophy (P. Brown et al., 2013; Scarlata et al., 2019), which allows for the increased respiratory strength during exercise (Scarlata et al., 2019). Conversely in people with BPD, hypocapnia and increased dyspnoea both at rest and during exercise has been described (Brat et al., 2019), thus making exercise more unpleasant, which is likely to lead to reducing activity levels - as was seen in the present study in the BPD group. This reduced exercise level in BPD is likely to contribute to a vicious cycle of increased dyspnoea with exercise, reduced exercise, reduced diaphragm loading, and finally diaphragm atrophy.

5.2 Diaphragm thickness in clinical populations

As this is the first study to examine diaphragm thickness, using USI, in females with a BPD, this precludes a direct comparison of this specific measure in this cohort to other studies. However, comparisons can be made with pathological conditions that have shown diaphragm dysfunction.

The results of the BPD and control groups (healthy BMI) are comparable to a previous study involving a low back pain population (Calvo-Lobo et al., 2019). The study population were athletes with low back pain (n=20) and healthy controls (n=20), and the sample size, primary outcome measure (USI assessment of diaphragm thickness) and aims (to measure diaphragm atrophy using USI) were similar to the present study. The study conducted by Calvo-lobo et al. (2019) used a observational methodological design, and had adequate blinding procedures, and a similar USI measurement technique, and found that diaphragm thickness was significantly reduced in the low back pain group, compared to controls, for the right diaphragm at Tvex (1.6±0.7 mm

vs. 2.3±0.6 mm [controls]; P=0.006) and left diaphragm at Tvex (1.3±1.2 mm vs. 2.0±1.0 [controls]; P=0.015) (Calvo-Lobo et al., 2019). These results are similar in scale to those in the present study, with significant bilateral diaphragm thinning at Tvex in BPD group. Therefore, right and left diaphragm atrophy at Tvex which is seen in the present study, is similar to the diaphragm atrophy at left and right Tvex seen in athletes with low back pain (Calvo-Lobo et al., 2019).

In stroke populations, unilateral diaphragm dysfunction has been described on the affected side (Jung & Kim, 2017; Kim et al., 2017). Kim et al. (2017) studied diaphragm thickness in a stroke population (n=45 stroke, healthy n=49), and aimed to show differences at Tvex and Tmax. This study utilised a large sample size, but had similar exclusion criteria regards to health and stroke population, to the present study. Observer blinding procedures were also similar to the present study. Measures of diaphragm thickness reported by Kim et al. (2017) showed a similar association to that seen in the present study, with pathological diaphragm atrophy evident for the diaphragm at Tmax (diaphragm thickness affected side Tmax 4.0±1.3 mm; non-affected side 5.4±1.7 mm; P<0.001; present study, BPD group 3.147±1.418 mm left Tmax; control group 4.83±1.523 mm left Tmax; P=0.001). However, the results for diaphragm thickness at Tvex in the Kim et al. (2017) study were not comparable to the present study (2.1±0.2 mm affected side; 2.2±0.2 mm non-affected side compared to the present study BPD group (left Tvex 1.583±0.341 mm). This suggests that stroke populations have a unilateral atrophy at Tmax on the affected side, that is similar to scale of the Tmax atrophy BPD (healthy BMI) population in the present study, but BPD populations also have atrophy at Tvex, which is not seen in all stroke populations. These differences between stroke and BPD are likely due to the different mechanisms affecting the diaphragm, where nerve innervation is reduced in stroke, but in BPD innervation is intact, but generally diaphragm atrophy, is comparable between stroke and BPD.

Further comparison can be made with the study by Jung and Kim (2017),which aimed to measure the correlation between diaphragm thickness, excursion, and lung function in a stroke population (n=114, females=55). Jung and Kim (2017) reported a diaphragm thickness at Tmax on the affected side diaphragm of 3.6±0.7 mm, which is comparable

with the BPD group in the present study (right Tmax 3.603±1.344 mm; left Tmax 3.147±1.418 mm). Diaphragm thickness at Tvex was 1.9±0.2 mm on the affected side and 1.8±0.2 mm on the non-affected side, is not comparable in scale to the present findings in the BPD group (left Tvex 1.583±0.341 mm; right Tvex 1.650±0.369 mm). In stroke, the diaphragm tends to atrophy due to impaired muscle innervation, and therefore it is used less, and further atrophy occurs (Kim et al., 2017). Therefore, while impaired nerve innervation is the cause of diaphragm atrophy in stroke, in BPD diaphragm atrophy is likely due to a mechanism of increased apical breathing, reduced diaphragm loading, reduced function and diaphragm atrophy (Clifton-Smith & Rowley, 2011; Hruska, 1997). However, again the theme that changes in the function and use of the diaphragm lead to atrophy is comparable between stroke and BPD.

The results of the present study can also be compared with studies that examined diaphragm thickness in phrenic neuropathy. One study by Caleffi-Pereira et al. (2018) aimed to examine the relationship between diaphragm thickness, gastric pressures and MIP in phrenic neuropathy (n= 27, females=12). This study had adequate measurement blinding, but included a sample of 29% ex-smokers, which may have created a heterogenous sample due to the association between smoking and COPD. Once again, as was seen in the present study, diaphragm atrophy was significant ($P<0.001$) in the pathological side (diaphragm thickness 1.1 mm at Tvex; 1.2 mm at Tmax; TF 4.4%) compared to the unaffected side (diaphragm thickness 1.7 mm at Tvex; 5.1 mm at Tmax; TF 196.1%) (SDs not reported). One study by Boon et al. (2014) aimed to evaluate the role of USI of the diaphragm in the diagnosis of phrenic neuropathy (n=66; aged 62.2±12 years). They found that while 67 affected diaphragms had had a TR lower than <1.2, only 28 affected diaphragms had a diaphragm thickness at Tvex below 1.5 mm. Therefore, studies of phrenic neuropathy show again that poor function through reduced innervation of the diaphragm muscle creates diaphragm atrophy, and this is similar to diaphragm atrophy in BPD that was seen in the present study.

Therefore, reduced use of the diaphragm muscle as seen in stroke, low back pain, phrenic neuropathy or BPD, leads to diaphragm atrophy reflecting its poor function. Furthermore, research is required to replicate the present study findings and to

understand the differences between in the mechanisms responsible for changes seen in diaphragm thickness of BPD, stroke, low back pain and phrenic neuropathy. A comparison of nerve function and MIP (which represent strength changes) to diaphragm thickness may be of use to understand if nerve innervation changes, or the proposed alterations in mechanical loading and strength are indeed the different pathological mechanisms behind diaphragm atrophy in BPD.

In contrast to those studies discussed above, diaphragm thickness research findings for other respiratory populations do not show consistent evidence of diaphragm atrophy, for example people with COPD and asthma (Baria et al., 2014; de Bruin et al., 1997), however, TR/TF is reduced in a small subgroup of people with severe COPD, when severe hyperinflation or severe lung disease is present (Antenora et al., 2017; Baria et al., 2014; Hafez & Abo-Elkheir, 2017). Diaphragm thickness and TF were assessed in people with COPD in a study by Hafez and Abo-Elkheir (2017), (n=113 COPD males, n=114 control males). This study utilised an adequate sample size, showed good intra-observer agreement with repeated measures, but did not utilise observer blinding. This study compared a subgroup of participants with severe COPD (as defined by GOLD classification) and evidence of diaphragm atrophy (that was arbitrary defined as diaphragm thickness at Tvex <1.8mm) (n=13) to people with mild and moderate COPD and normal diaphragm thickness (n=100). Diaphragm thickness in the severe COPD group (right diaphragm thickness at Tvex 1.8±0.03 mm, at Tmax 2.5±0.2mm, TF 23.9±2.3) was significantly lower (P=0.001) than the mild moderate COPD group (right diaphragm thickness Tvex 3.1±0.8mm, Tmax 4.0±0.8mm, TF 32.8±3.7). A pilot study by Antenora et al. (2017) (n=41) corroborated the results of Hafez and Abo-Elkheir (2017), and showed that significant diaphragm dysfunction was only present in 10 out of 41 patients who present to hospital with an acute exacerbation of COPD. Diaphragm dysfunction was again arbitrarily defined as a TF lower than 20%, and reduced TF was significantly associated with failure of non-invasive ventilation during a COPD exacerbation. The reason for failure of non-invasive ventilation is likely to be due to the difficulty in achieving adequate alveolar ventilation when the diaphragm is atrophied, and no longer producing adequate contractile force. This pilot study had a heterogenous population with many comorbidities included in the study sample, and no observer blinding.

A possible explanation for the different trends in diaphragm thickness and TF in COPD when compared to the current study, may concern the processes of lung disease and hyperinflation in COPD contributing to muscular shortening and thickening of the diaphragm fibres (De Troyer, 1997; De Troyer & Boriek, 2011). The pathological lung changes in COPD create hyperinflation that lead to chronic shortening and adaptation of the diaphragm, meaning its thickness and function is relatively preserved (De Troyer, 1997). However, if hyperinflation becomes severe the diaphragm cannot shorten any further and the function of the diaphragm is affected as seen in some studies (Antenora et al., 2017; Baria et al., 2014; Hafez & Abo-Elkheir, 2017). This process is unique to lung diseases such as COPD, as there no changes to the lung tissue in BPD, and this confounds the comparison of diaphragm thickness and TF in COPD populations to BPD populations.

In summary, reduced use of the diaphragm muscle as seen in stroke, low back pain, phrenic neuropathy, or BPD leads to diaphragm atrophy reflecting its poor function. The process of diaphragm thickness changes seen in severe COPD does not compare with the changes seen in BPD, due to the pathological changes seen within the lung in COPD. Further research is required to replicate the present study findings, and comparison of nerve function and MIP to diaphragm thickness may be of use to understand different pathological mechanisms behind diaphragm atrophy in BPD.

5.3 Diaphragm thickness in healthy populations

The findings for diaphragm thickness from the present study are not only interesting to compare to other clinical groups, but also to other data presented for healthy populations. In the present study, diaphragm thickness values at Tvex for the control group (healthy BMI) differed from the Tvex values described in other studies for healthy females (refer to Section 2.6.2 and Table 1) (Boon et al., 2013; Cardenas et al., 2018; Carrillo-Esper et al., 2016; Scarlata et al., 2019; Seok et al., 2017).

Diaphragm thickness at Tvex (right 2.013±0.617 mm; left 2.003±0.584 mm) in the present study was smaller than those reported by Boon et al. (2013) (right 2.7±1.0 mm; left 3.11 ±1.9 mm. mm). Boon et al. (2013) included a heterogenous population of non-smokers and smokers and had a population with a larger female BMI (26.6±6.8

kg/m^2), than the control group (healthy BMI) in present study (22.21±1.76 kg/m^2). The control BMI+ group had a similar BMI to those in study of Boon et al. (2013) and this is discussed in detail below in Section 5.4. As described in Section 2.6.2, limitations of the study by Boon et al. (2013) is that smoking is associated with lung damage or COPD, and possible lung hyperinflation, which creates shortening and adaptation in diaphragm muscle fibres (Antenora et al., 2017; De Troyer, 1997). A further limitation is that increased BMI and obesity has been correlated with increased thickness of the diaphragm (McCool, Benditt, et al., 1997; McCool & Tzelepis, 2012), thus reducing the comparability of these results to the control group in the present study.

In contrast, measures of diaphragm thickness at Tvex in the present control group (healthy BMI) were larger than the values described in many studies involving healthy female populations [see Table 1; Cardenas et al. (2018) right Tvex 1.79±0.3 mm; Carrillo-Esper et al. (2016) right Tvex 1.4±0.03 mm; Scarlata et al. (2019) right Tvex 1.67±0.4 mm; and Seok et al. (2017) right Tvex 1.7±0.3 mm]. The three lowest reported values for female normative diaphragm thickness at Tvex (Carrillo-Esper et al., 2016; Scarlata et al., 2019; Seok et al., 2017), had similar BMI values (22.9±3.18 kg/m^2 ; 22.3±2.4 kg/m^2 ; 24.2±3.5 kg/m^2) to the healthy BMI group in the present study (22.21±1.76 kg/m^2). Therefore, it is unlikely that BMI was a factor involved in these differences, and other possible reasons for the differences at Tvex between the present study and other published normative diaphragm thickness data may include: the use of a smaller sample size in the present study, differences in cultural morphology (Seok et al., 2017), differences between B- and M-mode ultrasound measurement [M-mode used in Scarlata et al. (2019)], and the increased exercise levels in the sample populations (refer to Section 5.1) (P. Brown et al., 2013; Scarlata et al., 2019). It may be that the control group in the present study from New Zealand represents unique ethnic morphological difference in diaphragm thickness when compared to other international samples as proposed by other authors (Carrillo-Esper et al., 2016).

At Tmax (or total lung capacity), the present study found diaphragm thickness values for the right diaphragm to be 4.494±0.8 mm, which was comparable with results from Cardenas et al. (2018) (right diaphragm thickness at Tmax 4.81±0.9 mm) and Seok et

al. (2017) (right diaphragm thickness at Tmax 3.2±0.8 mm), but was larger than the values reported by Scarlata et al. (2019) (right diaphragm thickness at Tmax 2.39±0.5 mm) which had BMI values that are comparable with our study population. However, the study by Scarlata et al. (2019) used a methodology of M-mode USI, but B-mode USI is a more accurate measure of diaphragm thickness, as the diaphragm fibre angle may change through the breathing cycle (Boon et al., 2013), thus leading to measurement differences as the fibres bend away from the reference line used to measure M-mode. Furthermore, the majority of studies use B-mode for diaphragm thickness USI, which is consistent with a recent review that recommended the use of B-mode for diaphragm thickness measurement (Laveneziana et al., 2019).

5.4 Diaphragm thickness in people with high BMI

Within the BMI+ group, poor recruitment and a small sample size (n=10) meant that statistical testing was not feasible. Therefore, no conclusions can be drawn from the BMI+ groups. Furthermore, comparison to other studies is difficult with only one study Boon et al. (2013) having a comparable BMI range (26.6±6.8 kg/m^2 females). The diaphragm Tvex values in the present study (right BPD BMI+ group 2.108 mm, control BMI+ group 1.912 mm) and the left side Tvex (BPD BMI+ group 2.002 mm; control BMI+ group 2.158 mm), are smaller than those reported by Boon et al. (2013)(3.1±1.9 mm (in females, left diaphragm), and the study by Boon et al. (2013) had limitations as described above in Section 5.3. As the baseline BMI data from the present study is not similar between groups this may also account for some of the diaphragm thickness differences seen between the BPD group and control group (BPD BMI+ group 30.2 ±3 kg/m^2 ; control BMI+ group 26.45 ±2 kg/m^2).

A right-side difference was observed in diaphragm thickness between the BPD BMI+ group and the control BMI+ group at Tmax and TF, with the control group diaphragm having greater increased diaphragm thickness and TF. The mean diaphragm thickness findings on the left side showed increased diaphragm thickness at Tmax and TF in the control group, but when the means were referenced to the standard deviations, the groups showed no differences. The pattern of more differences in diaphragm

thickness seen on the right diaphragm in the BMI+ group is the opposite to that seen in the healthy BMI groups which has more significant differences observed on the left side (Tvex, Tvin, Tmax and TF) than the right side (Tvex and Tvin). Animal modelling suggests that diaphragm fibres and diaphragm function changes due to increased BMI and increased central adiposity (Buras et al., 2019), and furthermore, the increased intra-abdominal pressure occurs in obesity (De Keulenaer, De Waele, Powell, & Malbrain, 2009). Therefore, increased BMI may lead to changes in diaphragm muscle fibres and function, and fat deposition and changes in intra-abdominal pressure may be the reasons for the left and right side differences between the BPD (healthy BMI) and BPD BMI+ groups. Therefore, further BPD diaphragm thickness research in a heathy BMI and BMI+ population may benefit from concurrent measurement of intra-abdominal pressure, and body fat measurement, as this may uncover the mechanisms of diaphragm thickness differences between these groups.

As per the healthy BMI groups a difference is seen between exercise levels in the BPD BMI+ and control BMI+ therefore this may be an important factor in the differences observed between the groups as has been shown by other studies (P. Brown et al., 2013; Scarlata et al., 2019) (Sections 2.6.2 and 5.1). It is also worth noting that increased BMI tends to increase symptoms of shortness of breath and respiratory discomfort making exercise less comfortable (Zammit, Liddicoat, Moonsie, & Makker, 2010). Therefore, increased BMI will likely worsen the vicious cycle of dyspnoea and BPD symptoms, accessory muscle use, reduced exercise, unloading of the diaphragm, and atrophy contributing to further reduced exercise levels in the BPD group.

5.5 The influence of BMI on diaphragm thickness

As described earlier (refer to 4.4), a small sample size within the BMI+ groups made comparison difficult to the larger sample in the healthy BMI groups. However descriptively, the expected trend of the BMI+ groups (BPD and Control) having increased diaphragm thickness was observed, when referenced to healthy BMI groups at measures of right Tvin, right Tmax, left Tvex, and left Tvin. Furthermore, an unexpected trend of right and left diaphragm paradoxical thickening was seen

consistently in the BPD group and BPD BMI+ group between the Tmin and Tvex measurements, and these findings are discussed in the following section (5.7).

It appears that the effect of increased BMI reduces or alters some of the differences observed in the healthy BMI groups in the measures of diaphragm thickness at Tvex and one potential reason may be fatty infiltration of the diaphragm in the BMI+ populations. As described in Section 5.4, in one mouse based modelling study, that had the aim of observing the effect increased fat diet on diaphragm function and fibre morphology, a high fat diet led to fatty infiltration and fibrosis of the diaphragm, and impaired diaphragm muscle function was observable with USI (Buras et al., 2019). Therefore, in the BPD BMI+ group the diaphragm may indeed be comparable in thickness to control BMI+ group at right and left Tvex, but may have increased adiposity and fibrosis that in future may be quantified using ultrasound. Therefore, future diaphragm thickness research should attempt to document and comment on the amount of fatty infiltration in the diaphragm in a manner that is consistent with other skeletal muscles (Somerson, Hsu, Gorbaty, & Gee, 2016).

5.6 Paradoxical diaphragm thickening in BPD

An unexpected finding from this study was the consistent paradoxical thickening of the left and right diaphragm from Tvex to Tmin in the BPD groups (both healthy BMI and BMI+ groups). This contrasts to the control groups (both healthy BMI and BMI+) where normal either thinning of the diaphragm or no change was observed in diaphragm thickness from Tvex to Tmin, which was the expected result. For clarity, the normal function of the diaphragm is to start at a resting thickness of Tvex, and to fully lengthen and thin during forced expiration to residual volume and this is the measurement Tmin; and conversely during inspiration the diaphragm maximally shortens and thickens to total lung capacity (TLC) and this is the measure Tmax (Ueki et al., 1995). There are three possible mechanisms that may explain the paradoxical thickening of the diaphragm from Tvex to Tmin: paradoxical breathing, hyperinflation and a Valsalva-type manoeuvre.

Paradoxical breathing (refer to Sections 2.1 and 2.4), has been described as complete reversal of the normal pattern of breathing, meaning the diaphragm contracts on exhalation instead of inhalation (Chapman et al., 2016; Perri & Halford, 2004), however, other authors report a delay in the diaphragm and chest contractions during inhalation (Fregonezi et al., 2018). A study by Calvo-Lobo et al. (2019) also described paradoxical breathing in athletes with low back pain (n=20) compared with controls (n=20), and this study used a similar sample size, blinding and USI measurement protocol to the present study. Paradoxical breathing was described as when the diaphragm thinned (instead of normal diaphragm thickening) from Tvex to Tmax. There were more cases of paradoxical breathing described in the low back pain group (left diaphragm: 7 participants low back pain paradoxical motion vs. 4 in the control group; right diaphragm: 3 participants low back pain paradoxical motion vs. 0 in the control group), but this was not statistically significant. A further study by Fregonezi et al. (2018) used optoelectrical plethysmography (which is used to measure lung volumes by motion capture software), to measure paradoxical breathing, which was defined as delayed timing of rib cage and diaphragm contraction, in a population with mild asthma. This study utilised a small sample size (n=21) and had no blinding procedures, but the results of this study indicated that mild asthmatics (as defined by spirometry) had greater dynamic hyperinflation ($P=0.002$) and greater paradoxical breathing or asynchrony than controls ($P<0.005$) during exercise. Therefore, paradoxical breathing has now been described in asthma, LBP and BPD indicating that paradoxical breathing may be a common feature of these pathologies.

The effect of hyperinflation on the diaphragm has been described in detail by De Troyer (1997), but has not been studied in BPD. Hyperinflation is described as abnormal increase in functional residual capacity or an increased lung volume at the end of tidal exhale (De Troyer & Boriek, 2011), and during hyperinflation all the inspiratory muscles must operate at shorter than normal lengths, which negatively impacts on the role of the diaphragm during inspiration. Therefore, the act of breathing is more dependent on accessory inspiratory muscles (De Troyer, 1997; De Troyer & Boriek, 2011). A hypothesis put forward by some authors, was that BPD are associated with hyperinflation (Clifton-Smith, 2014; Hruska, 1997), where the diaphragm is no longer able to produce normal inspiratory function and remains

hypertonic instead of completely relaxing on exhalation, and the ribs are elevated due to excessive chest and accessory muscle use. De Troyer (1997) described a process of change that occurs at extreme hyperinflation, where diaphragm contraction produces expiration, and rib descent with reduction of the thorax diameter. Therefore, in BPD it may be possible that during exhale to Tmin, diaphragm contraction may occur to allow the ribs to descend and to facilitate exhalation. Therefore, future research using motion capture software or chest circumference measurement with concurrent diaphragm thickness USI measurement may be used to assess the descent of the ribs during exhale and paradoxical diaphragm thickening in BPD.

Another possible explanation for the paradoxical diaphragm thickening from Tvex to Tmin seen in the present study, could be that participants in the BPD group may be using a Valsalva-type strategy or a dysfunctional strategy of intra-abdominal pressure during exhalation to Tmin. Diaphragm dysfunction has been implicated with abdominal muscle dysfunction (see Section 2.3.4) (De Troyer & Boriek, 2011; Hruska, 1997), and both weakness of the muscles of the abdominal wall and hypertonicity of these muscles has been described in BPD (Clifton-Smith & Rowley, 2011; Courtney, 2017; Whittaker, 2008). Previous experimental studies have demonstrated the diaphragm postural role may reduce in situations of increased respiratory drive, and the diaphragm may also reduce its normal postural contributions in conditions such as chronic low back pain (Hodges et al., 2001; Kolář et al., 2012). Therefore, it may be hypothesised that the BPD group in the present study had altered abdominal and diaphragm motor patterns of forced exhalation—which is normally described as a process of abdominal muscle contraction, and diaphragm relaxation (De Troyer & Boriek, 2011). However, there is no research in specific BPD populations to support or refute this hypothesis, and therefore further research is using diaphragm thickness USI and intra-abdominal pressure measurement is required to elucidate the mechanism creating paradoxical diaphragm dysfunction in the BPD groups.

5.7 Terminology of diaphragm thickness ultrasound measurements

The current terminology used for respiratory measurement points, where diaphragm thickness can be assessed, is not standardised and varies widely within the literature

(Boon et al., 2013; Harper et al., 2013; Laveneziana et al., 2019; Sferrazza Papa et al., 2016). For example, within the same research group (Boon et al., 2013; Hellyer et al., 2017), different terminology for the same lung volumes (i.e. end expiratory lung volume (EELV) and Tvex; TLC and Tmax) is used. Guidelines do not currently exist for standardised terminology for the measurement of diaphragm thickness (apart from TF and TR), with poor descriptions for many other data measurement points (Tvex, EELV, Tmin, inspiratory thickness, expiratory thickness). Furthermore, published literature reviews on diaphragm thickness (Sferrazza Papa et al., 2016) and recent European Respiratory Society guidelines (Laveneziana et al., 2019) provide no clear guidance on terminology, which contributes to confusion when reviewing the literature and designing studies measuring diaphragm thickness with USI.

The results from this study showed that in cases of BPD, the diaphragm may be significantly thicker at Tmin (residual volume) than at Tvex (tidal exhale). Furthermore, in three individual BPD cases, diaphragm thickness at Tmin was larger than at Tmax due to paradoxical diaphragm motion (two cases of right paradoxical thickening; one case of left paradoxical thickening) (refer to 4.2.8). In cases of paradoxical diaphragm activity terminology such as Tmin or Tmax may be misleading, and the current terminology needs to reflect known standardised respiratory parameters. Therefore, a new terminology system is proposed, to better reflect the points of measurement where the term Tmin should be replaced with Trv (thickness at residual volume), Tvex and Tvin should be retained and Tmax should be replaced with Ttlc (thickness at total lung capacity).

5.8 Limitations of this study

This study has limitations and the results should therefore be interpreted with some caution. Due to the nature of observational studies and confounding variables, although associations may be observed from the results, causation cannot be inferred in an observational study (Lederer et al., 2018). Furthermore, although pooling of data and regression analysis may seem appropriate between the BMI groups, regression analysis is not a currently favoured statistical technique in respiratory observational studies, and therefore this technique was not utilised in the present study (Lederer et

al., 2018). Therefore, it can be concluded that diaphragm atrophy was associated with BPD, but it cannot be stated that BPD causes diaphragm atrophy. Future prospective research using a randomised control trial design is needed to confirm if BPD is causally linked to diaphragm atrophy, and whether specific physiotherapy interventions can reverse diaphragm atrophy.

The present study involved a population that only included females, and although recruitment was sufficient in the healthy BMI range, the generalisability of the results is limited in >25 kg/m^2 BMI ranges, and male populations with BPD. Further BPD research should aim to control for gender, BMI, and exercise levels, as these variables are known to impact diaphragm thickness.

Another limitation of this study was that no lung function tests were conducted at the beginning of the study. Therefore, there may have been some participants who had altered lung function or a pathological process (e.g. mild undiagnosed asthma) that were included in the sample. As lung function tests are not part of standard physiotherapy assessment, they were not included in this study. Efforts were made to reduce the possibility of this occurring by excluding all participants who smoked or had a history of respiratory disease. A similar rationale can be applied to the lack of technical CO_2 measurements using tools such as end-tidal CO_2 or arterial blood gases, both of which are not part of standard physiotherapy assessment. However, efforts were made to reduce the possibility including patients with abnormal CO_2 levels in the control group by excluding all participants with BHT scores outside of the cut-off points (refer to Section 3.5).

Recruitment

During participant recruitment, two distinctive challenges arose: recruitment of participants in the BMI+ groups (both control and BPD participants), and the exclusion of multiple participants because of the strict inclusion and exclusion criteria (n=37, see Section 4.1, Table 2 and 3). Recruitment began on 5 April 2018 and was completed for the healthy BMI group in a straightforward manner (goal sample size n=34; final sample n=37). However, recruitment was challenging in the BMI+ groups, with only 10 of 42 targeted participants successfully recruited (goal sample size n=21 BPD BMI+

group, n=21 control BMI+). This low number was despite considerable effort and multiple strategies to recruit participants for the BMI+ group as outlined in Appendix 9.

Barriers that may have contributed to poor recruitment included factors such as: body image (particularly in the BMI+ group); the requirement for participants to roll up their shirt to reveal their abdomen for the ultrasound scan; male/female interaction (the primary researcher was a male and participants were females); the networks and location of the marketing may not have included high BMI populations; and people with a high BMI might have had secondary health issues that excluded them from participating in the study (i.e. high BMI is associated with diabetes)(Bays, Chapman, Grandy, & Group, 2007).

Recruitment was discussed multiple times at supervision meetings, with advertising strategies and techniques modified to facilitate recruitment for the BMI+ groups (for example, adverts were placed in gyms, university classes, and with female rugby teams). After 15 months of proactive recruitment efforts and in conjunction with thesis timeframes, it was decided to finalise the recruitment phase of the study. As such, this limits much of the interpretation of the data from the BMI+ groups.

The exclusion of participants (n=37) based on the strict inclusion and exclusion criteria was necessary for the design of this study, and was consistent with the current recommendations in the BPD literature (Chaitow et al., 2014; Courtney, Biland, Ryan, Grace, & Gordge, 2019). The most common reasons for exclusion at the physical exam were an apical dominant breathing pattern, during the Hi-Lo test (rather than an abdominal pattern) and poor BHT. The significant number of exclusions from the control group suggests that a broad spectrum of breathing health and dysfunction exists. This spectrum extends from BPD (symptoms and measures of dysfunction) to a ‘normal’ population (measures of dysfunction, but no symptoms), to the control group (no symptoms and no measures of dysfunction). Therefore, the control group in this study may represent ‘optimal’ breathing or people who have physiologically and biomechanically optimal patterns of breathing and are without symptoms (Figure 26).

A prospective observational study by Kiesel et al. (2017) showed a similar pattern as described above, with only five of 51 participants, showing normal breathing, while

13/51 had one measure of breathing dysfunction, 21/51 had two measures of breathing dysfunction, and 12/51 had a BPD or three measures of dysfunction) (Kiesel et al., 2017).

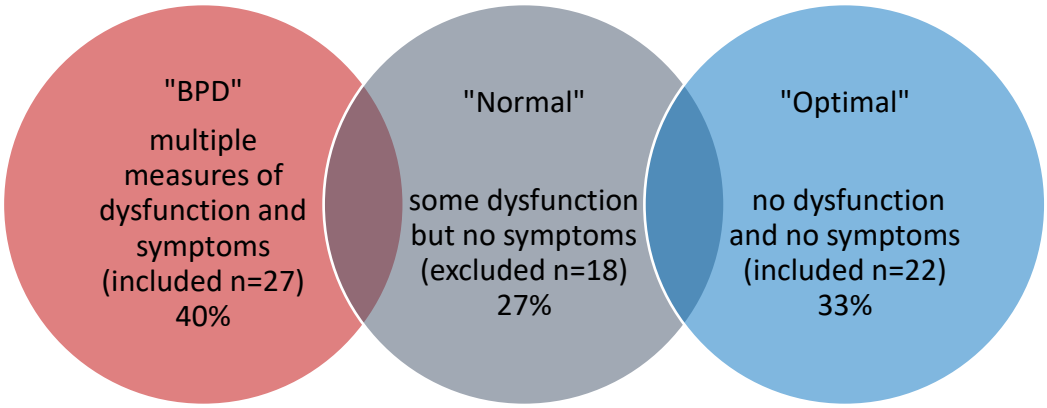

Figure 26. Hypothesised spectrum of dysfunction in the present study with the number of participants in each hypothesised group.

The exclusion of these 'normal' participants could be addressed in future studies by allowing for a third group of participants who have 1–2 markers of breathing dysfunction, such as apical breathing but normal RR and BHT, and are asymptomatic on the NQ. Research that includes this 'normal group' would be valuable to understand diaphragm morphology in more detail for a population of people that would appear to be well represented. Future scoping work in the area of definitions of BPD should include a new definition of the spectrum of breathing dysfunction, as the current terms of structural (i.e. as a defined pathophysiological process) and functional (i.e. no defined pathophysiological process) do not adequately describe variances in breathing that are apparent in the general population.

5.9 Future research

Future research should initially focus on the replication of the methodology used in this study in a male and female BPD population. Recruitment of a >25kg/m^2 BMI BPD sample would also be of value to provide further insights to the findings of the small sample of the present study. Replication of this BPD study would be of benefit in other

geographical locations to assess if these results are stable across cultural groups, due to reported variances in diaphragm thickness in different populations (Carrillo-Esper et al., 2016).

Further relationships of diaphragm thickness to other diaphragm measures, such as excursion of the diaphragm (Boussuges et al., 2009; Le Neindre et al., 2016), should also be explored in BPD, as alterations in excursion of the diaphragm, and identification of specific patterns of diaphragm dysfunction on USI, show promise as a further assessment technique in BPD.

As diaphragm atrophy is common amongst BPD, stroke, phrenic neuropathy and low back pain, comparison of nerve function and MIP (which represent strength changes) to diaphragm thickness may be of use to understand if nerve innervation changes or mechanical strength differences (to understand general respiratory strength) may be useful to understand the specific pathological mechanisms behind diaphragm atrophy in BPD.

To understand the relationship between adiposity, BMI and BPD diaphragm thickness research should attempt to describe the amount of fatty infiltration in the diaphragm in a manner that is consistent with other skeletal muscles, and other measurements of body fat analysis may be of use to uncover the association between adiposity, BMI and BPD.

Further research is required to elucidate which mechanism may be creating the observed thickening of the diaphragm during forced exhalation from Tvex to Tmin in the BPD group; this research may include other technical measures such as intra-abdominal pressures (which may indicate Valsalva-like manoeuvres), and chest circumference measurements (which may indicate hyperinflation changes) as part of the mechanism behind paradoxical thickening .

USI assessment of diaphragm thickness may also be studied in randomised controlled trials of BPD to determine if breathing retraining exercises or other interventions can produce a change in diaphragm thickness, and reduce atrophy, when compared to a with a control population. Furthermore, diaphragm thickness may assist in selecting

the most effective treatment techniques by allowing the comparison of changes in diaphragm atrophy before and after treatment.

Research into diaphragm thickness measures is warranted in other populations where diaphragm atrophy may be implicated such as GORD, chronic neck pain, chronic low back pain, anxiety and panic disorder. Diagnosis of diaphragm atrophy in these populations may lead to breathing based approaches to the treatment of these populations.

Chapter 6. Conclusion

This is the first research to examine diaphragm thickness and diaphragm thickening fraction using ultrasound imaging (USI) in females with a BPD and healthy female controls, and this study is the first study to establish that diaphragm atrophy is present in BPD.

A female BPD group (n=19) and female control group (n=18) with normal body mass index (BMI) were recruited in an outpatient setting. A second cohort of females (BPD group n=6, female control group n= 4) was recruited with an elevated BMI (BMI+). Significant differences were observed in the primary measure of diaphragm thickness between the BPD and control groups at Tvex on the left side and on the right side. Statistical significance was also observed at Tvin, Tmax and TF on the left side, and at Tvin on the right side. Parametric statistical testing was unable to be performed due to inadequate sample size in the BMI+ groups. A trend of paradoxical diaphragm thickening was observed in both the BPD and BPD BMI+ groups from the Tvex to Tmin measurements.

The findings of this research confirm that USI is likely to be a useful clinical tool in the diagnosis of diaphragm atrophy in BPD, and with further research may provide useful feedback in the treatment and management of BPD. Furthermore, these results are the first research show an association between diaphragm atrophy and BPD and thus link diaphragm atrophy to the biomechanical pathophysiological process in BPD. This is the first diaphragm thickness research of normative population of females in New Zealand, and furthermore new terminology was proposed for the measurement of diaphragm thickness.

Future research should initially focus on the replication of this study in a male BPD population, and recruitment of a >25kg/m^2 BMI BPD sample in would be of value to expand the results of this sample in the present study. Research is also warranted into in other populations where diaphragm atrophy is implicated. This research may reduce the burden of BPD as patients may be diagnosed more effectively, and this may reduce health system costs in hospital settings, and may also reduce treatment costs for patients with BPD.

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Appendices

Appendix 1: Nijmegen Questionnaire

Please fill out the following questionnaire. Do you experience any of the following symptoms and if so what is the frequency of each symptom.

Scoring: Never = 0, Rare = 1, Sometimes = 2, Often = 3, Very Often = 4.

Nijmegen Questionnaire (NQ) score ≥ 23 indicates the presence of a Breathing Pattern Disorder.

Total score = /64

Item	**Never**	**Rarely**	**Sometimes**	**Often**	**Very Often**
Chest pain					
Feeling tense					
Blurred vision					
Dizzy spells					
Feeling confused					
Faster/deeper breathing					
Short of breath					
Tight feelings in chest					
Bloated feeling in stomach					
Tingling fingers					
Unable to breathe deeply					
Stiff fingers or arms					
Tight feelings around mouth					
Cold hands or feet					
Palpitations					
Feelings of anxiety					
Total					

Appendix 2: Ethics approval

AUTEC Secretariat

Auckland University of Technology
D-88, WU406 Level 4 WU Building City Campus
T: +64 9 921 9999 ext. 8316
E: ethics@aut.ac.nz
www.aut.ac.nz/researchethics

29 March 2018

Richard Ellis
Faculty of Health and Environmental Sciences

Dear Richard

Re Ethics Application: **17/419 Examination of diaphragm thickness using ultrasound imaging in people with a breathing pattern disorder**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 29 March 2021.

Non-Standard Conditions of Approval

1. If all participants are female, this should be included in the study title.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by AUTEC before commencing your study

Standard Conditions of Approval

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining it. You are reminded that it is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard.

For any enquiries, please contact ethics@aut.ac.nz

Yours sincerely,

Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: scottpeirce@hotmail.com; Sarah Mooney

Appendix 3: Participant Information Sheet

Participant Information Sheet

Date Information Sheet Produced: 5.4.18

Project Title:

Examination of diaphragm thickness using ultrasound imaging in females with a breathing pattern disorder.

An Invitation: You are invited to take part in this valuable research project performed by Scott Peirce (Primary Researcher) whom is an expert in the area of Breathing Pattern Disorders and ultrasound imaging. The purpose of this document is to explain to you as openly and clearly as possible all the procedures involved in this project so that you can make a fully informed decision as to whether you are going to participate.

Participation is completely voluntary and you may withdraw from the study at any time without giving a reason or being disadvantaged. Please take the time to read this information sheet and feel free to ask questions about any information or what may be required of you. After one week, the researcher or a research colleague will contact you regarding your participation in the research; if you agree, an appointment will be made for you to attend the clinic. Once you have had the opportunity for your questions to be answered, you will be asked to sign the consent form. By signing the consent form, you indicate that you understand the information provided and that you give your consent to participate in the research project. You will be given a copy of the information sheet and consent form to keep as a record.

What is the purpose of this research? The aim of this project is to measure diaphragm muscle thickness using ultrasound imaging and to see how this compares between healthy participants and participants with Breathing Pattern Disorders. The diaphragm muscle is the primary breathing muscle and its thickness is an important measure of how this muscle functions. Success with this measurement could significantly expand research investigations in the area of Breathing Pattern Disorders and may help develop effective treatments for example inspiratory muscle training for strengthening the diaphragm.

How was I identified and why am I being invited to participate in this research? You may have been identified through any of three pathways; you may have:

1: Responded after reading an advertisement about the research, and can self-declare that you are currently not a patient or affiliate of Scott Peirce.

2: Been referred to a breathing clinic and the research was discussed by the receptionist with you.

3: You may have been attending another breathing clinic (such as a hospital clinic) and were then asked to consider participating in the research.

Exclusion criteria: In order to participate in the research, you should not have any of the following conditions: lung disease (e.g. asthma, COPD, bronchiectasis), current or previous smoking history, obesity, diabetes, kidney or liver disease, neuromuscular disease (e.g. stroke), pregnancy, cardiovascular disease (e.g. heart attacks or bypass), anaemia, chronic low back pain, chronic neck pain, chronic thoracic pain, recent upper respiratory tract infection or steroid use and recent surgery.

How do I agree to participate in this research?: Your participation in this research is voluntary (it is your choice) and whether or not you choose to participate will not disadvantage you. You are able to withdraw from the research at any time. If you choose to withdraw from the research, then you will be offered the choice between having any data that is identifiable as belonging to you removed or allowing it to continue to be used. However, once the findings have been produced, removal of your data may not be possible. All information collected will be anonymous.

What will happen in this research?: Firstly, you will be invited to volunteer. Some brief questions around smoking history and history of general health and lung disease will be discussed to see if you are eligible to be included in the study. The information sheet will be provided to you so you can take time to understand what is required of you, and decide if you would like to participate in the study. After one week, you will be contacted by research staff and asked if you would like to participate. If you agree, an appointment will be made for you to come to AUT Akoranga Campus or Breathing Works (Newmarket) whichever is more convenient to you. At the beginning of the session there will be time to answer all your questions and then provide informed consent. A copy of the information sheet and signed consent form will be provided to you. Testing will be completed over a 30-minute session and afterwards, there will be a 10-minute optional information session following the data recording whereby your results can be explained.

The 30 minute session will consist of completion of questionnaires on symptoms of breathing dysfunction (Nijmegen Questionnaire), and general details of age, smoking history etc. Your height and weight, respiratory rate and oxygen level will then be measured.

Visual observation of your breathing, and your ability to hold your breath will be assessed by the researcher.

In a lying position on your back, water-based transmission gel will be applied to the skin around the lowest part of the chest wall against your ribs. An ultrasound probe will be placed against your skin to look at your diaphragm muscle (the main muscle for breathing) so that its thickness can be measured. Measurements of the diaphragm muscle will be taken with you breathing at rest (normal, relaxed breathing) and also when taking maximum deep breaths. The measurement is completely pain free and

the deep breathing should be comfortable. You will only be asked to expose the thoracic area; all other areas will be covered.

What are the discomforts and risks? There are no risks or discomfort from the ultrasound scanning. The transmission gel is water-based thus precluding an allergic reaction.

What are the benefits? Participants: There will be limited benefits to you as the participant. However, although you will be from a healthy cohort, you will still benefit from this research from gaining knowledge of how the diaphragm works, also satisfaction gained from participating in a study that will inform future research. Participants with a Breathing Pattern Disorder will benefit from a clearer understanding of how the diaphragm works and also to the potential underlying mechanisms in this condition.

Researcher: The researcher will benefit from this research as the data is part of his Masters qualification. This qualification will provide him with improved recognition in this field and enhance his prospects for gaining professional specialist registration.

Wider Community: This research offers great potential and enhancement in the management of respiratory, mental health, gastrointestinal and musculoskeletal conditions. A better understanding of the role of diaphragm thickness as an adaptable structure will help clinicians in designing and implementing management and treatment programmes for conditions such as breathing pattern disorders, respiratory disease such as asthma, neck pain, low back pain, anxiety, and gastrointestinal issues.

What compensation is available for injury or negligence? In the unlikely event of a physical injury as a result of your participation in this research, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

How will my privacy be protected? Your privacy will be protected by allocating you by a number in the final report. Access to the data is restricted to the researchers. All data will be anonymized.

What are the costs of participating in this research?There is no monetary cost to you. It will however take up to approximately 30 minutes of your time to attend the session. You will receive a complementary $20 supermarket voucher for attending the testing session as recognition for your time.

What opportunity do I have to consider this invitation?Before volunteering, please consider carefully whether you are prepared to be part of the research. You will be contacted after one week of the information sheet being provided to you by the research staff. If you decide to take part in the research, an appointment will be made for you. There will be some flexibility around the appointment times for the data collection. It is of an expectation from both the researchers and participants that all parties are

both professional and punctual at all times. Please communicate clearly with us so convenience is optimized for all concerned, and appointments run smoothly and are on time.

How do I agree to participate in this research? You will need to read and sign a consent form in order to participate in this study. A consent form is enclosed with the participant information sheet. Please contact the researchers if you wish to participate in this study using the contact details provided at the end of this sheet.

What happens if anything unusual is found on the ultrasound scan in a healthy participant? If an unusual finding of a thin diaphragm is found on ultrasound, or you are found to have symptoms which may be attributed to a Breathing Pattern Disorder (healthy population group), with your consent, a referral will be made to a free of charge service in one of the Auckland Health services (hospital physiotherapy services) and your family doctor informed.

Will I receive feedback on the results of this research? Results will be made available to you at the completion of the research. A summary of personal data is available for participants as well as a summary of the overall findings on conclusion of the research. This will be in the form of a written summary. If you wish to receive this, please indicate on the relevant section of the consent form. Any papers that may be published arising from the research can be accessed on request.

What do I do if I have concerns about this research? Any concerns regarding the nature of this project should be notified in the first instance to:

Research Supervisor: Dr Richard Ellis ; Email address: richard.ellis@aut.ac.nz or

Phone: 921 9999 ext 7612.

Concerns regarding the conduct of the research should be notified to the Executive Secretary of AUTEC, Kate O'Connor, *ethics@aut.ac.nz* , 921 9999 ext 6038.

Whom do I contact for further information about this research?

Researcher: Scott Peirce; Email address: scottpeirce@hotmail.com

Phone: 522 1122

Approved by the Auckland University of Technology Ethics Committee on *29/3/2018*, AUTEC Reference number *17/419*.

Appendix 4: Verbal cues for ultrasound assessment

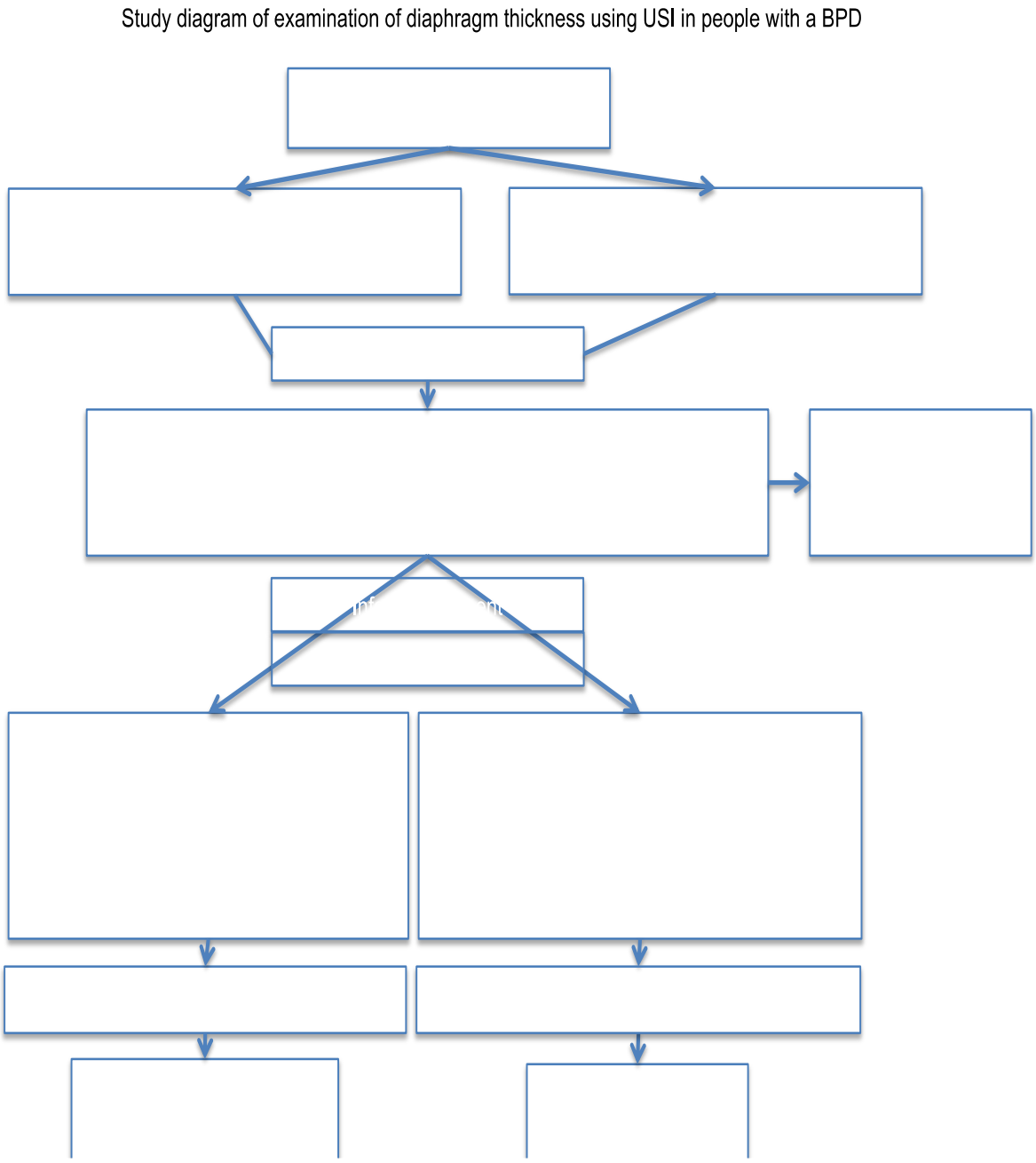
Tvex cue “Gently do a relaxed breath out” Quiet breathing exhale (Tvex), image freeze.

Tvin cue “Gently do a relaxed breath in” Quiet breathing inhale (Tvin) image freeze.

Tmin cue “Take a deep breath in then a full breath all the way out ” Maximal breathing exhale (Tmin) image freeze.

Tmax cue “Take a deep breath all the way in and hold” Maximal breathing inhale (Tmax) image freeze.

Appendix 5: Participant recruitment



Appendix 6: Run sheet for data collection

Advertising

Posters and flyers will be placed at AUT, BPD clinics in hospitals and at Breathing Works

Recruitment:

The potential participant will either call BW as a client or

May have responded after reading an advertisement about the study, and can self-declare that you are currently not a patient or affiliate of Scott Peirce or

May have been attending another breathing clinic (such as a hospital clinic) and were then asked to consider participating in the study.

The participant will then have some basic screening questions asked by receptionist at BW (Pathway 1) or by Scott Peirce (Pathway 2 or 3)

Phone script:

S: Hello is this (participant name …), This is (Cheryl Pilkington / Scott Peirce) from Breathing Works.

P: Hello,

S: I was wondering if I could discuss with you a research study we are conducting at breathing works and if you would consider being a participant in the study.

P: Ok? / Im not sure (CONTINUE WITH QUESTIONS)

OR

P: no I am sorry I am not interested

S: Ok Thanks for your time. Thanks again. (FINISH OF PHONE CALL)

(CONTINUE QUESTIONS)

S: Ok. It will take me about 2 minutes to discuss the study. Also I must state that this will not affect your treatment or your rights if you decline – and you can withdraw at any stage if you want.

P: ok fire away.

S: So the study uses a routine part of our current assessment of breathing– Ultrasound imaging- and it specifically is looking to see the thickness of your diaphragm and how this is related to good or poor breathing. The question we are trying to answer is –

whether people who breathe poorly have a thinner diaphragm when compared to people who breathe well?

P: Ok so you are trying to see if my diaphragm is thin?

S: Yes that is correct?

P: what will this mean if you do find it out?

S: well then we can give you some specific exercises to help strengthen and thicken this muscle. We will also refer you onto a physiotherapy service if this finding of wasting is found within a participant in the healthy group

P: ok makes sense? What do I need to do to do the study?

S: we will need to do a few other basic measures: height, weight, and other measures of your breathing : including your respiratory rate, your style of breathing, your ability to hold your breath, and your score on some questionnaires too.

P: how long will this take?

S: We think about 30-40 minutes total time.

P: ok sounds good (CONTINUE QUESTIONS)

OR

P: no I am sorry I am not interested

S: Ok Thanks for your time. Thanks again. (END OF CALL)

(CONTINUE QUESTIONS)

S: I just have a few more questions to see if you are the type of participant we are looking for ?

P: OK

S: Great so now I will read you a list and you can say yes or no to each of the conditions

Do you have lung disease (e.g. asthma, COPD, bronchiectasis)?

Are you a current or previous smoker?

Do you have diabetes, kidney or liver disease,?

Neuromuscular disease?

Are you Pregnant?

Have you had recent surgery?

Do you have heart or cardiovascular disease?

Do you have chronic low back pain, neck pain, thoracic pain?

Have you had recent cold or upper respiratory tract infection?

Do you use steroid drugs?

If **YES** to any of these then this means this person is not a suitable candidate for the study

S: Ok, thanks for your answers – unfortunately due to …….. condition we will have to say no to your involvement in the study. Thanks for your time!

If **NO** to all of these questions then person is a suitable candidate!

S: Great. Ok if you are keen to be involved I will send you through an information sheet and a consent sheet to consider for a week , to explain things a bit more and then we can set you up a time that suits you to do the study measurements. What is your email so I can send you a sheet or address so I can post it?

P: blah blah blah. 7 mickey mouse street.

S: Great do you have any questions???

P: no I think I am ok

OR

P: yes blah blah blah (if really technical discuss with scott or email questions through to bw@breathingworks.com)

S: Finally the location of the study is 81 Remuera rd Newmarket. Parking is free onsite.

OR AUT

S: thanks for your time!

Information sheet / Consent sheet sent out:

See attached forms sent via email.

Any return questions are replied to by Cheryl or Scott by email.

Booking of measurement session

Potential Participant followed up by phone + email next week after initial phone call and session booked.

Measurement session

(Participant shows up at: 81 Remuera Rd, Newmarket or AUT)

Screening measures and assessment: PARTICPANT IN SITTING

Participants will be assigned a randomly generated unique identifying code and this will be recorded on their personal details information sheet. This sheet will then be filed in a locked filing cabinet.

The participants then completes the further screening questionnaires (NQ, general health, smoking history, age, gender etc.)

Calibrated electronic scales will measure weight, and SECA stadiometer will measure height. Body Mass Index (BMI) will be calculated by the formula of: BMI= kg / m^2 (Ministry of Health, 2017).

The Hi–Lo test: the therapist asks the patient to place one hand on the abdomen and one on the upper chest (Chaitow et al., 2002, 2014) and then observes if the patient has an abdominal or thoracic dominant pattern of breathing.

Pulse oximetery is used to assess saturation of oxygen, and poor saturation (e.g. levels below 94%) may indicate the presence of lung disease (Chaitow et al., 2014).

BHT measurement occurs on a tidal exhalation; with the holding of the breath until the person feels a moderate-strong urge to breathe (Grammatopoulou et al., 2014). A low BHT below 30 seconds correlates with BPD, while normal is considered above 30 seconds (Grammatopoulou et al., 2014).

RR above 16 breaths per minute is considered to indicate a BDP, while a RR between 10-14 is considered ideal (Chaitow et al., 2002, 2014; Grammatopoulou et al., 2014).

Final Inclusion Criteria assessed:

Inclusion criteria for females with **BPD** of both BMI groups are as follows: Age 18-65 years old, Nijmegen Questionnaire (NQ) score ≥ 23, respiratory rate > 16 breaths per minute, Breath Hold Time (BHT) ≤30 seconds, presence of an upper chest breathing pattern on Hi-Lo assessment (Chaitow et al., 2014).

Inclusion criteria for **healthy** females of both BMI groups are as follows: Age 18-65 years old, NQ score <16, respiratory rate 8-15, BHT ≥ 30 seconds, presence of an abdominal dominant breathing pattern on Hi-Lo assessment.

USI of Diaphragm Thickness: PARTICIPANT IN SUPINE

Explanation of procedure one more time including depths of breathing on laminated sheets explained.

The participant is made comfortable in supine with pillows supporting head and under knees to maintain neutral spine.

Machine turned on – adequate paper towels – adequate gel etc

Participant is given paper towels to protect clothing

Flash drive inserted into ultrasound

Unique id number entered in area for ultrasound patient details

Small linear probe selected with gel applied – focus and gain appropriate

Palpation of rib 11, 10 , 9, 8 and small marking made on ribs 10 9 8 with anatomy pencil at anterior auxiliary line and line to ASIS

The USI technique uses a small linear ultrasound probe placed on the anterior auxiliary line between ribs 10 - 9 or 9 - 8 to allow for visualisation of the diaphragm (Boon et al., 2013).

Adjustment of image with gain and focus and movement to appropriate zone of apposition of lung.

Make sure image measures of thickness are covered on machine for blind measurement.

The participant will be asked to breathe to specific depths of breath, using verbal and or visual cues during measurement.

Initially measure **RIGHT SIDE**

"Gently do a relaxed breath out" Quiet breathing exhale (TVex), image freeze. Measure x 3 then save.

"Gently do a relaxed breath in" Quiet breathing inhale (TVin) image freeze. Measure x 3 then save.

"Take a deep breath in then a full breath all the way out" Maximal breathing exhale (Tmin) image freeze. Measure x 3 then save.

"Take a deep breath all the way in and hold" Maximal breathing inhale (Tmax) image freeze. Measure x 3 then save.

Re-measure **Left side** as per above protocol

"Gently do a relaxed breath out" Quiet breathing exhale (TVex), image freeze. Measure x 3 then save.

"Gently do a relaxed breath in" Quiet breathing inhale (TVin) image freeze. Measure x 3 then save.

"Take a deep breath in then a full breath all the way out" Maximal breathing exhale (Tmin) image freeze. Measure x 3 then save.

"Take a deep breath all the way in and hold" Maximal breathing inhale (Tmax) image freeze. Measure x 3 then save.

Check that all images have saved.

Thank participant – remove ultrasound gel

Healthy participant

Give thanks, card and Koha.

Unusual finding on USI

If unusual finding of wasting is evident in healthy group then refer onto hospital or free clinic, **details of BW charges**, **referral letter with copy to GP**. Give thanks, card and Koha.

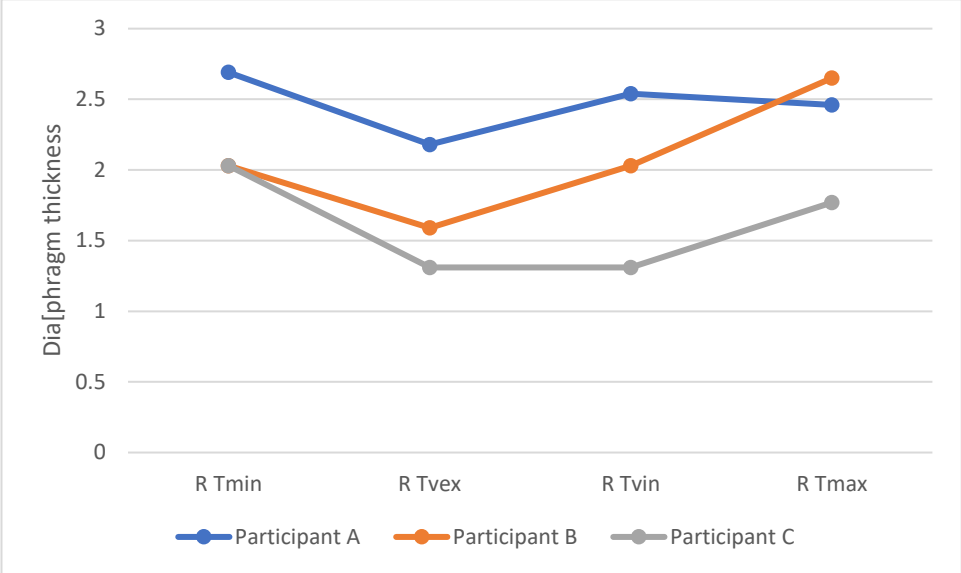
BPD Group

Participants in the BPD group will then be offered usual care for their condition. Give thanks, card and Koha.

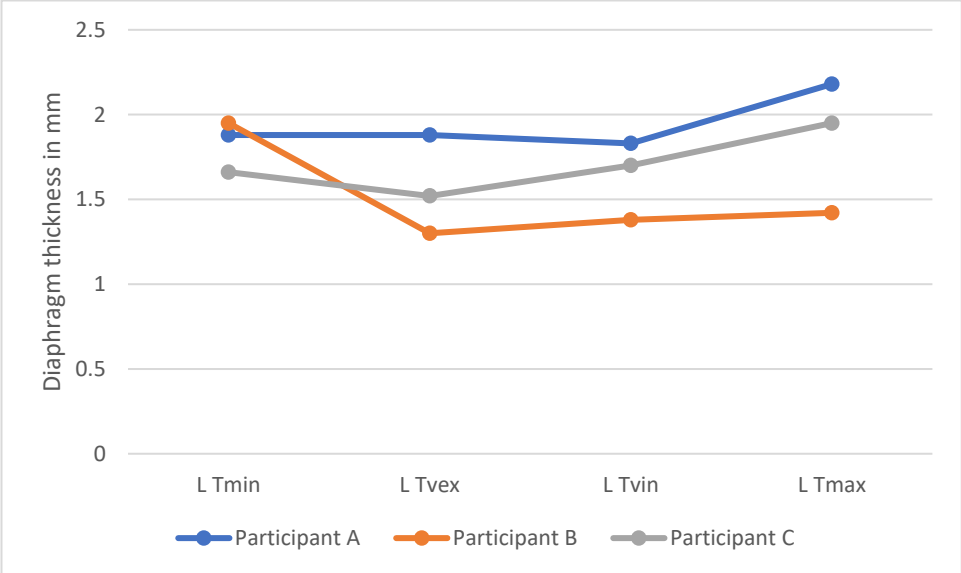
BPD from other referral source

Participants in the BPD group will then have a copy of their results of measurements and what this means explained and then referred back to original source. Give thanks, card and Koha.

Appendix 7: Additional graphs for paradoxical diaphragm thickening



Graph of paradoxical right diaphragm motion in 3 participants in BPD healthy BMI groups



Graph of paradoxical left diaphragm motion in 3 participants in BPD healthy BMI groups

Appendix 8: Strategies utilised to improve recruitment

Recruitment drives were created and promoted through multiple advertising mediums including:

- discussing the study at undergraduate lectures,
- message boards at AUT,
- discussing the study to other students at AUT,
- advertising with female rugby teams,
- paper advertising in physiotherapy clinics,
- via social media messages on twitter,
- advertising with videos and posts on Facebook,
- advertising within professional physiotherapy groups on Facebook.