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Faculty of Health Sciences

Breath by breath analysis of breathing pattern in health and disease: a potential outcome measure for breathing retraining?

by

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Thesis for the degree of Doctor of Philosophy

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Abstract

University of Southampton, Faculty of Health Sciences, Doctor of Philosophy

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Analysis of breathing pattern can quantify parameters of breathing such as rate, volume, timing and regularity/rhythmicity. This information can be useful to compare breathing patterns in those healthy and with disease, under different experiment conditions (such as rest versus activity) and to monitor changes over time. In this research, respiratory inductive plethysmography (RIP) was used to record breathing patterns in a group of healthy subjects and a group of severe asthma patients.

RIP is a leading technology for ambulatory monitoring of breathing, but traditional RIP devices suffered from poor signal quality under such conditions due to movement of the sensor. Several authors have also raised doubts about the existing calibration methods for RIP which can lead to inaccurate estimation of breathing parameters.

During the first phase of the programme, an instrumented garment (LifeShirt®) which had RIP embedded within was tested for its validity in comparison to a pneumotachograph (PT). The first study sought to validate the measurements obtained from the LifeShirt ®against PT when calibrated with a published but yet to be tested method for breath by breath analysis and to address the limitations of existing calibration methods. Eleven healthy individuals took part in this first study. Breathing patterns were simultaneously monitored by the LifeShirt ® and the PT during thirty minutes of rest and twenty minutes of exercise. Parameters of tidal volume, expiration time and tidal volume variability were recorded and compared between devices.

The analysis from the first study demonstrated that RIP recorded proportionate changes of tidal volume and expiration duration relative to PT during quiet breathing and exercise. Mean tidal volume and expiration duration between devices was strongly correlated for rest and exercise. No statistical difference in tidal volume variability was observed between devices during either period.

Significant differences in expiration duration between devices were observed in all participants at rest but not during exercise. Results of this first study demonstrated that valid breath by breath analysis using RIP without PT was feasible. This is clinically advantageous due to simplicity of set-up for RIP

In the second phase, measurement of breathing patterns was made in severe asthma patients with the LifeShirt® alone during thirty minutes of rest. It intended to add new knowledge with regards to the breathing patterns within this small population as compared to the healthy population.
Ten healthy individuals and ten patients diagnosed with severe asthma took part in the second study. Breathing parameters of tidal volume, inspiration time, expiration time, end tidal carbon dioxide levels, tidal volume variability and end tidal carbon dioxide levels variability were recorded by the LifeShirt®.

The analysis of the second phase shown no evidence that breathing pattern parameters could differentiate between the severe asthma patients and healthy volunteers in our small study. The symptoms of hyperventilation found more commonly in the severe asthma group were not associated with differences in breathing pattern parameters. However, considerable differences were found between individuals. This suggests the existence of individuality in breathing patterns between individuals. Such findings raised doubts as to whether there is a group ‘pattern’ that is common within the severe asthma population or within the healthy population. This programme calls for a change in paradigm to consider breathing patterns as an unique individual ‘trait’ rather than as a group characteristic.

Contents

Table of Contents

List of Figures

List of Tables

DECLARATION OF AUTHORSHIP

I, Wai Leung Ambrose Lo, hereby declare that the thesis entitled:

Breath by breath analysis of breathing pattern in health and disease: a potential outcome measure for breathing retraining?

and the work presented in the thesis is both my own, and have been generated by me as the result of my own original research. I confirm that:

this work was done wholly or mainly while in candidature for a research degree at this University;
where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
where I have consulted the published work of others, this is always clearly attributed;
where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
I have acknowledged all main sources of help;
where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
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Signed: ……..

Date:…….

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List of abbreviations

ATS	American Thoracic Society
AB	Abdomen
ACQ	Asthma controlled questionnaire
BTS	British Thoracic Society
CHF	Chronic heart failure
CO2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CV	Coefficient of variation
df	Degrees of freedom
EIT	Electrical impedance tomography
ETCO2	End tidal carbon dioxide
FVC	Forced vital capacity
FEV1	Forced expiratory volume in one second
HADS	Hospital anxiety depression scale
ICC	Intraclass correlation coefficient
LS	LifeShirt®
MLR	Multiple linear regression
NQ	Nijmegen questionnaire
PEF	Peak expiratory flow
PT	Pneumotachograph
QDC	Qualitative diagnostic calibration
RC	Rib cage
RIP	Respiratory inductive plethysmography
SEM	Standard error measurement
Sig	Significant
Stdv	Standard deviation
Ti	Inspiration time
Te	Expiration duration
Vt	Tidal Volume

Chapter 1: Introduction to the research

This document presents the research carried out to fulfill the requirements to obtain a PhD degree. The thesis will start with a brief introduction to and justification for the study, followed by an overview of this PhD thesis.

1.1 Background

At the beginning of the PhD, the main field of interest was the measurement of breathing pattern as a potential outcome for respiratory intervention such as breathing retraining. At that time, a garment called LifeShirt® had recently been developed by Vivometric in the US, which claimed to be able to record breathing pattern parameters noninvasively and in non-laboratory conditions, including during daily activities and into the extended environment. There were several published studies on this device, but the studies were funded and analysed by Vivometric. It was felt necessary to record and analyse the respiratory data independently to assess its validity and usefulness for recording breathing pattern parameters. The original intention was to conduct a series of studies involving the LifeShirt®.

However, in late 2010 Vivometric went into liquidation and was later declared bankrupt. The web page disappeared overnight and communication with the company became impossible. The intention of the PhD therefore moved away from assessing the validity of the LifeShirt® and focused instead on assessing breathing pattern with standard RIP technology (the same technology as in the LifeShirt®).

This thesis therefore reports the initial validity studies involving the LifeShirt®, and the second study on the breathing patterns of severe asthma patients.

1.2 Overview of the PhD thesis

A summary of each chapter in the thesis is provided below.

Chapter 2 reviews and discusses some of the key aspects of the normal mechanics and physiology of breathing, followed by a discussion of breathing patterns in the severe asthma population. It explores the types of changes in breathing pattern that have previously been reported in the mild/moderate asthma group, and highlights areas that have not previously been investigated in detail.

Chapter 3 discusses the complexity surrounding the monitoring of breathing pattern, the technology of respiratory inductive plethysmography, and the methodological flaws in existing studies using the LifeShirt®. This is followed by a discussion of the calibration methods for respiratory inductive plethysmography, and a description of the concept of assessing validity. Lastly, the research aims of the first study are described.

Chapter 4 describes the research methodology of the first study, which aimed to assess the validity of the parameters recorded by LifeShirt® in comparison with the laboratory gold standard equipment (the pneumotachograph). It also discusses the statistical tests that were used to test the validity of the LifeShirt®.

Chapter 5 presents the data recorded by the LifeShirt® and pneumotachograph during quiet breathing and exercise. It also presents the results of the validity analysis.

Chapter 6 discusses the results obtained for the first study. It also discusses the limitations of the first study.

Chapter 7 explains the research aims of the second study, which aimed to investigate the breathing pattern in severe asthma patients.

Chapter 8 explains the methodology for the second study, aiming to assess breathing pattern and its variability in severe asthma patients and how these factors are related to the same measurements in healthy controls.

Chapter 9 presents the results of the breathing patterns recorded by the LifeShirt® in the severe asthma cohort and healthy cohort.

Chapter 10 discusses the results obtained by the study. It also discusses the limitations of the second study. Finally, it provides a conclusion to the thesis and describes future research directions.

Chapter 2: Literature review – breathing pattern

Changes in breathing pattern carry great weight in diagnostic decisions, and disturbance of respiratory function can potentially be life threatening (Wilhelm *et al*., 2003). This chapter addresses the complexity of breathing patterns in humans, and describes the breathing patterns in healthy individuals and in patients diagnosed with asthma. The topics covered in this chapter are outlined below.

2.0 Introduction to breathing pattern

Breathing pattern can be considered as the result of a combination of various systems, including central and peripheral chemoreflexes, the cardiac system and the respiratory system. It has long been of interest in research and clinical practice (Wilhelm *et al*., 2003) because many pathologies present with abnormal, and sometimes characteristic, breathing patterns. However, the term 'breathing pattern' lacks a consensus definition and its meaning varies within the literature. For example, breathing pattern has been defined as chest wall movement and the number of apnoeas in a sleep disorder study by Dark *et al*. (1987). The most commonly described elements of breathing pattern are tidal volume, breathing rate, inspiration and expiration time, duty cycle, and rib cage and abdomen contribution of a breath cycle (Tobin *et al*., 1983a; Tobin *et al*., 1983b; Prys-Picard *et al*., 2006; Dark *et al*., 1987; Teramoto *et al*., 1995).

This chapter covers the main topic of the mechanics of breathing, the control of ventilation, breathing pattern, and how asthma may alter breathing pattern.

2.1 Mechanics of breathing

2.1.1 Overview of breathing mechanics

The lung itself does not have muscles that contribute to breathing, and it is not directly attached to the chest wall. Air will naturally flow from a region of high pressure to a region of low pressure, and airflow in and out of the lung is a result of changes in pressure created by the movements of the chest wall (Proctor & Hardy, 1949). In simple terms, inspiration is a result of increased intrathoracic volume and a reduction in intrathoracic pressure, while expiration is a result of reduced intrathoracic volume and an increase in intrathoracic pressure. This allows air to move into the lung via the conducting airways.

2.1.2 Movement of chest wall

Movement of the chest wall is brought about by a mechanical action of the respiratory muscles that displaces the rib cage (Ratnovsky *et al*., 2008). Each rib articulates at its head with the bodies of its own vertebra and of the vertebra above (at the costovertebral joint) (De Troyer *et al*., 2005). The head of the rib is connected to the vertebral bodies by radiate and intra-articular ligaments to allow slight gliding movements of the articular surfaces. The neck and tubercles of the rib are connected to the transverse process of the vertebra (the costotransverse joint) by short ligaments which allow slight cranial and caudal gliding movements at this joint. As a result the costovertebral and costotransverse joints form a hinge, allowing rib cage displacement of the ribs through rotation around the long axis. This axis is oriented laterally, dorsally and caudally. Since the ribs are curved and slope caudally and ventrally from the costotransverse articulations, the costal cartilages are more caudal than their dorsal end. Due to this arrangement, when the ribs are displaced in the cranial direction during inspiration, their ventral ends move laterally and ventrally as well as cranially, and the sternum is displaced ventrally. As a result, both the lateral and dorsoventral diameters of the rib cage usually increase. These chest wall movements are often referred to as ‘pump-handle’ and ‘bucket handle’

movements, as illustrated in the following figures.

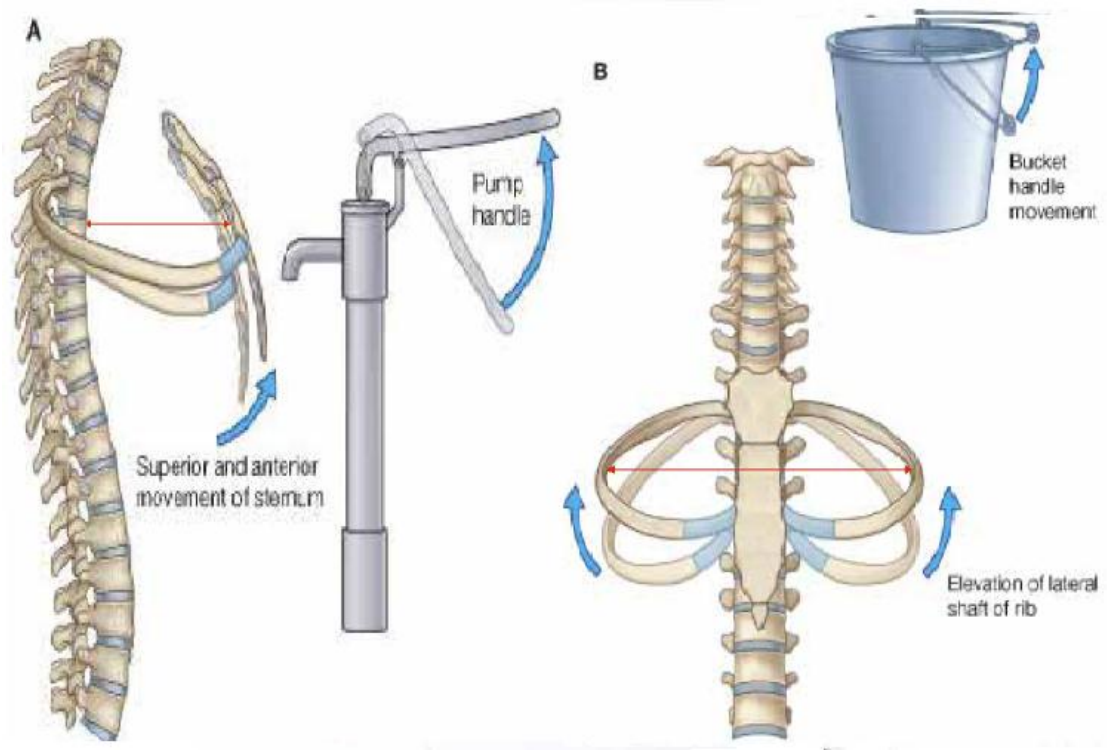


Figure 1: A demonstration of the 'pump-handle' and 'bucket handle' movements with which the ribs move cranially/caudally and sternum moves ventrally, thus increasing/decreasing diameters in the anterior-posterior direction during inspiration/expiration. B demonstrates the 'bucket-handle movement where cranial/caudal movement of the ribs at the ventral ends result in increasing/decreasing lateral diameters during inspiration/expiration (West, 2005).

2.1.3 Respiratory muscles

The group of inspiratory muscles includes the diaphragm and the external intercostal, sternomastoid and scalene muscles (Ratnovsky *et al*., 2008). Expiratory muscles include the internal intercostal, rectus abdominis, external and internal oblique and transverse abdominis muscles (Ratnovsky *et al*., 2008).

While the action of the respiratory muscles responsible for the chest wall motion described in standard text books appears to be clear, several authors have suggested that the individual roles of each respiratory muscle remain poorly

understood. Muscles that are involved in the act of breathing, such as the diaphragm, the external and internal intercostal muscles and the accessory respiratory muscles, are also skeletal muscles which participate in postural function and locomotor action. Therefore it is unclear which muscles should be considered proper respiratory muscles and which muscles perform an accessory function (Aleksandrova & Breslav, 2009). While the existing literature agrees that the diaphragm is the main inspirator and provides almost the entire amount of pulmonary ventilation, the action of the intercostal muscles remains a controversial area (De Troyer *et al*., 2005) due to their wide distribution throughout the rib cage.

2.1.4 Intercostal muscles

The intercostal muscles form two thin layers that span each of the intercostal spaces. The primary effect of their contraction is to displace the ribs and thereby alter the configuration of the rib cage. This action causes a change in pleural pressure and an expansion of the lung (De Troyer & Leduc, 2007). It is generally accepted that the external intercostal muscles are considered to be inspiratory and the internal intercostal muscles expiratory (Aleksandrova & Breslav, 2009). However, the exact role of internal and external intercostal muscles has been a controversial area throughout medical history. De Troyer *et al.* (2005) reviewed studies performed with dogs and human subjects, and summarised the mechanical interaction amongst the different portions of the intercostal muscles. They reported that in both dogs and humans, the external intercostals in the dorsal portion of the rostral interspaces have a large inspiratory mechanical advantage, but this advantage decreases in the ventral and caudal directions such that in the ventral portion of the caudal interspaces it is reversed into an expiratory mechanical advantage. The internal intercostals in the caudal interspaces have a large expiratory mechanical advantage, but this advantage decreases in the cranial and ventral directions.

The external intercostals are active only during the inspiratory phase of the breathing cycle. External intercostal activity is greatest in the dorsal portion of

the rostral interspaces, and it decreases both ventrally and caudally such that the muscles in the caudal interspaces remain silent. These muscles therefore have an inspiration function during the breathing cycle, acting to elevate the ribs. The internal intercostals are active only during the expiratory phase of the breathing cycle. Their activity is greatest in the caudal interspaces. These muscles therefore have an expiratory function during breathing, acting in concert with the abdominal muscles to deflate the chest wall and the lung.

Although the action of the internal and external intercostals provide opposite effects, they both may have inspiratory and expiratory functions in different portion of the muscles. This suggests the function of these muscles during breathing largely depends on the excitation of the muscle fibres during the inspiratory or expiration phases.

Electromyographic (EMG) studies in humans have found that the neural excitation of intercostal muscles maps the mechanical advantage of intercostal muscles. De Troyer *et al.* (2003) investigated the distribution of inspiratory drive in external intercostal muscles in humans using electromyographic (EMG) recordings. Six healthy individuals aged between 33 and 51 were recruited. EMG recordings were obtained from the dorsal and ventral parts of the rib cage during rest with an upright posture. Results demonstrated that the external intercostals in the dorsal portion of the third and fifth interspaces showed phasic inspiratory activity with every breath in every subject, whereas the ventral portion of the external intercostals at the same interspaces showed no activity in three subjects and only occasional activities in two subjects. The muscles in the ventral portion of the third interspace showed phasic inspiratory activity in only three of six subjects, and the muscle in the dorsal portion of the seventh interspace was almost silent.

The results from this study suggest that not all external intercostal muscles are excited during inspiration; rather, primarily the portions that have a mechanical advantage in inspiration are excited. The opposite actions from the external and internal intercostal muscles are largely as a result of selective regional

activation of the muscles, rather than different orientation of the muscle fibres as has been suggested in the literature (Allen, 2010).

2.1.5 Diaphragm

The diaphragm muscle is regarded as the most important inspiratory muscle of the respiratory system, providing almost the entire amount of pulmonary ventilation in humans (Aleksandrova & Breslav, 2009, Mierjedrzejowicz *et al*., 1988). The most commonly recognised action of the diaphragm is to decrease intrathoracic pressure, thus drawing air into the lungs. A large part of the abdominal contents actually reside within the thoracic cage and a substantial portion of the diaphragmatic area is apposed against the inner rib cage wall. When the diaphragm contracts, there is a substantial increase in intra-abdominal pressure, which increases the transverse dimensions of the lower rib cage. The area of apposition causes an upward vertical force on the lower ribs, causing them to move outward. This leads to the “bucket handle” effect which serves to increase lower rib cage transverse dimensions.

2.1.6 Accessory muscles

Accessory respiratory muscles are muscles that do not have significant contributions during respiration at rest, but will assist when required (Aleksandrova & Breslav, 2009). When the sternomastoid and scalene muscles are contracted, this raises the sternum and the first two ribs in a cranial direction, thus assisting in expanding the rib cage (Legrand *et al*., 2003). When the accessory expiratory muscles of the internal and external obliques and transverse abdominis contract, they pull the abdominal wall inward and cause the diaphragm to move cranially into the thoracic cavity. The lower ribs are also pulled caudally to further deflate the rib cage (Legrand *et al*., 2003).

2.2 Control of ventilation and the role of carbon dioxide

The basic respiratory rhythm is generated by the clustered neurons that are located at the pons and medulla oblongata (West, 2005). Rhythmic impulses are sent from the inspiratory centre at the medulla to excite the diaphragm and the intercostal muscles to expand the thorax. The impulse then switches off and expiration occurs passively as the inspiratory muscles relax and the lungs recoil. While the inspiratory centre generates the basic respiratory rhythm, the pons in the respiratory centre influences and modifies the activity of the medullar neurons. For example, the pons appears to smooth out the transitions from inspiration to expiration and vice versa. However, although there is no doubt that breathing is rhythmic the origin of the rhythm is still unclear (Smith *et al*., 2000). The most widely accepted hypothesis is that the rhythm is the result of reciprocal inhibition of interconnected neuronal networks in the medulla (West, 2005).

The respiratory centre controls breathing rate and depth in response to a wide range of receptors such as pulmonary stretch receptors and irritant receptors. The predominant receptors are the central chemoreceptors located in the bilateral ventrolateral medulla, and the peripheral chemoreceptors located in the carotid bodies found in the neck (Nattie, 2006). The most potent and closely controlled chemicals are carbon dioxide (CO_2) and arterial pH levels. Changes in CO_2 are detected by both the carotid bodies and the central chemoreceptors. Existing studies suggest that central and peripheral chemoreceptors have different roles. Central chemoreceptors detect interstitial fluid pH and monitor arterial carbon dioxide levels, cerebral blood flow and cerebral metabolism, while the peripheral chemoreceptors detect arterial carbon dioxide/pH and monitor alveolar ventilation (Dempsey, 2005; Forster *et al*., 2000; Smith *et al*., 2006). The central chemoreceptors therefore provide most of the steady-state tonic response, whereas the carotid bodies provide rapid responses to changes in carbon dioxide level. Carbon dioxide is regulated through negative feedback control (Khoo, 2000), whereby an increase in CO2 stimulates the chemoreflexes and elicits an increase in ventilation. Fluctuation in arterial

carbon dioxide therefore stimulates the chemoreflexes to produce ventilatory adjustments to restore carbon dioxide back towards its original equilibrium level (Khoo, 2000).

2.2.1 Summary

The above section described some of the key aspects of respiratory physiology as they relate to breathing pattern. Airflow is a result of changes of intra-thoracic pressure induced by the rhythmic contraction and relaxation of respiratory muscles that change the volume of the thorax. As expiration is a passive process, small airways are prone to premature closure in patients with respiratory conditions such as asthma, due to the reduction of airway wall elasticity as a result of the airway remodeling process.

Ventilation is predominately regulated by changes in carbon dioxide and pH levels via a negative feedback loop system, whereby a change in carbon dioxide level will stimulate ventilatory adjustments to restore equilibrium levels.

2.3 Breathing pattern

To date, there is no universally accepted definition for the term breathing pattern. The timing and volume components are the most common parameters that are described when considering breathing pattern. In this thesis, the elements of breathing pattern that will be studied are the tidal volume, breathing rate, inspiration time (Ti), expiration time (Te), respiratory rate and sigh rate. In addition to these, end tidal carbon dioxide level ($ETCO_2$) and the variability in tidal volume, $ETCO_2$ and expiration time were also recorded. These parameters were selected because there are some recognised differences in them between healthy individuals and individuals with respiratory disorders (Tobin *et al*., 1983b; Brack *et al*., 2002; Wysocki *et al*., 2006a; Tobin *et al*., 1983a). This makes them suitable for reflecting the respiratory status of patients, as well as a tool to monitor changes in breathing induced by experimental interventions (Valta *et al*., 1992).

2.3.1 Normal breathing pattern

Breathing is a rhythmic process of rib cage movements created by the respiratory muscles, which are driven by the central respiratory pattern generators located in the brainstem (Mangin *et al*., 2008). Normal values for breathing pattern parameters, measured by either a pneumotachograph or RIP, have been published as a guide for comparison between healthy and diseased individuals. A summary is provided in the following table:

	Parameters					
Study	Age	Vt (ml)	Ti (s)	Te (s)	Ti/Tot	Rate (breath/minute)
Tobin *et al*., (1983)	18–60	383 (85)	1.62 (0.3)	-	0.42 (0.03)	16.7 (2.7)
	60–81	382 (108)	1.68 (0.4)	-	0.41 (0.03)	16.6 (2.8)
Osborne (2000)	19–37	680 (130)	1.57 (0.5)	2.43 (1.02)	0.39 (0.05)	16.6 (3.8)
Parreira *et al*., (2010)	20–39	352 (133)	-	-	0.40 (0.04)	15.0 (3)
	40–59	302 (117)	-	-	0.40 (0.03)	15.0 (3)
	60–80	338(118)	-	-	0.38 (0.04)	16.0 (3)

Table 1: Summary of normal values of respiratory parameters for a healthy population. Ti/Tot: fractional inspiration time of the total of the respiratory cycle.

From the above table, it can be seen that a range of tidal volume values have been reported in existing studies. Although the difference may be due to different age groups being recorded in different studies, a considerable diversity in breathing pattern has also been documented in previous literature. The diversity in ‘normal’ tidal volume was first documented in Dejours *et al*. (1961),

where the tidal volume in healthy individuals ranged from 442ml to 1549ml. A wide range of respiratory rates has also been reported in healthy adults. The average respiratory rate in healthy adults is usually reported in text-books as twelve breaths per minute (Tortora & Anagnostakos, 1990), but higher values of –fifteen to twenty breaths per minutes have also been reported (Beckett, 1995; Bernardi *et al*., 1998). These reports suggest that considerable inter-individual variation in breathing parameters may exist in the healthy population.

2.3.2 Asthma breathing pattern

2.3.2.1 Changes to respiratory physiology in asthma

The condition of asthma is not a single entity. Rather, it is likely to be a heterogeneous condition that can be sub-divided into different phenotypes based on clinical, aetiological, physiological or pathophysiological features (Polosa, 2008). There is also no gold standard diagnosis for asthma, with diagnosis usually based on clinical symptoms (BTS, 2009). Asthma is characterised by airway hyperresponsiveness (Brown *et al*., 2006), airway inflammation (Pascual & Peters, 2005) and episodic or chronic air flow limitations (Stirling & Chung, 2001). It is believed that many of the observed pulmonary function changes, such as lung function decline (Pascual & Peters, 2005), premature airway closure (Tantucci *et al*., 2011) and airway hyperresponsiveness (Brown *et al*., 2006) are due to remodeling of the airways. Changes in the airway structures lead to a marked increase in airway resistance, which in turn leads to increased work in the process of breathing. As the degree of airway obstruction worsens expiration becomes active rather than passive, which further increases the work of breathing. As airway walls reduce elasticity and fail to recoil, airway collapse on expiration, resulting in air trapping and hyperinflation (Cormeir, 1990).

It is currently unclear exactly how airway remodeling may affect breathing pattern. However, it is hypothesised that airway hyperresponsiveness may result in bronchoconstriction and hyperventilation, which in turn lead to an increase in tidal volume and respiratory rate. Premature airway closure may also result in reduced expiration time.

Changes in breathing mechanics in asthma

Asthma is an obstructive disease characterised by increased airway resistance. High airway resistance from the airways, along with hyperinflation, will increase the load on the respiratory muscles (Laghi & Tobin, 2003). Hyperinflation often forces the respiratory muscles to operate at non-optimal lengths (Ratnovsky *et al*., 2008), meaning that respiratory muscles are required to generate higher forces with each breath (Hill, 2004). One of the adaptations to this increase in workload is the persistent use of accessory respiratory muscles to generate additional inspiratory force (Holley & Boots, 2009; Grover *et al*., 2011; Shaw & Shaw, 2011; Lavietes *et al*., 1988). In an early study by Martin *et al.* (1983) the activities of the respiratory muscles of seven asymptomatic asthma patient were studied. Progressive bronchoconstriction was induced with inhaled aerosolized histamine, and the results indicated a relative increase in the recruitment of intercostal/accessory muscles with a progressive increase in bronchoconstriction. The abdominal muscles, did not demonstrate such an increase, however, suggesting that the recruitment of intercostal/accessory muscles exceeds the recruitment of the diaphragm during acute bronchoconstriction.

The persistent use of the accessory muscles is referred to as apical breathing (Clifton-Smith & Rowley, 2011) and creates further expansion of the rib cage primarily in the cephalad direction (De Troyer & Kelly, 1984). Apical breathing is not as efficient as diaphragmatic breathing due to the higher lung compliancy of the basal area of the lung, which provides more surface area for gas exchange. The increased use of accessory muscles leads to increased work in breathing and consequent energy wastage (Gorini *et al*., 1999). The greater recruitment of the rib cage muscles also places them at risk of fatigue as their threshold for fatigue is lower than that for the diaphragm (Zocchi *et al*., 1993).

In patients with obstructive respiratory disorders such as asthma, paradoxical or asynchronous motion of the rib cage and abdomen have often been reported (Gilmartin & Gibson, 1984; Hammer & Newth, 2009; Aliverti *et al*., 2009). The

rib cage and the abdomen compartment move simultaneously in synchrony during breathing at rest (Hammer & Newth, 2009). An asynchronous breathing motion refers to non-coordinated motion of the rib cage and abdomen compartments. It is often characterised by a lag between the two compartments, or one compartment moving in the opposite direction to the other. The likely contributing factors for chest wall asynchrony in adults include diaphragm weakness (Hammer & Newth, 2009) and hypertrophy of the accessory muscles (Lavietes *et al*., 1988). Hyperinflation of the lung can also contribute to asynchronous motion. This is because as lung volume increases, the inspiratory muscles are passively shortened, placing then at a mechanical disadvantage (De Troyer, 1997). Therefore, patients with obstructive respiratory disorders frequently have a reduction of diaphragmatic mobility and its relative contribution to thoracoabdominal motion.

2.3.2.2 Literature on asthma breathing pattern

Published studies on the mild to moderate asthma population have reported no statistically significant differences in breathing pattern during the asymptomatic phase. A study by Delvaux *et al*. (2002) examined the breathing pattern in eighty mild to moderate asthma patients, according to the American Thoracic Society's criteria (1993). The mild to moderate asthma patients were aged between 16 and 60 years old and had asthma symptoms at the time of recruitment. A further forty healthy individuals, matched with the experimental group on the basis of sociodemographic characteristics, were recruited as a control group. The breathing parameters of tidal volume and respiratory rate were monitored by pneumotachograph in a seated position over three-minute recording periods. The difference between the groups was analysed using t-tests. The results showed that mild to moderate asthma patients had higher tidal volume and higher respiratory rate than healthy controls. However, the differences were very small (100ml difference in tidal volume, one breath per minute difference in respiratory rate) and did not reach statistical significance. This study appears to suggest that there is very little difference in tidal volume and respiratory rate between mild to moderate asthma groups and the healthy

population. However, the lack of significant differences may be due to a lack of power calculations for sample size, with the possibility that the sample did not have sufficient power to detect any differences. The recording period was short, and it is doubtful that such a short period of recording would be sufficient to capture the true extent of differences in breathing patterns.

Similar results were also reported in another study by Osborne *et al*. (2000). These authors investigated breathing pattern in mild to moderate asthma patients during the stable phase of the condition. Twenty-three participants with a history of asthma were recruited, and a further seventeen healthy participants with matching age, gender and height were recruited as controls. Breathing patterns were recorded via a laboratory standard heated pneumotachograph for five minutes, during which time tidal volume, inspiration time, expiration time, breathing rate and end tidal carbon dioxide were monitored. The differences between the healthy controls and the mild to moderate asthma patients were assessed by t-test.

The results showed that the patient group had a higher tidal volume, shorter inspiration and expiration times and a higher breathing frequency than the healthy controls. This finding seems to support the hypothesis that asthma patient groups may have different breathing patterns in comparison with healthy individuals. However, the observed differences did not reach statistical significant levels. Because a power calculation was not carried out, the lack of statistical significant difference in respiratory parameters between the two groups may be due to the sample size, which may not have been sufficiently large to have the power to detect small differences. The findings therefore remain inconclusive as to whether there is any difference in respiratory parameters between healthy controls and mild to moderate asthma patients. The recording period was short, consisted of only five minutes of recording. Also, given the complex nature of breathing patterns (Fiamma *et al*., 2007a), it is doubtful whether the short recording period would be sufficient to capture the true picture of the participants' breathing patterns, and subtle differences may not have been captured.

Another study by Tobin *et al*. (1983b) measured breathing pattern in 'symptomatic' and 'asymptomatic' asthma. The authors defined symptomatic asthma as having dyspnoea at rest or during moderate exertion, accompanied by wheezing. The exact measurement of dyspnoea or wheezing was not documented in the study. Seventeen asymptomatic asthma patients and fifteen symptomatic asthma patients were recruited, and their breathing patterns in a supine position were monitored by a respiratory inductive plethysmograph over a period of fifteen minutes. The results showed that the breathing rates were 16.6 breaths per minute in the asymptomatic asthma group and 16.0 breaths per minute in the symptomatic asthma group. However, the group mean tidal volume in the symptomatic group was markedly higher (679ml ±275) than in the asymptomatic group (386ml ±133). However, the mean tidal volume of the asymptomatic asthma group was not statistically significantly different from the mean tidal volume recorded from a healthy population (383ml±91). The mean inspiratory drive (defined as inspiration time divided by the total of inspiration and expiration time) of the symptomatic asthma group was also statistically significantly higher than for the asymptomatic group and the healthy group.

This study suggests that symptomatic asthma patients may have a larger mean tidal volume and a stronger inspiratory drive than either healthy individuals or asymptomatic asthma patients. The asymptomatic asthma patients, however, did not demonstrate differences in respiratory parameters in comparison with healthy individuals. The results for this study should be interpreted cautiously since there was no clear definition for inclusion within the asthma population. Given the wide presentation of clinical symptoms and the high incidence of co-existing conditions in asthma (BTS, 2009), it is difficult to draw conclusions from these results without a description of the sample population.

2.3.3 Carbon dioxide level

As mentioned in Section 2.2, carbon dioxide level plays a key role in the control of ventilation. Normal carbon dioxide levels are reported in various textbooks to be between 4.66 and 5.99 kPa (Esquinas, 2010; Murphy *et al*., 2009), with the lower threshold limit necessary to produce hypocapnia symptoms reported to be at 3.99kPa (Gardner, 1990; Rafferty *et al*., 1992). It has been suggested that 'hidden' hyperventilation is associated with asthma (Bruton & Holgate, 2005; Stalmatski, 1997). This theory has been given some support in studies that demonstrated hyperventilation symptoms in asthma patients. In a study by Thomas *et al*. (2001), 30% of the 219 patients with mild and moderate asthma were reported to have experienced symptoms of hyperventilation when surveyed with the Nijmegen questionnaire.

The primary cause of a reduction in carbon dioxide is over-breathing or hyperventilation, defined as alveolar ventilation that is inappropriately high for the metabolic production of carbon dioxide (Gardner & Lewis, 2005a). This leads to a reduction in arterial carbon dioxide levels, which in turn leads to respiratory alkalosis and the paraesthesia sensation. High arterial carbon dioxide level is reported to have a bronchodilatory effect in healthy individuals (Scichilone *et al*., 2001), and a reduction in arterial carbon dioxide level can lead to bronchoconstriction. It has also been reported that asthmatic individuals are more sensitive to carbon dioxide level, so that a small change in carbon dioxide level is likely to lead to bronchoconstriction. Van den Elshout *et al*. (1991) studied the effects of hypercapnia and hypocapnia on respiratory resistance in both normal and asthmatic subjects. It was found that in asthmatics, a reduction in end tidal carbon dioxide level of only 1kPa caused a 13% increase in airway resistance, while the same reduction in carbon dioxide level had no effect on healthy subjects. Conversely, an increase in end tidal carbon dioxide level of only 1kPa resulted in a significant fall in airway resistance in both asthmatic and normal subjects. It currently remains unclear as to why airway smooth muscles in asthma patients are more sensitive to changes in carbon dioxide than in healthy individuals.

There is currently a lack of strong empirical evidence that demonstrates the association between clinical signs of hyperventilation and carbon dioxide levels. A recent review by Meuret and Ritz (2010) reviewed several earlier studies that documented a reduction of carbon dioxide levels in asthma patients (Hormbrey *et al*., 1988; McFadden & Lyons, 1968) and concluded that some asthma patients have reduced carbon dioxide levels. However, most of the reviewed studies were based on acute asthma or 'symptomatic' asthma populations. Hyperventilation and hypocapnia are well-recognised features of acute asthma (Bruton & Holgate, 2005; Osborne *et al*., 2000). It is not certain why carbon dioxide level has any clinically relevant pathogenic role during the stable period of the asthma.

The study by Osborne *et al*. (2000), described in Section 2.3.2, had also investigated whether carbon dioxide level can be correlated with hyperventilation symptoms and respiratory parameters. The association between end tidal carbon dioxide and breathing rate was assessed using a Spearman rank correlation. A small but statistically significant difference in end tidal carbon dioxide was observed between healthy individuals and mild to moderate asthma patients (a difference of 0.39 kPa). No statistically significant correlations were found between end tidal carbon dioxide and other respiratory parameters, and the correlation coefficients were not presented. This study showed a small but statistically significant difference in end tidal carbon dioxide levels between healthy volunteers and mild to moderate asthma patients. It is currently unclear whether the observed small non-significant differences in respiratory parameters between the two groups were the reason for the reduced end tidal carbon dioxide level.

A small but statistically significant difference in end tidal carbon dioxide levels between healthy individuals and mild to moderate asthma sufferers (a difference of 0.29 kPa) was also reported in a study by Delvaux (2002). A weak but statistically significant negative correlation ($p<0.05$) was found between end tidal carbon dioxide levels and ventilation (Pearson $r = -0.37$). The author

concluded that increased tidal volume was responsible for the reduced end tidal carbon dioxide levels. However, this result should be interpreted with caution due to the large number of correlation comparisons (over ninety), which increases the chances of getting significant results by chance (a false positive, or Type I error). The correlation coefficient also demonstrated a weak correlation between the two parameters. It therefore remains unclear whether increased tidal volume is associated with reduced end tidal carbon dioxide levels.

To date, a limited number of studies have been found that investigate the relationship between breathing pattern and end tidal carbon dioxide in severe asthma patients. If carbon dioxide level indeed plays a pathogenic role, it would be logical to hypothesise that altered carbon dioxide levels can also be observed in severe asthma conditions.

2.3.4 Sigh

Sigh appears to be a vague notion; no universal definition of a sigh and of the 'normal' sigh rate could be found during the literature search. A wide variety of definitions for sigh in adults have been used in the literature. These include: twice the mean tidal volume (Wilhelm *et al*., 2001b); 2.5 times the mean tidal volume (Vlemincx *et al*., 2009b); three times the mean tidal volume (Bendixen *et al*., 1964); four times the mean tidal volume (Prys-Picard *et al*., 2006); and twice the mean inspiratory tidal volume (Wuyts *et al*., 2011). The reasons for these different definitions are unclear, since authors rarely report the rationale behind their choice of sigh definition in studies. In the author's opinion, the variation is possibly due to the wide range of tidal volume recorded in different sample populations, so that a particular definition from one population might not be an optimal definition for another population. However, the lack of a working definition means that comparison between studies is difficult, as it cannot be certain whether the same results would be obtained if different criteria were applied.

As a result of the different sigh definitions, there is no universal agreement for the normal sigh rate within the literature. It has been suggested that normal healthy adults have a sigh rate of approximately ten sighs per hour (Fredberg, 2001). However, a different sigh definition is likely to result in a different sigh rate. When sigh was defined as twice the mean inspiratory tidal volume, sigh rates of ten sighs per hour (Wuyts *et al*., 2011) and eight sighs per hour (Vlemincx *et al*., 2010) were reported in healthy individuals. When sigh was defined as breaths greater than twice the expiratory tidal volume, a sigh rate of twenty-two sighs per hour was reported (Wilhelm *et al*., 2001b). The lack of an agreed definition within the literature makes it difficult to compare the sigh rates between different studies.

An increased number of sighs is commonly believed to be a contributing factor to altered carbon dioxide levels in some asthma patients (Gardner & Lewis, 2005b). The relationship between sigh breaths and asthma patients has not

been previously investigated. There are some studies that investigated sigh and carbon dioxide levels in patients diagnosed with panic attack disorder. For example, Wilhelm *et al*. (2001b) investigated sigh in a group of patients diagnosed with panic disorder. The study recruited sixteen patients diagnosed with panic disorder, fifteen patients with generalised anxiety disorder and a further seventeen healthy controls. Tidal volume in a seated position was measured by respiratory inductive plethysmography, calibrated with the least squares method during a thirty-minute recording period. During the recording period, participants were instructed to keep still with their eyes open. $ETCO_2$ was measured via nasal prongs connected to a standard capnograph. The mean levels of $ETCO_2$, tidal volume and sigh frequency were calculated over all breaths for each individual.

The results showed that the panic disordered group had a higher sigh frequency (0.7 sighs per minute) than both the generalised anxiety disorder group (0.47 sighs per minute) and the control group (0.36 sighs per minute). There was a statistically significant correlation between $ETCO_2$ level and sigh frequency in the pooled results from all participants ($r = -0.47$, $p<0.02$). However, while the authors suggest that sigh is a contributor to altered end tidal carbon dioxide level, the correlation coefficient index suggests only a moderate correlation between $ETCO_2$ levels and sigh frequency despite reaching a statistically significant level. This casts some doubt over the significance of the association between sigh and $ETCO_2$ level.

To date, there is only a limited number of studies that investigate the relationship between sigh and carbon dioxide level in the severe asthma population. A case study reported a difference in sigh rate in a patient with difficult-to-treat asthma after breathing retraining exercise (Prys-Picard *et al*., 2006). In the same study by Prys-Picard *et al*. (2006), breathing pattern at rest was monitored by respiratory inductive plethysmography over a five-minute period. The recording conditions and the position of recording were not documented. The study reported that on average the patient had 1.2 sighs per minute over the five-minute recording period at baseline, and this was reduced

to 0.33 sighs per minute post-treatment. The number of sighs reported in Prys-Picard *et al*. (2006) was higher than the number of sighs previously reported in healthy individuals. Tobin *et al*. (1983a) reported that healthy individuals aged between18 and 60 had one or no sighs during a fifteen-minute recording period. Therefore, the results of Prys-Picard *et al*. (2006) appear to suggest that some asthma patients may have a higher sigh rate than healthy individuals. However, the results from the study cannot be applied to a wider population due to the fact that that data was from a single subject case study, as well as the lack of reported inclusion and exclusion criteria. In addition, a direct comparison between these two studies may not be fair due to the differences in the definition of sigh. Prys-Picard *et al*. (2006) defined a sigh as four times larger than the mean tidal volume, whereas Tobin *et al*. (1983a) defined a sigh as three times the mean tidal volume.

2.3.5 Summary

The evidence to date suggests that carbon dioxide levels may play a role in the pathophysiology of asthma, since a lower carbon dioxide level has been repeatedly observed in studies. While a low carbon dioxide level is likely to occur as a result of hyperventilation, existing studies have not reported an association between carbon dioxide levels and clinical signs of hyperventilation in terms of marked differences in tidal volume and respiratory rate between mild to moderate asthma patients and healthy controls during the stable period.

Sigh frequency has been proposed as a contributing factor to altered levels of carbon dioxide. To date, there are limited published studies investigating whether there is altered carbon dioxide levels or sigh frequency in severe asthma patients. If altered carbon dioxide levels and sigh frequency can be observed in severe asthma patients and sigh is found to be associated with altered carbon dioxide levels, it might then be possible to use these parameters as potential outcomes measures for intervention.

2.4 Breathing pattern variability

Variability is the term used to describe states of dynamic behaviour of a system, and can be defined as the amount of fluctuation within the system (Khoo, 2000). This study defines the term 'breathing pattern variability' as the level of fluctuation of individual parameters within the overall breathing pattern. The existence of variability is assumed to provide the respiratory system with flexibility to induce changes to response to changing environmental demands. There is a growing body of literature to suggest that normal healthy respiratory patterns are characterised by breath-by-breath variability.

2.4.1 Introduction

Variability in breathing pattern, in particular the variability of tidal volume, has been an area of research for many years (Kuratomi *et al*., 1985; Fiamma *et al*., 2007a; Wysocki *et al*., 2006a; Wuyts *et al*., 2011). It is proposed that the respiratory system is comparable with any engineering control system, in which it is necessary to have an appropriate balance between system stability and sensitivity to permit responsiveness to fluctuation in order to maintain function within tightly regulated limits (Baldwin *et al*., 2004). The purpose of the respiratory controller, however, is not only to achieve a particular overall output, but also to carefully adjust output on a breath-by-breath basis (Bruce, 1996). It is frequently assumed that measurements derived from an unstable system are likely to show a high degree of variability, whereas a stable system is expected to exhibit low variability (Khoo, 2000).

Excessive respiratory instability, characterised by large variations in tidal volumes, frequent respiratory pauses and increased frequency of sighing and/or gasping breaths, has often been suggested in individuals identified as having dysfunctional breathing (Courtney, 2011). Dysfunctional breathing is not well defined (Courtney, 2009); rather, it is an umbrella term to cover abnormalities in volume, timing, excessive or reduced variability, and dysfunction of the mechanics of breathing such as paradoxical movement between rib cage and abdomen (Courtney, 2009; Courtney, 2011). Empirical evidence to demonstrate

increased variability in the respiratory system in asthma patients is currently lacking. Despite this lack of empirical evidence, however, patients with asthma are often referred to breathing training programmes with the aim of 'normalising' their breathing pattern (Bruton & Lewith, 2005), with within-subject variability in tidal volume being one of the elements that may be manipulated (Bruton *et al*., 2011).

2.4.2 Variability in patients with respiratory conditions

Several studies have shown differences in tidal volume variability between healthy and non-healthy respiratory systems. Wysocki *et al*. (2006a) conducted a retrospective study to investigate whether breathing pattern variability might be used as an indicator to separate those who could be successfully weaned off a ventilator. Forty-six sets of data were recorded from patients who were admitted to hospitals with underlying diseases that required mechanical ventilation via an endotracheal tube for longer than twenty-four hours. Extubation decisions were made by the treating physicians based on a clear improvement of the condition and no administration of intravenous sedatives during the previous twenty-four hours. Separation from the ventilator was considered to be successful if spontaneous breathing could be sustained without any form of ventilatory support for greater than forty-eight hours after extubation. Cases of failure included patients who died, were re-intubated or required any form of ventilatory support within the first forty-eight hours post-extubation. Respiratory parameters of tidal volume, inspiratory time and expiratory time were measured via a heated pneumotachograph connected to the side port of the endotracheal tube.

The variability of each parameter was assessed by its coefficient of variation (CV). A statistically significantly higher variability was observed in all three parameters in the thirty-two patients who were successfully weaned off mechanical ventilator than in the sixteen patients who failed to be weaned off. The authors concluded that breathing pattern variability could be used as an indicator to predict the outcome of ventilator disconnection. However, the

results should be considered with caution due to the large difference in the sample size between the successful and failure group. The sample population in the success group was approximately 50% larger than the failure group. The between-group comparison of the mean variability is therefore not a fair comparison since the smaller group is likely to be affected by anomalies.

Brack *et al*. (2002) had also reported a reduction in breathing pattern variability in patients who were diagnosed with restrictive lung disease. Ten male patients (aged between 43 and 72) with restrictive lung disease and seven healthy age-matched male participants (mean age 60 years) were recruited. Tidal volume and expiration time were measured by inductive plethysmography over a one-hour period. The position and the activity during the recording periods were not documented. Variability in tidal volume and expiration time over the hour were assessed by coefficients of variation. The results demonstrated that the restrictive lung disease group had statistically significantly lower tidal volume and expiration time variability than the healthy group. The mean tidal volume variability and expiration time variability in the healthy group were 50% ±20% and 41% ±19% respectively, whereas in the disease group the results were 22% ±5% and 22% ±7% respectively. The authors suggested that this reduction in variability might be due to the adoption of a monotonous breathing style as a strategy to avoid the sensation of dyspnoea. The results of this study suggest that patients diagnosed with respiratory conditions might exhibit a different group trait of breathing variability in comparison with a healthy group.

The characteristics of variability in ventilatory flow are not well understood. It is believed that respiratory pattern variability is a normal response to a range of factors, including afferent information from mechanoreceptors, chemoreceptor reflexes and automatic ventilatory command (Del Negro *et al*., 2002). The source of variability is likely to be due to the control of breathing, and might be linked with subtle changes in arterial carbon dioxide levels.

2.4.3 $ETCO_2$ and breathing variability

Fiamma *et al*. (2007a) studied breathing patterns in eight healthy subjects under three different experimental conditions, i.e. normal breathing, induced hypocapnia and induced hypercapnia. The researchers achieved hypercapnia by asking the participants to inhale a hypercapnic gas mixture for fifteen minutes to increase $ETCO_2$ levels. The target $ETCO_2$ level in the hypercapnic state was not reported. A hypocapnic state ($ETCO_2$ level at 1.95kPa) was induced by semi-passive hyperventilation using mechanical ventilation. Tidal volume was measured using a pneumotachograph. The length of the recording period varied within each condition due to the different time each individual took to reach the target $ETCO_2$ level. On average, between 120 and 160 breath cycles were recorded from each participant under each condition.

The results demonstrated a decrease in tidal volume variability in hypercapnia (13%) and an increase in variability in hypocapnia (35%) when compared with a normocapnia state (20%). This study appears to suggest that variability in tidal volume might be associated with extreme $ETCO_2$ levels. However, it is unclear whether such findings would be applicable during normal breathing since the $ETCO_2$ levels induced during the study are unlikely to happen during normal breathing.

If the findings of Fiamma *et al*. (2007a) about the association between $ETCO_2$ levels and tidal volume variability are valid, and if it is accepted that some people with asthma have altered carbon dioxide levels, then it is logical to hypothesise that some asthma patients would also have altered tidal volume variability in comparison with healthy individuals.

A recent study demonstrated an increase in irregular airflow pattern in asthma patients with varying levels of airflow obstructions (Veiga *et al*., 2010). Airflow irregularity refers to instances of recognisable pattern within airflow that are different to tidal volume variability, which concerns fluctuations in tidal volume. The approximate entropy index is a non-negative number assigned to a time

series of measurements, with larger values corresponding to greater irregularity and smaller values corresponding to less irregularity. In Veiga *et al*.'s (2010) study, airflow irregularity was assessed in four groups of participants; healthy controls (n = 5), asthmatic patients with no airflow obstruction (n = 5), asthmatic patients with mild airflow obstruction (n = 5), asthmatic patients with moderate airflow obstruction (n = 6), and asthmatic patients with severe airflow obstruction (n = 5). Airflow obstruction was measured by FEV_1. Airflow pattern in a seated position was recorded for 120 seconds using a pneumotachograph with the first sixty seconds discarded, sampling at 16Hz. The between-group differences in approximate entropy index were assessed by t-test.

The results showed that approximate entropy indexes differed significantly between patients with mild airway obstruction and the healthy control group ($p<0.007$). The approximate entropy index showed that the mild obstruction group had a reduction of 31% and the moderate and severe obstruction groups had a reduction of 37% in comparison with the control group. A Pearson's correlation test also showed a significant correlation between approximate entropy and FEV_1 values ($r = 0.6$, $p<0.05$). The results of this study appear to suggest that airflow pattern is more irregular in an asthma population with airflow obstruction in comparison with healthy controls.

However, the results from this study should be interpreted with caution due to potential flaws in the methodology and statistical analyses. Pincus (1991) stated that approximate entropy should be to quantify irregularity on time series with at least a thousand data points. The time series obtained from each individual in this study contained 960 points (a sample frequency of 16 Hz over a sample period of 60 seconds). Approximate entropy is heavily dependent on the length of the recording period, and is often systematically lower than expected for short periods (Richman & Moorman, 2000). There is also doubt about the relative consistency of the measurement when applied to two separate time series. If the approximate entropy of one data set is systematically higher than that of another, it does not always remain higher for all conditions tested (Pincus, 1995). Therefore, given the methodological limitations of approximate entropy,

the interpretation of the results is limited. It is also unclear whether using such a short window for recording period (60 seconds) is sufficient to reflect the irregularity in airflow pattern.

There is also doubt in this study about the use of the t-test to test for between-group differences in approximate entropy index. Since the entropy index value is between zero and two, and the results show that the largest between-groups mean difference is approximately 0.1, it is unlikely that a sample size of six in each group would have sufficient power to detect such a small difference. Due to the methodological flaws, the findings in this study have limited interpretation and should be considered with caution.

2.4.4 Sigh and variability

Recent studies have suggested that sigh breaths may play a role in the loop feedback respiratory system, acting as a resetting mechanism to regulate variability in the system and to enhance information processing (Baldwin *et al*., 2004; Vlemincx *et al*., 2009b). Sigh is known to have a significant role in altering lung mechanical and airway wall function, and to relax the airway smooth muscle (Courtney, 2011). However, their role in respiratory control remains unclear. Baldwin *et al*. (2004) proposed that the negative feedback controlled respiratory system behaves like an engineering system that incorporates a resetting mechanism. When the level of variability of the respiratory system deviates from equilibrium or an optimal level, a sigh would occur to restore the previous equilibrium or optimal level. Baldwin *et al*. (2004) tested this hypothesis in preterm infants. Twenty-five healthy full-term infants were included in the study. Tidal breathing was measured by application of a compliant silicone face mask to the infant's mouth and nose for ten minutes. The mask was connected to an ultrasonic flowmeter. Sigh was defined as a tidal breath of at least double the mean tidal volume of the preceding ten breaths. When a sigh was identified from the time series, the twenty-five breaths before and after the sigh were selected. This formed a complete pre-post sigh series. Each pre-post sigh series was then divided into four blocks. Each block contained ten breaths with a 50% overlap from the previous block. A schematic below gives an illustration of how the blocks were divided. The variability of each block was calculated using a coefficient of variation (CV). CV is a measurement of variability over a period of time. It is calculated by dividing the standard deviation by the mean and then multiplying by one hundred. It is a dimensionless measurement used to assess overall variability.

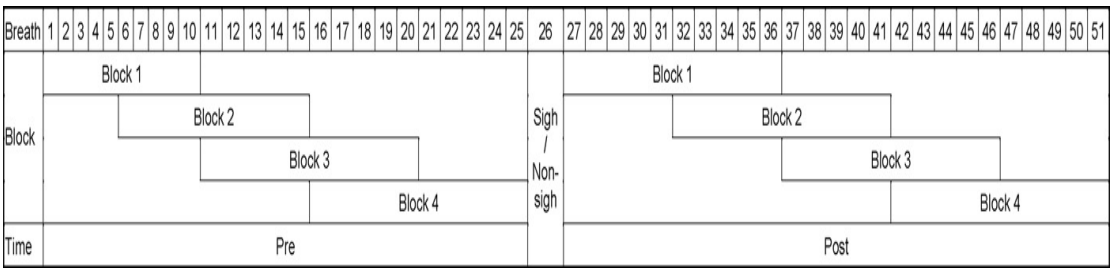


Figure 2: Schematic for block allocation

Comparisons were made between changes of CV in the period leading up to the sigh, changes of CV in response to the sigh, and the period required for a return to the baseline level of CV after the sigh. The results showed no evidence of an increase in tidal volume variability pre-sigh, but post-sigh there was a significant increase in variability of tidal volume for a period of fifteen breaths. After the fifteen breaths period, variability returns rapidly to a baseline level fluctuation.

This finding is in agreement with the study by Vlemincx *et al*. (2009a), which investigated whether sighing can reset respiratory variability in adults. Thirty-eight healthy individuals (eight men and thirty-four women) aged between 18 and 20 were recruited. Resting breathing pattern in a seated position was recorded by an ambulatory RIP device over a twenty-minute period. A sigh was defined as a breath with an inspiratory volume of at least 2.5 times the median inspiratory volume of a running baseline of two minutes before each breath. The methodology for variability analysis was the same as in Baldwin *et al*.'s (2004) study, using a moving window of coefficient of variation for eight blocks of ten-breath periods for each of the fifty breaths pre- and post-sigh, with a 50% window overlap between blocks. Vlemincx *et al*. (2009b) used an additional measurement of non-sigh series, which consisted of four pre-sigh blocks, a normal breath, and four post-sigh blocks. The ensembles of non-sigh series were the same as for the sigh series, but with a non-sigh breath instead of a sigh breath. A non-sigh breath series contained only breaths that were not included in the sigh series.

The results showed that in the non-sigh series no differences between CV pre- and post-non-sigh were found. However, the sigh series showed a significant increase in total variation from pre-sigh block 1 to pre-sigh block 4 and from pre-sigh block 4 to post-sigh block 1. Post-sigh block 1 to post-sigh block 2 showed a significant decrease in total variability. At post-sigh block 3 (fifteen breaths post-sigh), total variability was not significantly different from that in pre-sigh blocks 1 to 4. These results indicate that the breaths that lead up to a sigh have a gradual increase in variability, which contradicts the results of Baldwin's study where no increase in variability was observed. A potential explanation for the difference in results might be the difference in sample population, where Baldwin *et al*. (2004) used infants while Vlemincx *et al*. (2009) used adults. It is known that infants and adults have different breathing patterns.

However, both studies demonstrated that tidal volume variability returns to pre-sigh level after fifteen breaths post-sigh. These findings confirm that sigh breaths have a role in 'resetting' and reducing random variability within the respiratory system. However, comparison of the findings between the two studies might not be fair due to the different definitions of sigh used in each study. If the amount of variability in tidal volume in the asthma population is indeed different from that in the healthy population as commonly believed, it is possible that the altered tidal volume variability in the asthma population might be due to sighs failing to 'reset' the system. As variability in tidal volume remains high, the respiratory system continues to generate sighs in order to reduce variability. This would partly explain the increased sigh rate that is reported in published studies (Prys-Picard, 2008). This hypothesis is yet to be confirmed since to date there have been no studies that have investigated the relationship between sighs, $ETCO_2$ and variability in asthma patient groups.

2.4.5 Summary

Variability in breathing pattern within the asthma population has yet to be examined in detail, and therefore remains largely unknown (Veiga *et al*., 2010). From existing evidence on pathological conditions such as chronic restricted lung disease and panic disorder, it is reasonable to hypothesise that the severe asthma population also has altered variability in breathing pattern in comparison with healthy controls. It is possible that altered carbon dioxide levels may be the results of variability in breathing pattern.

Chapter 3 Literature Review – The monitoring of breathing pattern

The following section discusses and evaluates some devices which are available for the non-invasive monitoring of breathing pattern. A justification for the use of the equipment evaluated in this thesis is then provided. This is followed by a section on the calibration of this equipment, and a section on the methods for assessing the validity of any measurement tool. Finally there is a summary and justification of the research hypothesis.

3.0 Introduction

It has been described in previous chapters that severe asthma patients may have different breathing patterns in comparison with healthy individuals. It is recognised that there are some pathologies that cause subtle changes in respiratory pattern but are not associated with obvious external signs. For example, patients diagnosed with hyperventilation syndrome often have no clinically apparent over-breathing. Chaitow (2004)has suggested clinical tests for diagnosing hyperventilation using various observational methods, including the observation of movements of the chest wall or counting the number of breaths. These methods may be simple to perform but can lack objectivity and/or validity.

It is possible that the objective non-invasive monitoring of breathing pattern may enable the detection of subtle changes in breathing. However, until recently breathing pattern studies have required laboratory-based equipment using mouthpieces or face masks which affect breathing pattern, and this has restricted studies in this area.

The ability to perform long-term monitoring of breathing pattern outside a clinical setting is desirable for the study of conditions that are associated with respiratory abnormalities, as it would give a more relevant picture of patients' breathing pattern during real-life activities. This could lead to earlier diagnosis of developing complications, and result in appropriate treatment being given more

swiftly. The primary limitation in recording accurate breathing pattern non-invasively outside the laboratory has been reported to be technical difficulties with recording equipment (Tobin *et al*., 1983; Wilhelm *et al*., 2003).

The monitoring of breathing pattern has always been challenging. Various ways of monitoring respiratory pattern have been developed over the years. Despite the development in technology, it is believed that the goal of developing a simple non-invasive procedure to measure breathing pattern, which does not interfere with the dynamics of breathing and which can be used outside the laboratory, has yet to be achieved (Balleza *et al*., 2007). There are several ways to monitor breathing parameters either directly or indirectly. For example, expiration duration can be timed directly, while tidal volume can be estimated from measuring airflow or chest wall movement. The desirable features of a monitoring device are:

- Ambulatory: allowing long-term monitoring of breathing pattern during real life activities
- Non-invasive: causing minimal discomfort and changes to breathing pattern
- Simple set-up procedure: allowing users with minimal knowledge of electrical devices to operate the equipment

3.1.1 Opto-electronic plethysmography (OEP)

This monitoring device was introduced in 2001 to record breathing pattern (Aliverti *et al*., 2001). These authors reported the device to be highly accurate in the measurement of total chest wall volume variations in healthy individuals. The major advantages of this system are that it does not require direct connection to the individual and it can be used without a subject-specific calibration, which should theoretically allow respiratory monitoring in hospital or laboratory situations. The device involves the use of between forty-five and eighty-nine reflecting markers placed on the patient's front and back, over the chest wall from clavicles to pubis. Each marker is tracked in three dimensions by four video cameras, two in front of and two behind the subject. An image processor is used to measure the position of each marker, and volume is estimated by measuring changes in the chest wall surface. A three-compartment model of the chest wall is used to estimate lung volume (Aliverti *et al*., 2000). This model is made up of: 1) the pulmonary rib cage, defined as extending caudally from the markers placed on the clavicular line to those placed at the xiphoid level; 2) the abdominal rib cage, defined as extending from the xiphoid level to the lower costal margin; and 3) the abdomen, defined as extending from the lower costal margin to the anterior superior iliac crest line. The volume is calculated as the sum of the three compartments. The positions of the markers are placed to define the geometrical models that describe the whole surface or part thereof (see Figure 3). The volume and its changes due to movement can be calculated by surface integration, using Gauss's theorem to obtain a volume integral. A previous study has demonstrated that the use of an image processor to allow three-dimensional assessment of volume changes can produce accurate results in both healthy adults (n=11) and in patients with ventilation support (n=13) (Aliverti, 2000).

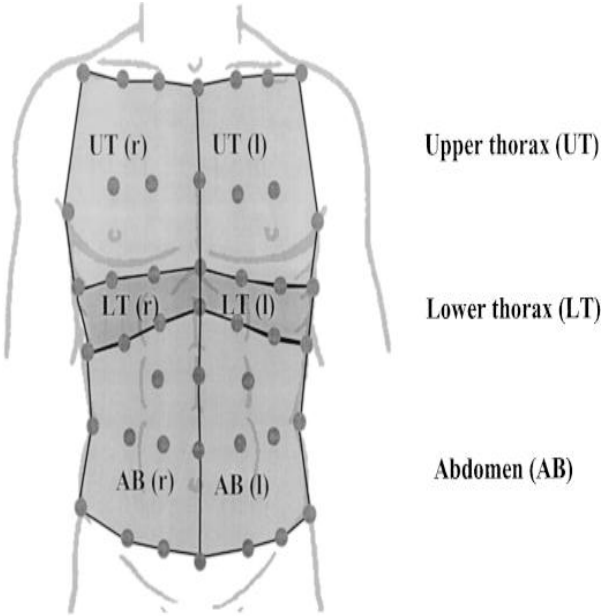


Figure 3: Marker positions of OEP and chest wall compartments (Aliverti, 2000)

Data from the opto-electronic plethysmography were assessed against a spirometer and a pneumotachograph using regression analysis and Bland and Altman analysis. Results showed that the average discrepancy between opto-electronic plethysmography and pneumotachograph was 1.6 % ± 5.4%, which was similar to the discrepancy between spirometry and opto-electronic plethysmography (1.7% ± 5.9%). Bland and Altman plots revealed no systemic bias.

This volume estimation technique has also been used to study the effects of pressure support ventilation on breathing pattern in patients with moderate to severe respiratory failure (Aliverti *et al*., 2006) and to detect changes in oxygen uptake at mouth level and at alveolar level (Wust *et al*., 2008). These two studies have demonstrated the feasibility of using the device to measure respiratory parameters in a laboratory setting. However, the use of such equipment is likely to be confined within the laboratory due to the technical requirements of the device (requiring the use of eighty-nine reflective markers and four video cameras equipped with infra-red ring flash to be set in four particular positions). This device also does not appear to be 'patient-friendly' because the reflective markers need to be placed on the skin, therefore involving the exposure of a large area of the patient's upper body. These drawbacks mean that OEP is not generally suitable for long-term continuous monitoring of breathing.

3.1.2 Electrical impedance tomography

Electrical impedance tomography (EIT) was originally developed as a non-invasive imaging technique in the 1980s (Frerichs, 2000). Its value as an imaging technique has been confirmed in a recent review (Serrano *et al*., 2002). It can be used to take chest images during spontaneous breathing by computing electrical conductivity within the participants (Vauhkonen *et al*., 1999). It works on the principle of applying sinusoidal electrical currents around the surface of the chest using electrodes. Impedance signals are collected via sixteen electrodes placed around the chest. The internal resistivity distributions are calculated and images of the lung can then be computed.

EIT has been applied primarily to obtain information on lung function, as well as to collect information about other organs, such as gastric, brain and cardiovascular function (Frerichs, 2000). One of the advantages of EIT over other imaging devices such as simple impedance pneumography is its ability to provide regional lung function information (Frerichs, 2000).

An example of EIT application in the monitoring of regional lung function in mechanically ventilated patients was described in a study by Frerichs *et al*. (1998). Ten patients were monitored in wards, operating rooms and intensive care units during spontaneous breathing and in different modes of mechanical ventilation. EIT identified a shift in ventilation distribution from the dependent toward the non-dependent lung regions on the transition from spontaneous to mechanical ventilation, and also the recruitment of the dependent lung areas due to the addition of the positive-end-expiratory pressure. The latest generation of EIT is capable of calculating tidal volume from a graphic depiction of a cross-section of the chest during spontaneous breathing (Balleza *et al*., 2007). These authors consider the latest EIT equipment to be a viable means of measuring breathing pattern non-invasively because it involves a relatively easy set-up procedure (Balleza *et al*., 2007). A diagram of the set-up is shown in Figure 4.

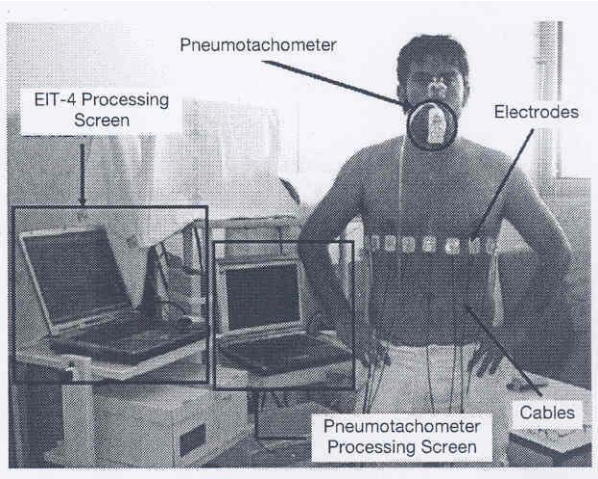


Figure 4: A diagram to show the set-up of the EIT equipment (sourced from Balleza *et al*., 2007)

The study recruited thirteen healthy volunteers for whom tidal volume and respiratory rate were simultaneously monitored by pneumotachograph and an EIT device. The tidal volume for the participants at rest (position not documented) was recorded for periods of thirty seconds with a three-minute rest period between measurements. Between five and eight breaths were recorded for each measurement, and a total of twenty to twenty-five breaths were analysed for each participant. The results showed no significant difference between the measurements obtained by the different devices, and the correlation coefficient between the two tidal volume measurements was 0.923. The authors concluded that the EIT and the pneumotachograph are interchangeable in measuring tidal volume.

However, despite the apparent positive results their interpretation is limited due to insufficient statistical comparison. The agreement in measurement between the two devices was analysed using a t-test on the mean of the tidal volume. A statistically significant difference in the means is not considered to be sufficient analysis of agreement between the measurements (Rankin & Stokes, 1998; Bland & Altman, 1995). This is because it gives no indication of potential bias, or the range or precision of error. A Bland and Altman analysis involves plotting the difference against the average of the measurements, and would show the presence of bias. The 95% limits of agreement would indicate an error range and the magnitude of the disagreement between measurements. This would complement the findings of the t-test. As insufficient statistical analysis was

performed, it is therefore difficult to decide on the validity of the EIT-obtained tidal volume measurements based on this study.

Another potential issue with EIT is that electrode movement or cable contact can cause measurement error. In Balleza's (2007) study, it was observed that electrode movement or cable contact were responsible for over 10% of errors in some cases. This problem would probably be exacerbated during any activity that caused the electrode cable to move to a greater extent. As the application of EIT in estimating lung volume changes is still relatively new, only a small number of published studies that have employed this method have been identified. The validity of the EIT in estimating lung volume changes is yet to be established. On closer examination, the set-up and calibration procedures do not appear to be as simple as the authors claim (see Figure 4). This might affect its potential to be used as a routine clinical examination tool. The device used in the published study was a prototype which does not appear to be commercially available.

3.1.3 Pneumotachograph

The pneumotachograph is regarded as the gold standard in measuring air flow (Witt *et al*., 2006). A pneumotachograph measures changes in pressure across a transducer, caused by air movement in response to the air moving in and out of the lungs. These pressure changes are then converted to air flow measured in litres per second. The flow signals are integrated to give the tidal volume. Measurement requires the use of a face mask or a mouthpiece to form a sealed coupling to the airway. The use of face masks is considered to be 'invasive' (Witt *et al*., 2006) and has been reported to introduce unnatural breathing patterns during data collection (Askanazi *et al*., 1980; Perez & Tobin, 1985). In the study by Askanazi *et al*. (1980), twenty-eight participants were recruited to participate in a study using a canopy-computer-spirometry system (a transparent head chamber with a neck seal). Breathing parameters were monitored under three conditions: a) with a mask; b) with a mouthpiece plus a nose clip; and c) without any attachments to the face or airway. The results showed that when participants were not breathing through either the mask or the mouthpiece and nose clip, there was a significant decrease of tidal volume and minute ventilation. No change in breathing frequency was observed in the study.

A study by Perez and Tobin (1985) used respiratory inductive plethysmography (see next section) as well as pneumotachography to record breathing pattern. Data was acquired with both RIP and PT and then with RIP alone. These authors also observed a significant increase in tidal volume as well as a reduction in frequency when using the mouthpiece. The results of the two studies described above may not be comparable since different types of apparatus were used to monitor breathing patterns. In the study by Perez and Tobin (1985), adhesive tape was used to seal the nose in order to simulate the condition of a mouthpiece and nose clip. This mechanism was not a true reflection of the laboratory apparatus and was likely to have caused changes in breathing pattern, but not necessarily the same changes as would be seen with the standard laboratory equipment.

Another study by Bloch *et al*. (1995) provided further evidence to support the theory that use of a face mask can alter breathing pattern. In their study six healthy men were asked to perform incremental bicycle exercise to volitional exhaustion on two separate occasions, with and without a face mask. Respiratory inductive plethysmography (RIP) was used to record ECG and breathing pattern. The results showed an increase in tidal volume and respiratory cycle time by up to 63% and 33% respectively in face mask breathing during mild exercise. No significant differences were observed between face mask and RIP-recorded breathing pattern during maximal exercise.

However, the application of the results from this study may be limited as there is some doubt about the validity of the data collected by RIP during exercise or when body movement is introduced. Several studies have shown that when body movement occurs, the signal from traditional RIP tends to degrade and the variation in volume estimation by RIP tends to increase (Caretti *et al*., 1994). This may account for some of the differences observed between measurement with and without face masks. A recent study by Fiamma *et al*., (2007) provided further evidence to demonstrate a difference in breathing pattern when it is not simultaneously recorded by the RIP and a face mask. These studies have provided evidence to confirm the theory that monitoring apparatus that involves a mouthpiece or nose clip affects breathing patterns. This technical limitation means that such apparatus may not be ideal for monitoring breathing patterns, and therefore direct evidence for clinically significant changes in breathing pattern is difficult to obtain.

3.1.4 Traditional respiratory inductive plethysmography

The three methods for monitoring breathing pattern that have been described above are laboratory-based, and do not allow monitoring outside a clinical setting. Respiratory inductive plethysmography (RIP) is a standardised, well-validated technology for the accurate estimation of both timing and volumetric respiratory variables (Clarenbach *et al*., 2005). RIP is an indirect measurement of ventilation and is based on Konno and Mead's (1967) principle that the chest wall possesses two compartments, the rib cage and the abdomen, and the summation of the calibrated rib cage and abdomen signals equates to the tidal volume. During normal breathing, airflow is a result of changes in intra-thoracic pressure brought about by the movement of the chest wall. Movement of the chest wall is generated by the respiratory muscles. A detailed description of chest wall mechanics was given in Section 2.1.

Konno and Mead (1967) proposed that since the movement of air is generated by chest wall movement, the amount of air moving in and out of the lungs must equate to the amount of chest wall movement. Therefore, by measuring the displacement of the rib cage and the abdomen, lung volume can be estimated. RIP is a method to estimate lung volume changes by recording the surface inductance changes in current-carrying wired coils applied to the abdomen and to the rib cage (Loveridge *et al*., 1983). Traditional RIP devices consist of two bands, with one band located at mid-chest level and another at the umbilical cord level. Each band is elastic and is embedded with wire arranged in a zigzag fashion to allow for expansion and contraction when it is worn around the chest and abdomen. Alternating current is passed through the wire to generate a magnetic field. The motion of breathing (i.e. chest and abdomen movement) then causes a change in the cross-sectional area of the enclosed field, thus inducing an opposing current which alters the frequency of the applied current. The signal produced is linear, and is a fairly accurate representation of the change in cross-sectional area. A calibration procedure is required to convert the changes in measured cross-sectional area into volume. Methods of calibration will be discussed in later sections.

RIP has a number of advantages over other monitoring methods, including its simplicity to set up. The latest version of RIP, embedded within a garment (e.g. the LifeShirt®), is claimed to offer accurate monitoring outside a laboratory or clinical setting. It is widely use in clinical and research settings to monitor respiratory pattern non-invasively, and several studies have demonstrated close agreement between ventilation measurements from laboratory standard equipment and those from RIP in healthy individuals (Bloch *et al*., 1995) and in patients with cardiac and pulmonary disease (Bloch *et al*., 1997). Inspiration and expiration time may be extracted from recorded signals, as well as the different contributions of the ribcage and abdomen to tidal volume.

This property of measuring the different contributions of ribcage and abdomen enables their relationship during the respiratory cycle to be studied. Teramoto (1995) used RIP to investigate whether age affected the relative contributions of the ribcage and abdomen in the respiratory cycle during exercise. In this study, twelve young (aged between 21 and 60) and twelve elderly (aged between 65 and 78) normal male volunteers were recruited. Breathing was simultaneously monitored by pneumotachograph and RIP during resting and during three minutes of exercise, measured at 20 watts and 40 watts on a cycle ergometer. Only data from the last minute of exercise were analysed, as it was revealed in the pilot study that the breath-to-breath variations of measurements were significant in the first minute of the exercise. The results demonstrated that the fractional contribution of chest and abdomen was similar in both age groups during the resting period. However, during exercise the increase in abdominal contribution during exercise was greater in the elderly than in the young. Although it is recognised that chest wall compliance in the elderly is lower than in the young, the relative abdominal and thoracic contribution during exercise has not been assessed quantitatively. The ability of RIP to record relative rib cage and abdomen contributions to a breathing cycle may be valuable in quantifying breathing pattern for patients with breathing disorders such as asthma, disproportionate breathlessness and hyperventilation syndrome.

Despite its widespread use, many studies have demonstrated artefactual problems with the traditional RIP band since signal qualities tend to degrade as a result of the movement of the individual bands around the chest and abdomen caused by locomotion (Witt *et al*., 2006). Previous studies on ventilation monitoring with RIP technology during sleep and exercise have demonstrated poor agreement with values obtained from a standard pneumotachograph (the laboratory gold standard for measuring flow and volume). In particular, one study investigated the accuracy of RIP in patients with sleep apnoea by comparing the results of RIP and a pneumotachograph (Cantineau *et al*., 1992). Twenty patients with a history of sleep apnoea syndrome were recruited from a hospital. Data from only thirteen of the patients were included because the thoracic belt of the RIP failed intermittently during the course of the study, suggesting the vulnerability of the equipment.

In this study calibrations were performed during voluntary changes to thoracic and abdominal efforts during tidal volumes of different size. This was to calibrate the equipment during different amounts of thoracic and abdominal movement and hence during different tidal volumes, which should result in more accurate calibrations. The results suggested that there were measurement errors in tidal volume. The margin of error was positively correlated with the level of body movement and with undetected body position changes.

Similarly, another study also demonstrated that the agreement of measurement between RIP and simultaneously measured flowmeter volumes was low (Caretti et al., 1994). In this study eight healthy volunteers were recruited and tidal volumes from RIP were compared with simultaneous flowmeter volumes during five incremental work rates (60W, 90W, 120W, 150W and 180W) during cycling and treadmill exercise. Results showed that when intensity was below 150W, the measurements for approximately 17% of the participants did not differ significantly between the two methods. As the intensity increased to 180W, 50% of the participants demonstrated a significant difference between methods. This suggested that the reliability of the instrument decreased as the level of work intensity increased, possibly due to slippage of the RIP elastic bands or as the

calibration factor became unsuitable (calibration will be discussed in Section 3.2). Taken together, these studies have demonstrated the limitations of the traditional RIP system and confirmed that the application of RIP in ambulatory patients is problematic because the sensors not only respond to respiratory activity but also to body movement and postural changes.

3.1.5 LifeShirt®

The LifeShirt® system is based on the RIP technology, but it claims to overcome the artefactual problems described above by having sensors embedded into a wearable garment. The manufacturer claims the garment is able to 'collect laboratory-quality data in any environment to reflect true physiological response from the real world' (VivoMetric user manual, VivoMetric). The RIP sensors in the LifeShirt® consist of a sinuous arrangement of electrical wires that are excited through an extremely low current in an electrical oscillator circuit. One sensor is sewn into the shirt at the level of the rib cage (at the fourth intercostal space) and one at the level of the umbilicus. The signals are written to a removable memory card in a handheld computer (Visor^{TM}, Handspring Inc., California), and then analysed offline. From these signals, a variety of calibrated respiratory pattern measures are extracted, such as minute ventilation, tidal volume, respiratory rate, fractional inspiratory time, peak inspiratory flow, thoraco-abdominal co-ordination, and change in end-expiratory lung volume.

The advantage of garment-based RIP is illustrated in a case study of disproportionate breathlessness that is associated with deep sighing breathing in a patient with asthma (Prys-Picard *et al*., 2006). The LifeShirt® was used to monitor breathing pattern during baseline and during an incremental shuttle walk test. Deep sigh was monitored as an outcome measure. Frequent deep sigh (defined as four times the magnitude of tidal volume) was observed at baseline for the participant (six sighs during a five-minute period). An increase in respiratory rate, tidal volume and the number of sighs (at least twenty-one sighs over five minutes) was observed during the shuttle walk test. After undergoing breathing retraining (which consisted of five monthly sessions of physiotherapist-led breathing control and re-education, relaxation and stress management, as well as a graduated activity/exercise program), the patient's condition improved. A decrease in resting sigh rate was seen (to fifteen sighs per fifteen minutes), and breathlessness also decreased.

The generalisability of these findings in terms of the effect of breathing retraining on sigh pattern is limited as it was a single case study and the experimental protocol was not documented in detail. The calibration procedure of the garment-based RIP was not documented, and this might have had an effect on the accuracy of the RIP signal (see Section 3.2 on calibration). However, this study did demonstrate the feasibility of using the LifeShirt® in clinical settings. It was hypothesised by the author of the study that this technology may be useful in a clinical setting as an outcome measure tool, to monitor physiological changes as a result of an intervention, or to identify abnormal breathing patterns which are difficult to quantify or observe during clinical examination.

3.1.6 Summary

The above section described several methods for monitoring breathing parameters. It highlighted certain issues with the laboratory-based gold standard equipment. Currently RIP is the leading technology in ambulatory monitoring, despite the drawbacks that have been reported. The latest version of RIP, the LifeShirt®, claims to have addressed some of the issues of the traditional RIP bands. The next section will critically discuss and evaluate some studies investigating the reliability and the validity of the LifeShirt®.

3.1.7 Reliability and validity of the respiratory parameters measured by the LifeShirt®

This section will critically discuss and evaluate studies that have investigated the reliability and the validity of the respiratory parameters measured by the LifeShirt®.

To date, there are a small number of published studies that have investigated the validity and reliability of the LifeShirt® in monitoring respiratory parameters. A study by Witt *et al*. (2006) investigated the validity of the LifeShirt® during exercise by comparing the data from the LifeShirt® with a standard reference pneumotachograph. In this study ten healthy young male volunteers (aged 23.4 ±2.3 years) were recruited, and their breathing patterns were simultaneously monitored by RIP and pneumotachograph. Data were recorded continuously during five minutes of standing rest, four five-minute stages of incremental treadmill test to exhaustion, and up to four minutes of standing exercise recovery. The parameters monitored were breathing frequency, tidal volume and minute ventilation. Agreement between methods was assessed by two-way repeated-measures analysis of variance, and linear regression between the two instruments using the minute mean values of tidal volume and breathing frequency. All the RIP signals were used for data analysis. Agreements and bias between methods were also analysed by the Bland and Altman method (Bland & Altman, 1986).

No statistically significant differences between the methods were observed in terms of breathing frequency, tidal volume and minute ventilation at any exercise stage ($p>0.05$), and the Bland and Altman tests revealed no systematic bias for the parameters. This study seems to suggest that the LifeShirt® device can produce close estimations of ventilation parameters in relation to the pneumotachograph. However, there are a number of methodological weaknesses with the study. Firstly, since this study only involves male participants, the results are not generalisable to a female population. The quality of the recorded signals may be prone to degradation due to the female

upper body shape, which might cause acceleration forces that could produce artefactual problems. Secondly, this study lacks external validity as the sample was limited to individuals from a university community who may not be typical of the wider population. Furthermore, despite the apparent positive result, these findings may be due to a potential flaw in the calibration procedure. The RIP was calibrated by the simultaneous use of the pneumotachograph, to which the RIP was then compared. This was done because the calibration process converts the chest wall area into a volume estimation, and the reference value obtained from the pneumotachograph scales the RIP signals to match the signals from the pneumotachograph – i.e. x amount of change in chest wall area equals x amount of changes in tidal volume. Therefore, by calibrating and then comparing the RIP with the same pneumotachograph, the RIP signal is very likely to be a close match with the pneumotachograph because they were matched together during the calibration process.

Another study by Clarenbach *et al*. (2006) also evaluated the validity of the LifeShirt® in a pathological population, by comparing respiratory variables recorded with the respiratory data from a pneumotachograph in healthy adults and patients with congestive heart failure (CHF) and chronic obstructive pulmonary disease (COPD). A total of thirty-one participants were recruited, including twenty healthy volunteers (mean age 32 ±7 years), five patients with CHF (mean age 57 ±22 years) and six patients with COPD (mean age 62 ±8 years). Patients were recruited from referrals after a cardiopulmonary exercise performance evaluation. After calibration of the instruments, breathing patterns were simultaneously monitored by RIP and the pneumotachograph during progressive treadmill exercise until exhaustion. The speed of the treadmill was increased approximately every five minutes, and the exercise period was divided into phases of low, intermediate, submaximal and maximal effort.

After data collection twenty breaths were selected from each intensity period for data analysis. The level of agreement between the two monitoring methods was evaluated by computing the bias and limits of agreement using a Bland and Altman analysis (Bland & Altman, 1986). The results demonstrated no

significant bias between the LifeShirt® and flowmeter-derived breath-by-breath inspiratory volume, inspiratory minute ventilation and duration of respiratory cycle in any group of subjects. Comparisons of the mean values from the two methods also showed close agreement, with all values of tidal volume and the respiratory rate of all included respiratory cycles falling between 5% of each other.

The results appeared to suggest that the LifeShirt® provided comparable measurements of breathing patterns in relation to the laboratory standard. However, an element of bias may exist in the data which could affect the strength of the evidence. The process or the criteria for breath selection was not documented in the article. The author selected twenty breaths for breath-by-breath analysis, and stated that these twenty breaths would represent approximately thirty seconds, the period recommended by American Thoracic Society (ATS) guidelines for analysing data obtained in exercise testing. However, in fact the published recommendation was to use the mean value averaged over a period of not shorter than thirty seconds for analysis. The study by Fiamma *et al*. (2007a) demonstrated that breathing is a complicated process and large variability may exist between individual breaths. Without a description of the breath selection process, it is impossible to fully assess the validity of the study. There was a period of 'stabilisation', which was the period from when data recording began until breathing pattern had stabilised. No explanation was given to explain what constituted the state of being 'stabilised'. This could introduce a degree of subjectivity into the data, which may affect validity of the results.

The authors also concluded that the LifeShirt® was equally accurate in measuring ventilation parameters in healthy individuals and in patients with COPD and CHF. However, the comparison between groups may not have been representative because the number of participants in each group was not equal; there were more healthy individuals (n=20) than CHF (n=5) and COPD (n=6). The unequal distribution between groups means it would be more likely to achieve better agreement in the healthy group than the CHF (Northridge *et al*.,

1990) or the COPD groups, since there were a higher numbers of breaths in the sample population.

In a more recent study (Kent *et al*., 2009), both the validity and the reliability of the LifeShirt® were investigated. Sixteen healthy individuals aged between 18 and 30 (six males, ten females) were recruited to the study. Breathing patterns were simultaneously recorded by the LifeShirt® and a pneumotachograph during incremental exercise and exercise at a constant work rate (recorded on different days). The incremental exercise protocol was a standardised exponential exercise protocol (Northridge *et al*., 1990), which starts at a low work load and increases exponentially every minute. All participants started at stage 3 (the lowest level) and continued until volitional fatigue. The work level during the constant work rate test was determined by a target heart rate equivalent to 65% of peak oxygen uptake, estimated from peak heart rate recorded during the incremental test on the first visit. Mean values for the last thirty seconds of each stage on the incremental test were used for analysis. This was because the initial analysis of respiratory pattern on a breath-by-breath basis between the LifeShirt® and the pneumotachograph revealed a large amount of variability in both devices, and the data were therefore deemed unsuitable for breath-by-breath analysis.

The validity and reliability of the parameters of tidal volume, respiratory rate and expiratory time were assessed using repeated-measures ANOVA, and the results were reported in terms of the significance of the bias and coefficients of variation (CV). Eight stages of the incremental exercise protocol were completed by all participants (stage 3 to stage 11). For incremental exercise, the results showed no significant differences in tidal volume ($p=0.055$) between the spirometer-calibrated LifeShirt® and the pneumotachograph from stage 3 to stage 6. However, from stage 7 onwards the difference between the systems became significant and increased progressively. The largest differences were observed at stage 11. The CV of tidal volume between devices was 3.8%, increasing to 9.5% at stage 11. No significant bias was observed over the two days for the LifeShirt® data. A significant bias was observed in expiratory time

between the systems, but as exercise intensity increased the difference between the systems became smaller. The CV of expiration time between the systems was 2.5% at stage three, and the mean CV was 2.2%. No significant bias was observed over the different testing days.

For the constant work rate test, a significant bias was observed in tidal volume, respiratory rate and expiratory time between the devices, but no significant bias was observed between days. The authors concluded that the LifeShirt® can provide valid and reliable respiratory data across multiple assessments. However, the results do appear to suggest that respiratory parameters measured by the LifeShirt® differ significantly from those measured by the pneumotachograph when breathing intensifies from moderate intensity to higher intensity. Kent *et al*. (2009) did not offer any explanations for the difference observed. It is therefore hypothesised by the author of this study that the difference may be due to the unsuitability of the calibrating factor, which was obtained at the beginning of recording period during quiet breathing, and its applicability to a changed breathing strategy adopted during exercise.

The results from Kent *et al*. (2009) should also be interpreted with caution because they had similar weaknesses to those from the study by Clarenbach (2005) in that not all the recorded data were analysed. Although the American Thoracic Society recommended that respiratory data from exercise testing procedures should be averaged over an interval of not less than thirty seconds (ATS/ACCP, 2003), the use of only a thirty-second window might be not be a suitable method to validate the LifeShirt® with a pneumotachograph. Although the use of interval averaged data would give a stable measurement, some of the details may be lost. For example, if the LifeShirt® recorded three consecutive breaths of tidal volume of 450ml, 700ml and 650ml, this would give a mean value of 600ml. However, if the pneumotachograph recorded the same three breaths as having a tidal volume of 300ml, 500ml and 1000ml, this would also give a mean value of 600ml. This demonstrates that even when the mean values are the same, it does not follow that the LifeShirt® is measuring a valid tidal volume relative to the pneumotachograph. While it might be acceptable to

use interval averaged data for measurement of physiological trends, it may not be suitable to use interval averaged data for investigating validity. Previous LifeShirt® validity studies have stated that it is appropriate to follow the ATS guidelines of using the mean value over an interval for analysis; however, the same ATS guidelines also made a recommendation that all breaths collected should be used in the processing of the data. Neither of the studies by Kent *et al*. (2009) and Clarenbach *et al*. (2006) included all the recorded data in their analysis. This casts some doubts over the validity of their findings.

The justification for not doing breath-by-breath analysis is that it is fundamentally open to variation. In their preliminary analysis, Kent *et al*. (2009) reported the coefficient of variation for tidal volume of the pneumotachograph and the LifeShirt® calculated on a breath-by-breath basis to be 17.5% and 18.9% respectively. The data set was then deemed to contain too much variability, and subsequently the mean values of the final thirty seconds of each stage were used for validity analysis. However, two other studies have reported a mean CV for tidal volume of 23.96% ±9.07% (Wysocki *et al*., 2006b) and 20% (Fiamma *et al*., 2007b) for healthy individuals at rest breathing through a pneumotachograph. This seems to indicate that variability is normal in any breathing pattern, as is the case with other physiological systems such as cardiac rhythm (Poon & Merrill, 1997).

There are studies which have successfully demonstrated the use of breath-by-breath comparisons between RIP and pneumotachograph (Cantineau *et al*., 1992; Fiamma *et al*., 2007a; Poole *et al*., 2000). Cantineau *et al*. (1992) investigated the accuracy of the RIP during sleep in thirteen obese patients with obstructive sleep apnoea. Respiratory pattern was monitored during wakefulness and sleep by RIP and pneumotachograph simultaneously. The recording period was between two and four hours. The results showed that the mean correlation coefficient between devices was greater than 0.9 when the person was awake. A tendency to greater dispersion was observed at volumes greater than 600ml. During sleep, the difference between devices was significantly different from zero. However, mean RIP-measured tidal volume

was between 10% and 20% in eleven participants and between 20% and 30% in two participants. A clear tendency for greater dispersion was observed at volumes greater than 800ml. Specific care was taken to measure the volume displaced by movement recorded by RIP only between the times of onset and end of the inspiration recorded by the pneumotachograph. This study demonstrated that breath-by-breath analysis is possible in sleeping adults.

Another study by Fiamma *et al*. (2007) also demonstrated that it is possible to examine breath–by-breath variability of breathing. Eight healthy individuals were recruited, and breathing signals from the LifeShirt® and a pneumotachograph were simultaneously and consecutively recorded and compared. After a fifteen-minute stabilisation period data were collected over ten-minute epochs under randomly selected conditions of simultaneous or consecutive acquisition of LifeShirt® and pneumotachograph signals. Signals from both devices were analysed in MatLab using custom routines for analysis. The tidal volume signal obtained by RIP was differentiated to give the flow signal and then compared with the flow signal obtained from the pneumotachograph. Noise titration techniques (Poon & Barahona, 2001) were used to identify chaos. The indices of noise limit (the index for chaos), the largest Lyapnunov exponent (the index of the sensitivity of the system to its initial conditions) and the correlation dimension (a fractal dimension reflecting the irregularity of the system) all demonstrated that the flow signals between the devices were interchangeable when breathing was simultaneously monitored. Mean tidal volume, mean expiratory and inspiratory time and duty cycle were all significantly correlated during simultaneous monitoring. The results of this study suggest that significant breath-by-breath variability exists in normal breathing patterns, and this is comparable between measurements from the LifeShirt® and the pneumotachograph. In this study, the results for ventilatory parameters should be interpreted with caution because only the mean value was reported. However, the differentiation of the RIP volume to get the flow demonstrated the robustness of the method, because this would tend to add more noise into the signals than the integration of the flow from the pneumotachograph to get the volume. If the flow signal is comparable on a breath-by-breath basis, the volume

should also be comparable on the same basis since it is obtained by the integration of the flow signal.

One of the possible reasons for the significant difference between devices when using breath by breath analysis could be the associated commercial software from the two devices using different criteria to define a breath. Although both the pneumotachograph and RIP present data related to tidal volume, they measure fundamentally different aspects (as described briefly in Section 1.1). RIP measures the cross-sectional area changes in the chest wall, whereas a pneumotachograph indirectly measures tidal volume from the speed of air flow. Therefore, their associated software might well use different criteria to define a breath. This might explain why Teramoto (1995) and Kent *et al*. (2009) reported significant differences for breath-by-breath analysis (which subsequently led to the use of interval averaged data), whereas Fiamma *et al*. (2007) demonstrated that flow signals were interchangeable between the pneumotachograph and RIP when assessing the signals using separate software. To date, there are few published studies that have compared the LifeShirt®'s signals for respiratory parameters to the pneumotachograph signals using third-party software. The advantage of analysing respiratory signals is that this type of analysis can minimise the potential error due to different interpretations of what constitutes a breath.

In most of the published LifeShirt® studies the authors depended on the company who manufactured and marketed the LifeShirt® for the analysis of their LifeShirt® data. While this is not in itself indicative of any irregularity, positive findings would be more convincing if the data were independently analysed by researchers with no vested interest in a positive outcome. Therefore, there was a perceived need for an independent appraisal of the LifeShirt® equipment and software to validate the equipment before it could be recommended with confidence.

3.1.8 Summary

This section described several available methods for the non-invasive monitoring of breathing pattern. The LifeShirt® was believed to have the potential to give accurate monitoring outside the laboratory. Despite a few studies that have investigated the validity of the LifeShirt®, there were still many questions to be answered. This was due to: 1) the use of interval averaged data in the majority of studies, which may not give a representative picture of the LifeShirt®'s measuring capabilities; 2) the potential for differences in breath definition between the LifeShirt® and any comparative device, which may affect breath-by-breath analysis; and 3) the dependence on a commercial company and their software for the analysis of the LifeShirt® data.

The next section describes the issues surrounding the calibration of the LifeShirt® and RIP systems, as this has a major impact on validity.

3.2 Introduction to calibration

A calibration procedure is required when any parameter is being measured indirectly – for example, when tidal volume is being deduced from movements of the chest wall or from the speed of air flow, depending on the device used. This section discusses methods of calibration for the pneumotachograph and the respiratory inductive plethysmography.

3.2.1 Calibration of pneumotachograph

The calibration of the pneumotachograph is straightforward since it involves a standard operating procedure, injecting a known volume of gas into the transducer using a calibrating syringe. The software can then perform a calibration with the known volume of gas. There is no other recognised method to calibrate a pneumotachograph.

3.2.2 Calibration of RIP

RIP technology estimates lung volume by measuring changes in the cross-sectional area of the chest and abdomen. A calibration procedure is required to quantify the changes in volumetric measurement derived from the change in area (Springer *et al*., 1996). Without such calibration, RIP can only provide qualitative changes of parameters (Brown *et al*., 1998).

RIP is based on the principle that the chest may be divided into two separate volumes associated with the rib cage and the abdomen respectively (Konno & Mead, 1967). Konno and Mead observed that although these compartments move together as a unit, there is also a considerable amount of independence between the compartments; i.e. one could inspire mainly with the rib cage or with the abdomen, and even cause outward displacements of one part while moving the other inward. This manoeuvre takes advantage of the unitary behaviour of the rib cage and the abdomen, and the ability to move each compartment separately. It is proposed that the rib cage and abdomen, with the

diaphragm separating the two, can be considered as two independent parts, so that the system contains two degrees of freedom.

A system can either be closed or open. In a closed system, volume changes take place among the parts but a constant volume is maintained within the system. The number of degrees of freedom in a closed system is one less than the number of parts in the system. An open system can exchange volume with its surroundings, and the number of degrees of freedom is equal to the number of parts within the system. In a respiratory system with two degrees of freedom, the summation of the movements of the chest and abdomen is equal to the tidal volume measure obtained by the pneumotachograph:

$$\Delta V_T = \Delta V_{RC} + \Delta V_{AB} \qquad (1)$$

where ΔV_T is the change of tidal volume (V_T) measured by the pneumotachograph, and ΔV_{RC} and ΔV_{AB} are changes in the rib cage (RC) and abdomen (AB) compartments respectively.

According to equation 1, the change in tidal volume can be derived from the area change of the chest and abdomen regions. RIP applies electromagnetic waves sinusoidally across the chest and abdomen, and from changes in these waves the changes in the area of each compartment can be measured. By measuring the changes in area within each compartment with external sensors and calibrating these changes against a spirometer, an absolute volumetric measurement can be calculated (Martinot-Lagarde *et al*., 1988). As each compartment is capable of moving separately, they do not always move by the same amount. A calibration coefficient must be obtained to adjust for this and to give the rib cage and abdomen an equal weighting. The changes in area of the combined rib cage and abdomen will therefore equate to the tidal volume measure at mouth levels. The Konno and Mead model has the assumption that the two compartments remain at a constant height during breathing and only considers the change in area within the two compartments. This is a weakness of this model since the chest wall is capable of vertical movement. Any distortion of this assumption e.g. apical breathing, changes of posture, sitting,

standing, or slump may cause the model to fail and affect the volume estimation.

The calibration methods for RIP consist of two steps: firstly, establish the electrical proportional relationship of the chest and abdomen change measurements; secondly, apply an external reference value to obtain the absolute values of volume change. There are four calibration methods that have been proposed by various authors: i) isovolume manoeuvre calibration (Konno & Mead, 1967); ii) quantitative diagnostic calibration (Sackner *et al*., 1989); iii) multiple linear regression (Loveridge *et al*., 1983); and iv) single K constant (Banzett *et al*., 1995). All four methods are based on the volume estimation model proposed by Konno and Mead (1967), but they use different mathematical methods to perform the two steps.

3.2.3 Isovolume manoeuvre calibration (Konno & Mead, 1967)

The isovolume manoeuvre was the earliest proposed method for estimating lung volume by measuring the displacement across the rib cage and the abdomen. The manoeuvre involves the subject moving as much gas as possible back and forth between the rib cage and abdomen while the upper airway is occluded and without flexing and extending the spine. The manoeuver is performed slowly, taking about five to ten seconds to complete one cycle. During the manoeuvre the system has one degree of freedom (because it is a closed system), and the volume within the system remains constant. It assumes that the height of the other two compartments stays constant during a breathing cycle. The change of volume within one part must therefore be equal and opposite to the volume change in the other part. The volume changes, as recorded by a spirometer or a pneumotachograph, correspond to the motion changes of the rib cage and the abdomen. Since the total volume change is known, once the change in one of the two parts is known, the volume change of the other part may be obtained by subtraction. The proportional relationship between chest and abdomen is calculated using a X-Y recorder through a transducer. The signals collected are then used to plot the volume-motion displacement, and the proportional relationship of chest and abdomen can be calculated.

Konno and Mead demonstrated in their original paper that the anterior posterior motions of the rib cage and abdomen are close to linear and the volume-motion relationship of the rib cage and abdomen is also close to linear during breathing at rest. However, when breathing at extreme volume (100% of total lung capacity) the linearity between rib cage and abdomen decreases. It was thought that the reduction of linearity is likely due to the chest wall having more than one degree of freedom, and breathing at maximum volume causes distortion of the chest wall beyond the limits of ordinary breathing. This distortion of the chest wall at extreme lung volume is likely to be due to the use of accessory muscles, which produce vertical as well as lateral movement of the rib cage. However,

the effect of vertical movement of the chest wall on volume estimation was not further investigated in the original study.

No other subsequent studies were found during the literature search that had investigated the impact of vertical movement of the chest wall on this calibration model.

The theory of volume method estimation is widely accepted and forms the basis of various calibration methods. However, the isovolume manoeuvre method itself has not been used in many research studies due to its disadvantages. The main disadvantage is that this calibration procedure requires the participants' co-operation to perform a manoeuvre. This may not always be possible with infants or young children, or with those who are critically ill or uncooperative (Sackner *et al*., 1989). It is also widely accepted that only individuals who have received specific training can perform the isovolume manoeuvre correctly, making it not possible for everyone and impractical for many others. Due to these disadvantages several other variations have been proposed.

3.2.4 Qualitative diagnostic calibration (Sackner *et al*., 1989)

The qualitative diagnostic calibration (QDC) method was proposed by Sackner as an alternative to the isovolume calibration method. It is a computerised method that can be performed during natural breathing (Sackner *et al*., 1989), which makes it an attractive method because it does not require any participant cooperation to perform a specific manoeuvre, and it can be performed during natural breathing with a single posture. It became very popular and is now widely accepted as a calibration method for RIP (Bar-Yishay *et al*., 2003).

Based on the theory developed by Konno and Mead (1967), Sackner *et al*. (1989) used the following formula to demonstrate the relationship between volume and chest wall:

$$\Delta V_T = M(K(\Delta V_{RC}) + \Delta V_{AB}) \qquad (2)$$

where K refers to the electrical proportional relationship between the uncalibrated signals from RIP. The term M put the value of $(K(\Delta V_{RC}) + \Delta V_{AB})$ into the scale equivalent to the change of tidal volume as measured with a spirometer. When ΔV_T remains constant and the constant M can be treated as a unit gain, then equation 2 can be rewritten as:

$$K = -\Delta V_{AB} / \Delta V_{RC} \qquad (3)$$

Instead of using the isovolume manoeuvre to produce zero change in ΔV_T in order to calculate K, Sackner *et al*. (1989) proposed that the collection of a large number of breaths (a period of five minutes was deemed sufficient) with the exclusion of breaths with a large deviation from the mean (i.e. a standard deviation of more than ± 1.0) might provide an approximation for a constant volume. K could then be solved by breath-to-breath standard deviation of ΔV_{AB} and ΔV_{RC} signals:

$$K = -SD(\Delta V_{AB})/SD(\Delta V_{RC}) \qquad (4)$$

This method is therefore based on two assumptions: 1) the respiratory system is assumed to move solely within two degrees of freedom of motion (based on Konno and Mead's model); and 2) statistical selection of breaths from a

calibration period is assumed to provide a constant tidal volume when tidal volume is in fact near-constant. Despite the assumptions, Sackner (1989) performed several experimental protocols to demonstrate the use of the QDC method for the estimation of K to set the electrical gain of the RC and AB, which in turn allowed the close estimation of tidal volume using the isovolume manoeuver.

In the first protocol of the original validation study, the value of K calculated by the QDC method was compared with the K obtained from the isovolume manoeuvre during the collection period of regular breathing and varied tidal volume amplitudes as exemplified by simulated Cheyne-Stokes respiration. Cheyne-Stokes respiration is a disorder characterised by a crescendo-decrescendo pattern of tidal volume and the recurrence of central apnoeas during sleep (Naughton, 1998). Tidal volume obtained by RIP (calibrated by QDC and the isovolume manoeuvre) and spirometer in a supine posture during both normal breathing and Cheyne-Stokes respiration were also compared (n=10). Calculations of K using QDC and the isovolume manoeuvre were obtained during one, three and five minutes of regular breathing and during Cheyne-Strokes breathing.

The results showed no significant difference among the means or the variance of these trials between the values of K obtained from QDC and the isovolume manoeuvre. The comparison of tidal volume showed that all RIP values had a deviation within 10% from the spirometry values for all trials. Five minutes of regular breathing and Cheyne-Stokes breathing had mean deviations of 3.4% ±1.6 and 4.9% ±3.1 respectively. The isovolume manoeuvre had a mean deviation of 2.6%. The percentage of the deviation of the absolute values of volume obtained by RIP increased as the length of the calibration period decreased. There was no significant difference between the tidal volumes obtained by RIP and spirometer in all QDC trials. A significant difference was observed between the isovolume manoeuvre and spirometer values, despite the isovolume values having the lowest percentage deviation from the spirometer values. Results from this protocol seem to confirm that QDC is at

least as good as other methods to adjust the electrical gain for RC and AB for tidal volume estimation during quiet breathing and Cheyne-Stokes breathing in a single posture.

In the second protocol of the original validation study, QDC was tested in six subjects to evaluate whether it remains stable in different positions (supine, right-lying, left-lying, prone and sitting), for different breathing strategies (natural breathing, preferential thoracic breath and preferential abdominal breathing) and at different volumes (voluntary changes of breathing volume from 300ml to 2200ml). RIP was calibrated with QDC using five minutes of regular breathing in a supine posture. The values of K and M obtained during the calibration period were kept constant during positional changes and varying tidal volumes.

The results demonstrated that all values of RIP for natural tidal breathing were within 20% of the spirometer volume in all four positions. The plot of the tidal volume measured by spirometer against the tidal volume measured by RIP showed a greater scatter for preferential abdominal than for natural breathing, but less scatter for abdominal than thoracic breathing. This seems to suggest that changes in breathing strategy may decrease the agreement between devices. Changes in position increased the deviation to between 30% and 40% between the tidal volumes obtained by spirometer and RIP. All values fell within a 20% deviation for varied amplitude. The supine position gave the lowest percentage deviation (10% ±8.3%), while sitting produced the biggest percentage deviation (20% ±14.6%).

These results suggest that the QDC method is capable of producing K values that permit accurate setting of electrical gains of rib cage and abdomen during natural breathing and varied amplitude breathing when subjects remained in the calibration position. However, the results also indicate that when breathing pattern is recorded during changes from the condition of calibration (a change of body position, breathing amplitudes, preferential thoracic and abdominal strategy and postures), the accuracy of the tidal volume estimation deteriorates. One possible explanation for this is that the K value obtained during the

calibration period do not necessarily apply to the rest of the recording period (Millard, 2002).

A similar situation was also observed in a recent study (described earlier) that used QDC as a calibration method. Kent *et al*. (2009) compared the tidal volume between a RIP device and a pneumotachograph during incremental exercise. No significant difference was observed between the tidal volumes recorded by RIP and pneumotachograph during low intensity exercise, but differences became significant when the exercise level intensified. Similar results were also reported in a study by Caretti *et al*. (1994); when exercise level increased, the agreement between measurements decreased. These studies appeared to suggest that when conditions change (e.g. during activity), the original estimation for K may no longer be appropriate.

The statistical analysis in Sackner *et al*. (1989) regarding the validity of the QDC method was not sufficient to demonstrate the agreement between the two calibration methods. The difference between the measurements made by QDC and a spirometer were expressed as a percentage deviation. This analysis did not show any other property of the QDC method, e.g. whether it is overestimating or underestimating, and under what circumstances. The absolute differences between tidal volumes obtained by QDC and spirometer were not documented in any except the first protocol. This information is important to determine whether QDC is a suitable calibration method. Sackner *et al*. (1989) used a t-test to compare the means and standard deviations of the tidal volumes obtained from RIP and spirometer. As described previously in Section 3.1.2, the use of t-tests is not sufficient to determine the agreement between measurements (Bland & Altman, 1995; Rankin & Stokes, 1998). Although no statistically significant difference was observed, the mean and standard deviation were not sufficient to demonstrate the validity of QDC, especially when the sample population was relatively small (n=10).

Results from recent studies have raised some doubts about the reliability of the QDC method of calibration (Brown *et al*., 1998; De Groote *et al*., 2001;

Thompson, 1999). Brown *et al*. (1998) conducted a study to evaluate the QDC method with twenty-three anaesthetised infants, comparing tidal volumes obtained by QDC-calibrated RIP signals under various measurement conditions: natural breathing, mechanical ventilation, and sighs. Calibration factors were obtained from the simultaneous use of a pneumotachograph. A section of five minutes' recording was isolated for QDC calibration. After five minutes, recording for natural breathing began. The recording of intermittent positive pressure ventilation (IPPV) commenced approximately fifteen minutes after calibration. Data were recorded by Respitrace systems (a form of RIP not in a garment), and QDC was performed with the associated software. The agreement between the two methods was assessed by a Bland and Altman test, and 95% limits of agreement were calculated.

The results demonstrated no significant differences between volumes obtained by RIP and pneumotachograph under all circumstances. However, despite the apparently positive result, these findings may be due to a potential flaw in the calibration procedure, as was also the case with the study by Witt *et al*., (2006). This problem is that the RIP was calibrated by the simultaneous use of the pneumotachograph, to which the RIP was then compared. This is not appropriate because the calibration process quantifies the chest wall area into volume estimation, and the reference value obtained from the pneumotachograph scales the RIP signals to match the signals with the pneumotachograph. Therefore, by calibrating and then comparing the RIP with the same pneumotachograph, the RIP signal is very likely to be a close match with the pneumotachograph.

Although there was no significant difference between tidal volume measured by RIP and pneumotachograph, there was a marked difference in the 95% limits of agreement immediately after calibration (-2.3-3.0%), ten minutes after calibration (-9.6-10.2%) and at high IPPV (-38.1-31.0%). This suggests that the variance of the data increases either over time or with varying breathing pattern. It is recognised that the induction of anaesthesia is associated with changes in thoracoabdominal asynchrony (Benameur *et al*., 1993). Breathing strategy from

spontaneous to mechanical ventilation during anaesthesia is also associated with alterations in chest wall mechanics (Froese & Bryan, 1974). These two factors may have contributed to the increase in the 95% limits of agreement between devices. Although the potential bias in the calibration process means that the results must be interpreted with caution, this study highlights the importance of maintaining the condition of the calibration period during the monitoring period to allow QDC to remain valid.

A similar conclusion was also reached by De Groote *et al*. (2001). These authors mathematically assessed the QDC method and evaluated the limits of its application. In their study, simulated data with constant tidal volume, quasi-constant tidal volume (dispersion is added) and natural breathing volume (irregular) were used. The results showed that under constant and quasi-constant tidal volume conditions, the approximation of tidal volume was acceptable and yielded an accurate calibration factor K. When simulating the natural breathing volume, it was found that that accuracy of K decreased, especially if variability of the contributions of thoracic and abdominal compartments decreases. This is explained by the fact that QDC assumes a constant volume regardless of whether the volume is actually constant. The authors argued that in healthy adults, the variability between chest and abdomen motion is very small. Hence the volume estimation is more dependent on the choice of M (the value that calibrates the signal to give the absolute value), which may explain why all calibration techniques, including the one using a standard ratio (Banzett *et al*., 1995), produced good estimations of tidal volume. When changes in breathing pattern occur, such as natural preferential thoracic or abdominal breathing and voluntary changes of tidal volume, a greater discrepancy in tidal volume is observed because the K value no longer holds.

The study by De Groot *et al*. (2001) provided a much-needed mathematical assessment of the QDC method, and confirmed that if breathing pattern during the recording period deviates from the calibration period, the precision of the volume estimation decreases. The conclusions of this study may be open to

debate because the study was based on simulated data rather than data collected from real people. However, since the QDC method is based on a statistical model, it is logical to evaluate its limitations with a mathematical assessment.

Millard (2002) has also voiced concerns about whether applying a pre-determined K value calculated from the calibration period is suitable for continuous monitoring. Millard conducted a theoretical test to illustrate the potential problems with QDC and other calibration methods (Millard, 2002). It was proposed that in order to produce the ideal K value to suit the latest situation, K should be computed for each breath cycle. This would minimise the problem of a K value obtained during a calibration period becoming unsuitable during a monitoring period, either due to a change in breathing pattern, posture or drift, because the K value would be evolving appropriately to suit the developing conditions. This evolution could track the relative changes in tidal volume over a period of time.

To date, only one study has been found to have used this calibration method (Millard & Black, 2004). Twenty-nine patients who had undergone major abdominal surgery were monitored during the first twenty-four hours post-surgery. Relative changes of tidal volume and the degree of asynchrony of chest and abdomen movement were assessed. The waveform produced by RIP was compared with the waveform obtained from a flow meter using the quality of fit (R^2). The waveform for least squares calibration had a quality of fit of 0.9958 and the semi-calibrated waveform had a quality of fit of 0.9944 in comparison to the flow meter wave form. This shows that this calibration method can produce a quality of fit as good as the least square method. However, these results should be interpreted with caution as the reported figures constitute the summary statistics for the analysis from the twenty-nine patients. Data for individuals were not reported, and there was no indication as to whether all participants had the same level of agreement. Data for tidal volume was averaged over an interval between 100s and 200s, as convenient, due to the presence of central apnoea lasting up to 40s. Again, this might not be

a full assessment of the validity of this calibration method, as discussed in Section 2.1.7. The condition of breathing did not change throughout the recording period, and most patients breathed synchronously and therefore give little indication as to whether the calibration method remains as good when there is a change in breathing strategy. Although this study provided some evidence to suggest that this calibration method is at least as good as the traditional methods, further investigation is needed to fully explore the possibility of using this as a calibration method.

3.2.5 Multiple linear regression calibration

Multiple linear regressions (MLR) were proposed by Loveridge (1983) as a method to obtain the calibration factor. The relationships between volume, rib cage and abdomen are proposed to be as follows:

$$\text{Volume} = a + b_1 RC + b_2 ABD \quad (5)$$

where a is the intercept and b_1 and b_2 are the regression coefficients for the ribcage (RC) and abdomen (ABD) respectively. Loveridge *et al*. (1983) tested this method using eight healthy subjects (six males and two females). Data were collected while participants were seated in a dental chair with back, arm and foot supports, which limited spontaneous movements of the participants. Calibration was performed initially and verified at twenty, forty and sixty minutes, and the calibration period for all runs was one minute. Participants wore a nose clip and breathed through a pneumotachograph, and only the inspiratory data were analysed. Data were divided into ten-second blocks and multiple linear regression analysis was carried out on each block of inspiratory data to calculate the intercept, regressions, multiple correlation coefficients, and the standard error of the estimate (SEE) for that block. These statistics were calculated cumulatively for six blocks for the entire data collection period. Results showed no significant change with time for the regression coefficients or the SEE, and the correlation coefficients between RIP and the pneumotachograph were above 0.9 and the mean slope was 0.995 ±0.074 in all calibration and verification periods, suggesting high stability in the calibration. The range of SEE was very small (0.015 – 0.034) for all subjects, indicating small errors in volume estimation.

This study demonstrated that the multiple linear regression calibration technique is highly stable. However, as all participants had limited spontaneous movement and remained in a single position, it gives no indication of how the calibration would behave if movements were introduced or if breathing patterns changed. This limits the practical use of such a calibration technique and reduces the ambulatory possibility of RIP. It also does not appear to be superior to the QDC

method as it has not been shown that MLR can produce a calibration factor that would remain appropriate when breathing pattern has changed.

3.2.6 K constant calibration (Banzett *et al*. 1995)

The fixed K method was first introduced by Banzett *et al*. (1995). This method was proposed as a way to minimise the error of statistical estimation of QDC to derive the gain ratios. It involves pre-setting a value for K and applying it across the entire duration of the monitoring period. Banzett *et al*. (1995) validated this method of calibration in normal subjects using RIP along with spirometric volume and anthropometric measurements. Six men aged between 22 and 46 and five women aged between 23 and 31 were recruited. Calibration was tested in standing and sitting positions, and was based on the following equation:

$$\text{Volume} = K\,[(\sigma/\beta)\,RC + AB] \qquad (6)$$

where σ/β is a dimensionless number representing the gain of rib cage to abdomen and K is a constant. A range of fixed values (8, 4, 2 and 1) were tested as the standard gain ratio. These values were chosen on the basis of the anatomy of the chest wall. The K value was also determined using the isovolume manoeuvre.

The volumes obtained with this method, along with volume obtained by the linear regression method, were compared with spirometer measurements. Three different types of error were compared: the ‘mean absolute error’, ‘mean error’ and ‘maximum error’. The results showed that using a standard ratio during quiet breathing the median error of tidal volume was approximately 35ml, which equates to 1% to 8% of spirometric tidal volume. However, infrequent errors approaching 100% were observed. During mixed breathing the errors were larger than during normal breathing, but the median error remained less than 100ml. The error was generally less than 10% of the spirometric tidal volume. Errors with the standard ratio were similar to, or less than, errors with the ratio determined by the isovolume manoeuvre.

This study shows that the use of a standard ratio may be able to produce good estimation of tidal volume, and the findings are in agreement with other calibration methods. In Loveridge *et al*.’s (1983) study it was concluded that 95% of breaths fall within 30ml to 50ml of the spirometric values. In Sackner

(1989) a mean error of 5% during quiet breathing was observed. This suggests that the standard gain ratio is at least as good as the other two proposed methods. However, the idea of using an arbitrary choice of gain ratio seems rather irrational, and it has been suggested that the volume estimated by this method is crude (Millard, 2002), and has the potential for misidentification of obstructive sleep apnoea (Poole *et al*., 2000).

3.2.7 Which calibration method to use for RIP?

Recently Poole *et al*. (2002) conducted a study to compare three calibration methods (the constant K method, QDC and MLR) in infants during quiet and active sleep. Data were simultaneously recorded using RIP and pneumotachograph. A five-minute calibration period was used to calculate a proportionality constant with each method. The absolute difference between the pneumotachograph and RIP was measured on a breath-by-breath basis and expressed as a percentage of tidal volume. The mean absolute percentage error was then calculated for each minute of the data. The 95% limits of agreement were also calculated to test the agreement between two methods.

The results demonstrated that during quiet sleep the percentage error was significantly greater in the fixed K method than in MLR ($p=0.01$). QDC gave higher mean percentage errors than MLR, but the differences were not statistically significant. The 95% limits of agreement were widest when MLR was compared with the fixed K method. The narrowest range was found between MLR and QDC. During active sleep, the percentage errors were greater in all measurements. MLR again had the lowest mean percentage errors and the fixed K method had the highest. The observed mean percentage errors between methods were not statistically significant. The narrowest range of 95% limits of agreement was found between MLR and QDC, and the highest between QDC and the fixed K method. This study demonstrated that the three methods provide varying levels of tidal volume estimation in infants during sleep, but all methods produced similar results, with the fixed K method containing the most variability.

The agreement between RIP and pneumotachograph and the QDC method did not deteriorate when a paradoxical breathing pattern (altered use of ribcage and abdomen) was observed during active sleep. This contradicted other studies, which had reported that when paradoxical breathing occurs it will cause greater discrepancies between QDC-calibrated RIP and pneumotachograph tidal volume. The narrow range of 95% limits of agreement between QDC and MLR

indicated a low level of variation between the two methods. It can be concluded that both QDC and MLR can produce a good level of calibration accuracy.

Although all the recognised calibration methods seem to produce reasonably good volume estimates, the issues that Millard (2002) raised regarding the application of pre-set K values cannot be ignored. The results of the comparison study (Poole *et al*., 2000) might not apply to adults as they have different chest wall mechanics. Also, data collected during sleep studies might not accurately reflect the data collected from awake individuals or when movement is introduced. The method proposed by Millard (2002) shows potential for avoiding some weaknesses inherent in traditional methods. To date, there are few published studies that have employed the calibration method proposed by Millard (2002) to track relative changes in tidal volume, so it is logical to conduct a study to assess this calibration method.

The common problem for all existing methods for the calibration of RIP is that if the chest wall movement during the recording period is different from the calibration period, the original calibration factor will not hold and therefore the measurements will not give the correct volume estimation. The calibration method proposed Millard (2002) should address this issue by calculating the K values for each breath, to ensure that the calibration factor is suitable for the breathing strategy. It is worth noting that all existing calibration methods for RIP are based on the model proposed by Konno and Mead (1967) where the chest wall has two degrees of freedom (two moving parts in an open system). The changes of area within the chest and abdomen region will therefore equate to volume measured at mouth level.

Although all the recognised calibration methods based on this model seem to produce reasonably good volume estimates, the model itself is not flawless since it only measures changes of cross-sectional area and does not take into account the vertical movement of the rib cage. It is known that the rib cage is capable of vertical movement, especially when the scalene and sternomastoid accessory muscles are contracted to lift the sternum and the first two ribs in

cephalad (vertical) and dorsal (frontal) directions. This may lead to poor estimation of tidal volume due to a violation of the assumption of the Konno and Mead model, as suggested in their original paper. To date, no published study has been found which has discussed this issue or suggested the impact of vertical chest wall movement on various calibration methods.

3.2.8 Summary

This section has discussed and evaluated methods of calibration for the RIP system. The main issue with calibrating RIP is that the scaling factors obtained during the calibration period may not be suitable to be applied during the monitoring period, either due to changes of breathing strategy, changes in breathing amplitude or changes in posture. The method suggested by Millard (2002) has a strong theoretical background and it is logical to keep the scaling factor updated so that optimal scaling factors can be used for every breath throughout the monitoring period. In theory the method should be able to give a valid estimation of relative tidal volume even with changes in breathing strategy. Therefore, the calibration method proposed by Millard (2002) has been used within this thesis.

The next section will discuss issues relating to measurement validity and the estimates used to describe this concept.

3.3 Assessment of validity

Validity refers to how well an instrument truly assesses the characteristic it is intended to measure (Karras, 1997a). Assessing validity is related to assessing the extent to which an instrument measures what it is intended to measure (Sim & Arnell, 1993). In order to decide if a measurement tool is valid, it needs to be assessed against an external reference. This is different to reliability, since reliability refers to technique's internal consistency with repeated trials and indicates the extent to which differences in measurement are due to variability within the instrument rather than to actual differences in the parameters being studied (Karras, 1997a). However, validity can be considered to incorporate elements of reliability as any instrument with poor reliability is also likely to be invalid (Karras, 1997b).

There are four types of validity: face validity, content validity, construct validity and criterion-related validity (Sim & Arnell, 1993; Karras, 1997c). Face validity, content validity and construct validity are related primarily to qualitative studies or questionnaire/survey design. These aspects will not be discussed within this thesis. Criterion validity often underpins quantitative research because it quantifies the relationship between measurement scores and scores from a standard indicator (Sim & Arnell, 1993; Sarfit, 1989). When comparing results obtained from two methods, it is unlikely that they will agree with each other completely. Appropriate analysis is essential to quantify the level of agreement between methods.

A wide range of indices has been used in the literature to investigate validity, and the choice of which index to use would depend on the situation. The commonly-used indices in the literature are simple correlation coefficients (Bowling and Ebrahim, 2005), intra-class correlation coefficients (Deyo, 1991), Bland and Altman analysis (Bland & Altman, 1986) and standard error of the measurement (Diamond & Jefferies, 2008). Some of these indices are also commonly used for reliability studies. However, these indices concern the differences between measurements, and as suggested by Karras (1997b),

techniques that measure difference are meaningful in validity assessment. The following sections discuss each type of index individually.

3.3.1 Simple correlation coefficients

Correlation coefficients give information about the degree of association, not the agreement, between two sets of data. The application of correlation coefficients to reliability studies has been regarded as inappropriate by several authors (Bland & Altman, 1986; Rankin & Stokes, 1998; Haas, 1991). This is because correlation coefficients give an indication of how two sets of scores vary together, not the extent of agreement between them. They will not detect any systematic bias, and it is possible to have two set of scores that are highly correlated but in poor agreement. However, they can be a useful tool to assess the association or relationship between measurements obtained by a new method and the criterion measure. For example, if the LifeShirt® was calibrated using the method proposed by Millard (2002) to record relative changes of tidal volume, and these measurements then compared with tidal volumes obtained from a pneumotachograph, then it would possible to use a correlation coefficient to assess the relationship between the two devices. It would give an indication as to how well the LifeShirt is tracking relative changes of tidal volume compared to the pneumotachograph.

A correlation coefficient measures the linear association between two continuous variables and correlations can be negative, positive or no correlation (Healey, 1996). The coefficient ranges from -1 to +1, where -1 indicates a negative correlation, +1 indicates a positive correlation and 0 indicates no correlation. The closer the coefficient is to +1, the closer the relationship is to a perfect positive linear correlation. There is no universally accepted meaning of ‘satisfactory’ or ‘unsatisfactory’ regarding the coefficient of correlation (Thomas *et al*., 2005a). It has been stated that a correlation coefficient value of 0.5 indicates a ‘moderately strong’ positive linear relationship between variables, and coefficients approaching 0 can be described as ‘weak’ and those approaching 1 as ‘strong’ (Healey, 1996). Values in between these extremes

are difficult to interpret directly, and their assessment very much depends on the purpose of the correlation. The interpretations of the simple correlation coefficients for this study follow the guidelines recommended by Krishef (1987). This author recommended five levels of interpretation of correlation coefficients: 'slight' (0.0 to 0.2); 'low' (0.2 to 0.4); 'moderate' (0.5 to 0.7); 'high' (0.8 to 0.9); and 'very high' (0.9 to 1.0) (Krishef, 1987).

3.3.2 Bland and Altman analysis

Bland and Altman analysis is an approach for assessing agreement between two different methods of measurement. This statistical method was first published in 1986, when the authors discussed the inappropriateness of the use of correlation coefficients in measuring agreement between methods. In their paper the authors stated that a high correlation does not mean that two measurements agree. The Bland and Altman analysis involves calculating the mean for each pair of measurements obtained from the two measurement methods and plotting these calculations against the difference of the pair of measurements. This allows the identification of any systematic bias. The 95% limits of agreement are calculated as the mean difference plus or minus two standard deviations of the mean difference. The limits of agreement indicate the range of error, which may relate to clinical acceptability. Although Bland and Altman analysis is very useful in identifying agreement, it presents a similar problem to those that occur with other indices, such as the ICC, in that there is no known 'cut off' score for the 95% limits of agreement. The analysis gives clinicians or researchers an idea of the spread of the data, but it is then up to the researchers or clinicians to decide whether this spread is acceptable.

3.3.3 Intra-class correlation coefficient (ICC)

The ICC value ranges from 0 to 1, and is an attempt to overcome some of the limitations of simple correlation coefficients (Bruton *et al*., 2000). It was designed by Bartko (1966) specifically to examine reliability. It is a single index calculated using variance estimates obtained through the partitioning of total

variance into between- and within-subject variance. It therefore reflects both the degree of consistency and the agreement among ratings.

There are several formulae for calculating ICC (Shrout & Fleiss, 1979), and each formula is appropriate to specific situations. There are six different formulae for ICC: (1,1), (1,k), (2,1), (2,k), (3,1),(3,k). When choosing which ICC models to use, there are three main considerations: 1) one or two way models; 2) are the difference between the judges mean rating is relevant of interest; 3) fixed of random effect.

For model 1, each subject is assumed to be assessed by a different set of raters than other subjects. These raters are assumed to be randomly sampled from population of possible raters so that raters are a random effect. Model 2 assumes each subject is assessed by the same group of raters, and these raters were randomly sampled from the population of possible raters. In this case the rater are also considered a random effect. Model 3 assumes each subject was assessed by the same group of rater, but these particular raters are the only raters of interest and that one does not wish to generalise the ICC beyond the confines of the study. This is considered to be fixed effect.

The ICC is given a second designation of 1 if the scores in the analysis are from single scores from each subject for each trial (or rater if assessing interrater reliability). If the scores in the analysis represent the average across the trials from each subject, then the ICC is also given a second designation of k.
The third consideration when choosing which ICC mode is the fixed or random effect model. Fixed effect is one in which all error of interest in the trials are included in the analysis whereas random effect is one in which the levels of the error in the trial are a sample of the possible error. Hence the analysis of fixed effect does not attempt to generalise the reliability data beyond the confines of the study whereas analysis of the random effects may be used to generalise to other levels. Model 1 and 2 only consider random effects and model 3 only consider fixed effects.

One of the disadvantages of the ICC is that there is no universal acceptable level of reliability. For example, Ghinn (1991) recommends that any measure should have ICC of a minimum of 0.6 to be useful. However, Rankin and Stokes (1998) suggested that if the ICC is 0.7 or above, then the measure is deemed to be acceptable. However, there are situations where a higher ICC value is desirable. For example, if clinical decision is based on the measurement, then ICC value should be at least 0.96 (Portney and Watkins, 2000).

The ICC is also affected by the variance between subjects. The ICC is the ratio of between-subjects variance to true score variance plus error (Rankin & Stokes, 1998). If the ICC is applied to the data from a wide range of the measured characteristic, the ICC values will appear to be higher than when it is applied to a group with a narrow range of the same characteristic. Another disadvantage of the ICC is that it gives little information on the magnitude of the disagreement between measurements (Rankin & Stokes, 1998). This makes clinical interpretation difficult as clinicians are unable to identify the real difference in variation. These disadvantages mean that the ICC should not be quoted in isolation.

3.3.4 Standard error of measurement

The standard error of measurement (SEM) can also be used to assess agreement between measurements (Diamond & Jefferies, 2008; Karras, 1997b). SEM refers to the difference between two measurements of the same parameter, and it can give an indication of the precision of the measurement (Franzen, 1954). For example, for expiration duration, the smaller the standard error of measurement, the less difference in expiration duration between the LifeShirt® and the pneumotachograph. SEM is expressed in the units of measurement (i.e. in seconds for expiration duration). The SEM is calculated with the formula:

$$SEM = stdv \times \sqrt{1 - r} \quad (7)$$

where *stdv* is the standard deviation of the mean difference and *r* is the reliability index, which is usually the intra-class correlation coefficient (Morrow and Jackson, 1993).

3.3.5 Paired t-test

The paired t-test is used to compare the difference between the means of two sets of measurements. The result of the t-test, the *p* value, is used to decide whether to accept or reject the null hypothesis (Krishef, 1987). The null hypothesis (H0) assumes that there will be no difference between the sets of measurements. There is also an alternative hypothesis (H1), which assumes that there are differences between the measurements (Field, 2005). Some of the differences may be due to chance alone, and the confidence level will indicate how likely it is that the difference is due to chance. The confidence level of the paired t-test is traditionally set at the 0.05 level (Field, 2005). If the *p* value is less than 0.05 then the null hypothesis is rejected and the alternative hypothesis is accepted – i.e. there is a statistically significant difference between the means of the two sets of measurements. If the *p* value is greater than 0.05 then the null hypothesis is accepted and the alternative hypothesis is rejected – i.e. there is no significant difference between the means of the two sets of measurements.

3.3.6 Summary

This section has discussed the concept of validity, and evaluated various methods of assessing validity and the interpretation of the different indices. With all the validity indices, there is no universally accepted cut-off point or 'satisfactory'/'unsatisfactory' classification. The requirements of the study and the judgment of the researcher will determine whether the validity indices are acceptable. Therefore, several statistical analyses for validity should be performed so that they might complement each other.

3.4 Research aims –validity of the LifeShirt® during quiet breathing and exercise

Measuring breathing patterns non-invasively and unobtrusively is desirable to acquire data that reflect normal respiratory behaviour. One accepted technology to achieve this is RIP, which theoretically can be improved by embedding it within a garment (like the LifeShirt®) to reduce artefactual motion. However, before such garments can be used for evaluating clinical interventions, or for long-term disease monitoring, it is first essential to assess the validity of the data generated. The preceding literature review highlighted the methodological weaknesses in previous validity studies of the LifeShirt®, i.e. the fact that the use of interval averaged data may not give a representative picture, and the potential for differences in breath definition, which may contribute to differences observed in the studies. The validation study reported in this thesis aimed to address these issues by: 1) carrying out a breath-by-breath comparison between the LifeShirt® and the pneumotachograph; and 2) analysing raw respiratory signals using third-party software, which should minimise potential error due to differences in breath definition.

The preceding review also highlighted the primary problem with traditional calibration methods for RIP technology, i.e. that the calibration factor obtained during the calibration period does not necessarily remain appropriate for the entire period of recording. It has been argued that the calibration factor should be calculated for each breath cycle (Millard, 2002), but currently there is little published data to support this. Therefore, this current validation study also aimed to validate the calibration method proposed by Millard (2002). As such, the first phase of the study sought to address the following aims:

- To establish whether the LifeShirt® can estimate realistic and predictable relative changes in tidal volume on a breath-by-breath basis during quiet breathing and exercise when calibrated with the method proposed by Millard (2002), thereby addressing the problem of data averaging.
- To establish the level of agreement between the LifeShirt® and the

pneumotachograph in measuring expiration on a breath-by-breath basis duration during quiet breathing and exercise, when calibrated with the method proposed by Millard (2002).

- To measure the variability in tidal volume recorded by the LifeShirt® and the pneumotachograph during quiet breathing and exercise.
- To determine whether the LifeShirt® can detect significant changes in tidal volume and expiration duration (using the pneumotachograph as the reference standard).

Tidal volume and expiration duration were chosen to be tested as they were the two parameters that could be simultaneously recorded by both devices. A previous study had demonstrated a measurable difference in tidal volume between healthy individuals and patients with respiratory conditions such as asthma (Tobin *et al*., 1983b). Expiration duration was also chosen because previous studies reported premature closure of airways in respiratory conditions, which could lead to changes in expiration duration (see Chapter 2 for a detailed description).

Chapter 4: Methodology for measuring the validity of the LifeShirt® during quiet breathing and exercise

Introduction

This chapter provides a description of the equipment and methodology used in the validity study. A general overview of the study is presented first, followed by specific details of the procedures. Aspects of the methodology will be described as follows:

4.0 Research hypotheses

This study was designed to establish whether there is a predictable relationship between changes in tidal volume that are measured by the pneumotachograph and the estimates of tidal volume measured by the LifeShirt®, and to validate the parameter of expiration duration. These two parameters were chosen because they were parameters likely to be affected as a result of respiratory conditions, and because they can both be validated against the gold standard of the pneumotachograph. A number of specific hypotheses were developed to enable these aims to be achieved:

Research hypotheses:

H1a The LifeShirt® measurement of relative tidal volume correlates with the absolute tidal volume of the pneumotachograph during quiet breathing

H1b The LifeShirt® measurement of expiration duration agrees with the pneumotachograph measurement of expiration duration during quiet breathing

H1c The LifeShirt® measurement of expiration duration correlates with the pneumotachograph measurement of expiration duration during quiet breathing

H1d The LifeShirt® measurement of relative tidal volume correlates with the absolute tidal volume of the pneumotachograph during exercise

H1e The LifeShirt® measurement of expiration duration agrees with the pneumotachograph measurement of expiration duration during exercise

H1f The LifeShirt® measurement of expiration duration correlates with the pneumotachograph measurement of expiration duration during exercise

H2a The relative tidal volumes measured by the LifeShirt® are significantly different between exercise and quiet breathing when there is also a significant difference in tidal volume recorded by the pneumotachograph between exercise and quiet breathing

H2b: The expiration durations measured by the LifeShirt® are significantly different between exercise and quiet breathing when there is also a

significant difference in expiration duration recorded by the pneumotachograph between exercise and quiet breathing

H3a: The LifeShirt® records similar breath-by-breath variability to the pneumotachograph during quiet breathing

H3b: The LifeShirt® records similar breath-by-breath variability to the pneumotachograph during exercise

4.1 Study design

A quantitative experimental design was suitable for this study since it aimed to measure and quantified breathing parameters objectively.

4.1.1 Validity of respiratory parameters measured by the LifeShirt®

This study used a concurrent design to assess the validity of relative tidal volume and expiration duration measurements derived from the LifeShirt® signals. The tidal volume and expiration duration calculated from the LifeShirt® data were compared to the tidal volume and expiration duration obtained by the laboratory standard equipment, a pneumotachograph. This type of validity testing is suitable since a pneumotachograph is regarded as the gold standard for measuring respiratory parameters Akre *et al.,* (2000), and hence the validity of the LifeShirt® data can be assessed by recording data from each device simultaneously.

4.2 Research governance

4.2.1 Ethical approval

Ethical approval was sought from the Ethics Committee of the School of Health Sciences, University of Southampton. Professional indemnity insurance was obtained from the University of Southampton in order to provide liability in case of injury to study participants. The study was approved by the Research and

Development Department of the University of Southampton, and the Data Protection Officer was also informed.

4.2.2 Ethical consideration

Verbal and written explanations were given to all participants regarding the aims of the study, and an information sheet regarding the study was given to them. All participants were informed that the data would be used for the purpose of the study. Voluntary consent was obtained via a written consent form, and participants were reminded that they had the right to withdraw from the study at any time without giving any reason. A number was allocated to each participant to ensure anonymity throughout the study. As the study required people to remove some clothing, a chaperone was offered and provided if requested by the participants. The researcher was a qualified physiotherapist and had received training in operating the equipment to a safe standard. The Chartered Society of Physiotherapy Code of Conduct was strictly adhered to at all times. There are no known side effects from the wearing of the instrumented garment or the face mask, and this study presented minimum potential harm to participants.

4.2.3 Health and safety

A risk assessment was carried out prior to the study to identify any major hazards. The face mask and the circuit tubing used by participants were disinfected after each participant's data collection period, according to the requirements of the local Trust infection control and manufacturer's instructions for avoiding cross-infection. The Southampton University Hospital Trust Hand Hygiene Policy and Infection Control Policy were strictly followed. Disposable filters were placed in the pneumotachograph circuit to prevent saliva entering the circuit. The LifeShirt® garment used for recording was washed in accordance with the manufacturer's instructions.

4.3 Sample population

A power calculation was considered to be unsuitable for this study as insufficient data generated by the LifeShirt® are already in the public domain. Although the embedded RIP technology has been available for many years in other forms, the main focus of this study was to examine the specific data generated by the LifeShirt®. The Bland and Altman analysis requires a minimum sample size of fifty for the test to be significant (Bland & Altman, 1986). As the data would be analysed on a breath-by-breath basis, a sample size of ten participants would provide approximately 3600 breaths during a thirty-minute recording period (based on an average respiratory frequency of twelve breaths per minute), which would give sufficient data for a valid Bland and Altman analysis. However, this would limit the generalisability of the findings due to the small sample size.

The study included healthy individuals between the ages of 18 and 35. Limiting the age range reduced the likelihood of age-related pathologies and increased the homogeneity of the sample population. The inclusion/exclusion criteria were set to include as many individuals within this age range as possible.

Inclusion criteria:

- Aged 18 to 35
- Non-smokers (those who have never regularly smoked one or more cigarettes per day)

Exclusion criteria:

- History of serious cardiovascular complications, pulmonary disease, neurological conditions
- History of smoking
- Recent upper respiratory infection (within the last four weeks)
- Currently receiving treatment from a health practitioner
- Inability to ride an exercise bicycle

The exclusion criteria were designed to exclude those for whom the study may present a risk. It also reduced the likelihood of age-related pathologies and enhanced the internal validity of the results by improving the homogeneity of the sample population.

4.4 Recruitment process and method

The sample population was recruited from the community of the University of Southampton. Participants were recruited from the student and staff population at the University of Southampton via word of mouth, posters and internet forums. Advertising posters were displayed in the School of Health Sciences (Buildings 45 and 67), around the Highfield Campus, at Boldrewood and at Southampton General Hospital. Advertisements were also placed on the Chinese (www.uker.net) and Taiwanese (www.hellouk.org) students' forums on the internet. This was an attempt to maximise the number of potential participants.

Interested volunteers were asked to contact the researcher via email or telephone. Information sheets about the study were then sent out and any questions answered. Responders then had as long as they needed to decide if they wanted to participate in the study, and had the opportunity to contact the researcher for further information or to answer any queries. If individuals decided to participate, they contacted the researcher to arrange a visit to the hospital.

Convenience sampling was used to recruit the sample population based on the accessibility to the researcher (Clifford & Harkin, 1997). Although it would have been ideal to collect a random sample from the population, time and financial constraints meant that this was not practical. A randomly selected sample population would have enhanced the validity of the results because the findings from the sample could be generalised to a wider population. The convenience sampling method poses threats to the external validity of the study since results might not be generalisable to the wider population. Based on the number of

breath samples needed to conduct a Bland and Altman analysis, it was intended to recruit a convenience sample size of between ten and twenty people. A similar sample size had also been used by others in research studies about the validity of the LifeShirt® (Clarenbach *et al*., 2005; Witt *et al*., 2006).

4.5 Preparation

The researcher received training in the use of the data acquisition systems and the dedicated software used to interpret the data. There was also ongoing technical support from the manufacturers of the two systems, ADInstruments and VivoMetrics. The researcher attended a course on signal processing to gain an understanding of how data acquisition systems operate and how to perform signal processing using MatLab. Some preliminary data collection sessions were carried out on the researcher to test the feasibility of the collection procedure and to experiment with an appropriate means of signal synchronisation. This preliminary work also helped to identify any potential risks and hazards, which were then minimised for the study.

4.5.1 Pilot work

Prior to carrying out the study, pilot work was carried out to ensure the feasibility of the protocol. One of the issues to arise was the synchronisation of the pneumotachograph and the LifeShirt®. Because the pneumotachograph and the LifeShirt® recorded signals onto separate devices, there was a need to create a suitable marker for synchronisation in the signals in order to align the signals for breath by breath analysis. Several synchronisation markers were tested, including coughs, tap on the chest and breath holds. The suitability of the markers was tested by the researcher by wearing the LifeShirt® and the face mask while performing a cough, a tap on the chest and breath holds. The signals were then visually inspected in MatLab, and it was determined that the most easily identifiable marker was a breath hold. It was therefore decided to use two breath holds ten seconds apart as the synchronisation marker.

During the pilot phase, it was also determined which parameters could be measured by both devices. The parameters that can be measured by both pneumotachograph and the LifeShirt® were expiratory tidal volume and expiration duration. Tidal volume had been shown to have some differences between healthy people and patients with respiratory conditions (Tobin *et al*., 1983b).

4.6 Equipment for recording respiratory signals

The respiratory signals were recorded by the LifeShirt® system (VivoLogic TM, VivoMetrics®, Ventura, California) and the pneumotachograph (PowerLab®, ADInstruments Ltd., Oxfordshire). Data from the LifeShirt® were recorded onto a removable memory card via its associated data logger for offline analysis. The PowerLab® system recorded data directly onto a computer. All respiratory signals were then imported into MatLab for signal processing. Tidal volume and expiration duration were calculated in MatLab using algorithms developed by the Institute of Sound and Vibration, University of Southampton. The LifeShirt® sampling frequency was pre-set at 50Hz. The sampling frequency of the pneumotachograph was set at 400Hz to avoid aliasing effects, and at a range of 200mV to avoid clipping of the signals.

The pneumotachograph only measured expired airflow, and flow rate was measured in litres per second. Tidal volume was calculated by the integration of the flow signal, and the integrated signals were imported into MatLab for analysis.

The LifeShirt® recorded movement during both the inspiration and expiration phases. According to Konno and Mead's (1969) model, the summation of the chest and abdomen movements is equal to the tidal volume. The individual and summated signals of chest and abdomen were imported into MatLab for analysis.

Detailed descriptions of the calculation of the parameters are provided in a later section (Section 3.8.1: Signal Processing).

4.7 Experimental procedure

4.7.1 Calibration

4.7.1.1 Calibration of pneumotachograph

The flow head pneumotachograph was calibrated prior to each data collection period (before commencing recording of quiet breathing, and again before the exercise breathing period). The flow head is a pressure transducer which consists of a membrane in the centre; the difference in pressure before and after the membrane is measured. The changes of pressure were then converted into airflow, and the airflow signals were integrated to give the tidal volume. The first step in calibrating the pneumotachograph was to zero the flow head. This was to ensure that the pneumotachograph reading was at zero when there was no air flow. After it was zeroed, a three-litre calibrating syringe was used to inject gas into the gas analyser of the pneumotachograph through the flow head. The process of injecting a known volume of gas through the flow head is to provide a reference for a known resistance. This method was chosen as it is the recommended calibration method proposed by the manufacturer, ADInstruments. This type of calibration method is a standard operating procedure for the pneumotachograph.

4.7.1.2 Calibration of the LifeShirt®

As discussed in the previous chapter, several methods of calibration procedure have been proposed by various authors. The respiratory signal from the LifeShirt® was calibrated offline in MatLab. It was calibrated using a variation of the QDC method proposed by Millard (2002), which has theoretical advantages over the traditional QDC method. To recap the traditional QDC technique,

Sackner *et al.* (1989) proposed that the collection of a large number of breaths (a period of five minutes was deemed sufficient) with the exclusion of breaths with a large deviation from the mean (i.e. a standard deviation of more than ± 1.0) can be assumed to provide a constant volume. The calibration factor, K, could then be obtained by calculating the breath-to-breath standard deviation of the ΔVAB and ΔVRC signals. The major weakness of this method is that the selection of breaths is assumed to be of constant volume, when in fact it is not constant. De Groote *et al.* (2001) demonstrated a reduced accuracy in tidal volume approximation when comparing values of K obtained during natural breathing to constant or near-constant volume. The traditional QDC method also assumes the chest wall motion-to-volume coefficient obtained during the calibration period will remain the same during the recording period, when in fact it may vary (Millard, 2002). There is sufficient evidence to support the theory that when breathing strategies during recording period differ from calibration period, e.g. showing an increased tidal volume range or preferential thoracic/abdomen movement, the accuracy of the estimation of tidal volume will reduce (Kent *et al.,* 2009; De Groote *et al.*, 2001; Thomson, 1999; Brown *et al., 1998*).

Sackner's calibration method works on the basis of underlying variations in the chest and abdomen contributions. One of the aims of breathing retraining is to improve synchrony of chest and abdomen, thereby reducing the variation of the contributions. This might cause the QDC method to fail, and therefore it would not be a suitable method if the LifeShirt® were in future studies that involve breathing retraining intervention which aims to 'normalise' the breathing pattern and might affect the natural variations of chest and abdomen. There is also some doubt as to whether, when using the traditional methods (QDC, MLR or the fixed K method), the calibration factors would remain adequate throughout the entire period of recording, as participants might change their breathing strategy.

Millard's calibration method (2002)

The key difference between Millard's method and the traditional QDC method is that K is calculated for every cycle, using the standard deviation of all the

sample points within a respiratory cycle, whereas the traditional QDC method calculates K from a selection of breaths based on the assumption of constant volume. Such an assumption often cannot be met. The advantages of Millard's method are twofold: 1) K is updated for each breath to suit the latest breathing pattern, thus minimising the issue of K being unsuitable due to changes of chest wall motion; 2) it addresses the assumption from the traditional QDC method that the statistical selection of breaths from a calibration period is of a constant tidal volume when tidal volume is in fact not constant. However, as this calibration method is also based on two degrees of chest wall motion, it will also have the inherent weakness of being affecting by vertical chest wall movement.

The calibration method used in this study was based on the original calibration equation of Sackner *et al*. (1989), such that $\Delta V_T = M(K (\Delta V_{RC}) + \Delta V_{AB})$, but with the omission of the M value (M value set to equal 1) which is the reference value obtained externally from either spirometer or pneumotachograph. The value of K is calculated from the equation below:

$$K = -SD(\Delta V_{AB}) / SD(\Delta V_{RC})$$

In plain terms, the K factor is calculated from the standard deviation of all the sample points within a respiratory cycle. The following figure illustrates how the standard deviation of all sampling points from each breath cycle was calculated.

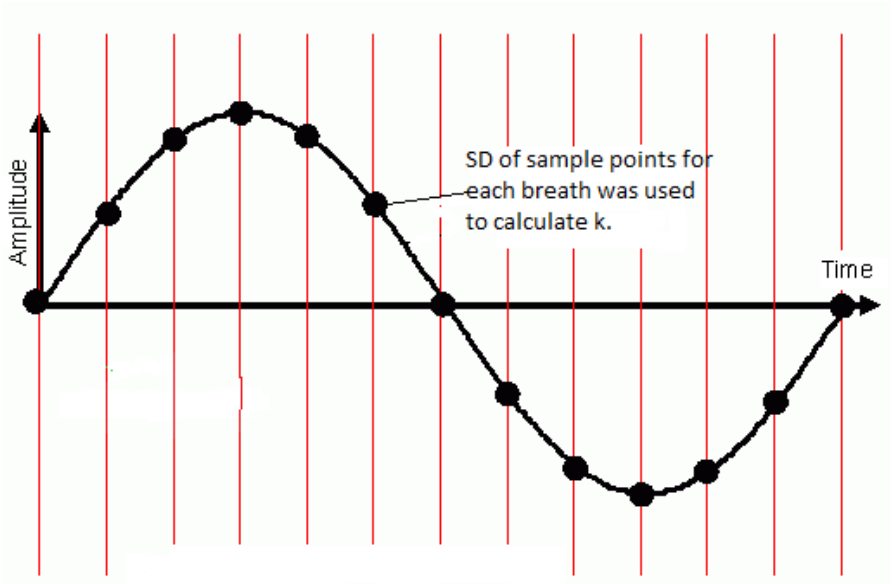


Figure 5: An illustration to demonstrate how K was calculated for each breath cycle. The mean and standard deviation of all the sample points recorded from the AB and RC traces during a breath cycle were calculated. The standard deviations were then used to calculate K, according to formula (1).

The optimum K value for each breath cycle was calculated as the negative of the standard deviation of the changes in magnitude of the uncalibrated AB and RC traces of each respiratory cycle. Once the K value was obtained, the original equation from Sackner *et al*. (1989) was applied to obtain the tidal volume measurement. As M needs to be obtained from an external reference (i.e. a spirometer), it is not possible to obtain the M value for each breath and therefore this calculation is not suitable for breath by breath analysis. The equation used in this study to calculate the tidal volume is as follows:

$$\Delta V_T = [\ -SD(\Delta V_{AB}) / SD(\Delta V_{RC}) \times (\Delta V_{RC})\ ] + \Delta V_{AB}$$

By using the standard deviations from the rib cage and abdominal traces as the K factor, this gives the two signals an equal weighting. The reason for setting the RC and AB traces to equal weighting was that all RIP calibration methods were based on the two degrees of freedom model proposed by Konno and Mead (1967). As any volume change of one compartment must be equal and opposite to the other compartment, it is essential to give them equal weighting. The summation of the equally weighted AB and RC traces will therefore equate

to the volume measured at mouth level when an external reference value is applied.

The calibration procedure proposed by Millard (2002) has the advantage that it calculates the electrical gain for the abdomen and rib cage for each breath cycle, which minimises the problem of a constant calibration factor not being suitable when breathing strategy changes. If discrepancies between devices are observed, then they would be more likely to be due to a real difference between devices than a result of any drift in the calibration or due to the calibration factor becoming unsuitable.

However, this method had the disadvantage that it is only capable of tracking relative changes of volume – i.e. measurements are in the correct ratio, but in different units to the pneumotachograph. If information about absolute volume was required, then an external source would be needed to calibrate each breath cycle. It was the intention of this study to establish whether the LifeShirt® could detect relative changes of tidal volume to the same extent as the pneumotachograph. If the LifeShirt® demonstrated a sufficient degree of accuracy in measuring tidal volume and expiration duration when calibrated with the method proposed by Millard (2002), this would be useful in a clinical setting as clinicians would not need to carry out complicated external source calibration procedures. This would enhance the ambulatory nature of RIP. The unit for the tidal volume calculated by the LifeShirt® is ‘assumed litres’. This terminology is adopted from the manufacturer, VivoMetrics, who used the term ‘assumed millilitres’ for uncalibrated output (VivoMetrics user manual). The output was converted to ‘assumed litres’ by dividing the output by one thousand to give it the same order of magnitude as the pneumotachograph data.

4.7.2 Experimental procedure

Participants were invited to attend the Gait Laboratory at Southampton General Hospital on one occasion for approximately one and a half hours. The session was divided into two parts: a quiet breathing period and an exercise period. On arrival at the site, participants were greeted by the researcher and were given the opportunity to ask questions about the study before signing the consent form. Demographic data about age, weight, height and body mass index were collected. Abdominal and chest girth measurements were taken and were used to find the appropriate size of the LifeShirt® garment. Participants were shown how to wear the LifeShirt® and were then allowed to get changed in private. The researcher checked to make sure a satisfactory snug fit was achieved, and some time was given to allow participants to acclimatise to the LifeShirt®. Participants were fitted with the face mask of the pneumotachograph and were seated on a comfortable chair. Recording then began. Data were recorded simultaneously by the LifeShirt® and the pneumotachograph.

4.7.2.1 Quiet breathing period

For the quiet breathing period, participants were asked to sit quietly for thirty-five minutes during which a video of nature scenery was played. This was to distract the participants from thinking about their breathing. It has been suggested that watching a video or television programme may induce changes in breathing pattern (Hark *et al*., 2005). However, because only the concurrent validity of the simultaneously recorded data was of interest, a change in breathing pattern during the recording period should not affect the results of the validity analysis. This is because of the fact that if there were any changes in breathing, they should be recorded by both devices.

Respiratory data were recorded simultaneously by the LifeShirt® and the pneumotachograph for thirty-five minutes (see Figure 6). A breath hold of ten seconds was used as a marker in the signal, since this had been identified during the pilot work as the easiest identifiable marker when the signals were

viewed in MatLab. Two breath hold periods of ten seconds each were used. Participants were asked to do the first ten-second breath hold to mark the synchronisation of both devices when recording began. After five minutes participants were asked to do another ten-second breath hold to mark the beginning of data recording that would be used for analysis. The first five minutes of the data (recorded in between the two breath holds) were not analysed since they were likely to have high variability due to participants' nervousness about being recorded and getting used to the sensation of breathing through a face mask. A visual inspection was performed on all the data set to check for abnormality of the recording. After thirty-five minutes recording ceased, the face mask was removed and participants were given a break of five minutes.

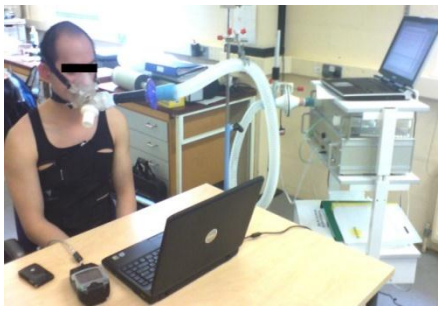


Figure 6: Simultaneous recording of tidal volume and expiration duration during quiet breathing

4.7.2.2 Exercise period

Respiratory data collection during exercise commenced after the break. Participants were asked to sit on the cycle ergometer and the face mask was re-applied. The flow head of the pneumotachograph was zeroed and calibrated again before the start of recording. Again, a ten-second breath hold marked the synchronisation of the signals. Participants initially remained relatively still for five minutes while sitting on the cycle ergometer. After five minutes, participants were asked to perform a ten-second breath hold and then they began to exercise, continuing for twenty minutes (see Figure 7). Exercise was performed at mild to moderate level; participants were asked to exercise at a level that was described as feeling ‘very light’ (between 9 and 11) and ‘somewhat hard’ (around 13 to 14), according to the Borg Scale of Perceived Exertion Borg (1982). The scale was enlarged to a page A4 size,. and was presented to the participants prior to data recording and at the beginning of pedaling.

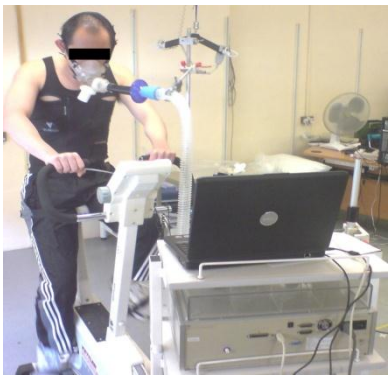


Figure 7: Simultaneous recording of tidal volume and expiration during exercise

Data were again recorded simultaneously by the LifeShirt® and the pneumotachograph for twenty-five minutes. The first five minutes were discarded because they were likely to have high variability as participants got used to the ergometer. At the end of the exercise period the participants were asked to cool down by cycling gently at their own pace in order to minimise muscle soreness. At the end of the exercise period, recording ceased and the LifeShirt® and the face mask were removed. Participants were then free to leave the laboratory.

4.8 Data analysis

The section describes the process of data analysis. It first describes the signal processing method, followed by the statistical analysis to test the hypotheses described previously (see Section 3.0). The data analysis focused on the parameters of interest that could be measured indirectly by both devices, i.e. tidal volume and expiratory duration. The recordings of the parameters obtained from the signals collected by the two devices were compared against each other in MatLab.

4.8.1 Signal processing

The algorithms to perform the calibration and calculate the tidal volume and expiration duration from the respiratory signals recorded by the LifeShirt® and the pneumotachograph were developed by Dr Anna Barney from the Institute of Sound and Vibration Research at the University of Southampton. Figures 8 and 9 below show the respiratory signals recorded by the LifeShirt®. Figure 8 shows the individual traces of the abdomen and the rib cage, and Figure 9 shows the summation of the two traces.

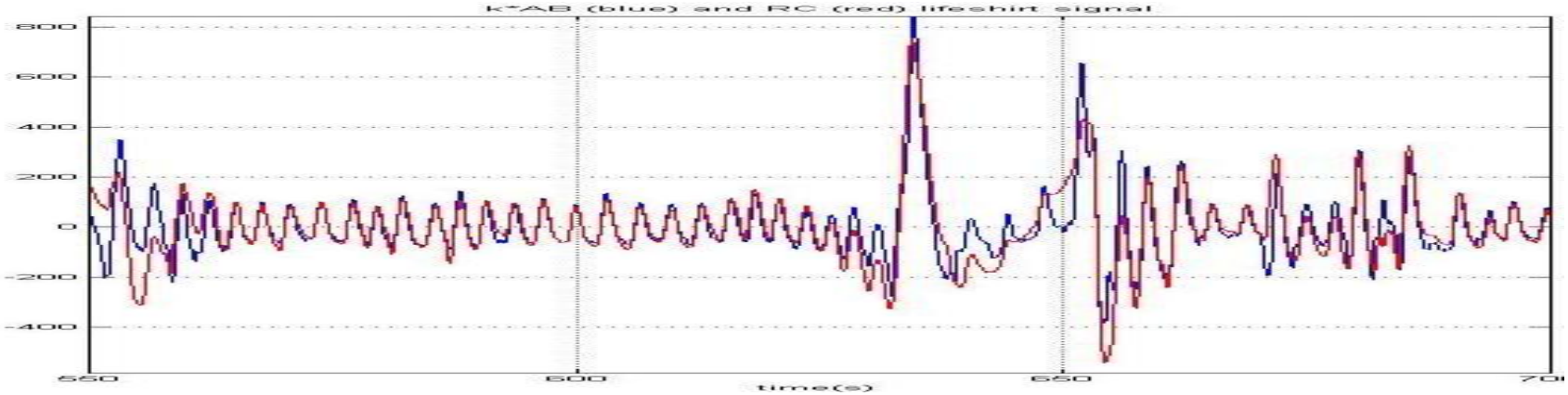


Figure 8: Rib cage and abdomen traces from the LifeShirt®. The blue signal represents the abdominal trace and the red signal represents the rib cage trace.

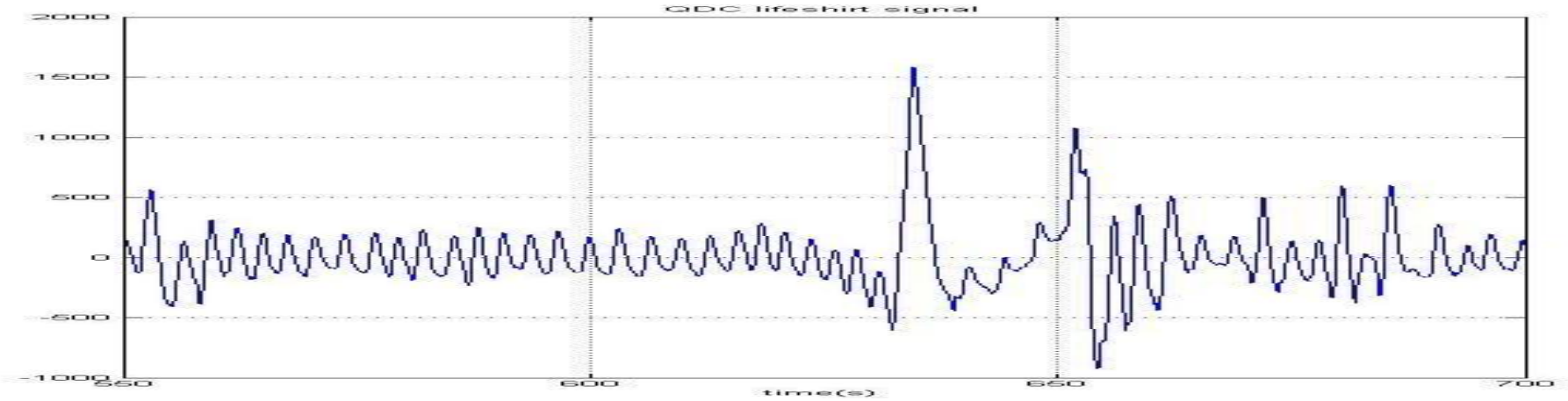


Figure 9: Tidal volume was derived from the summation of the two traces, in accordance with Konno and Mead's model.

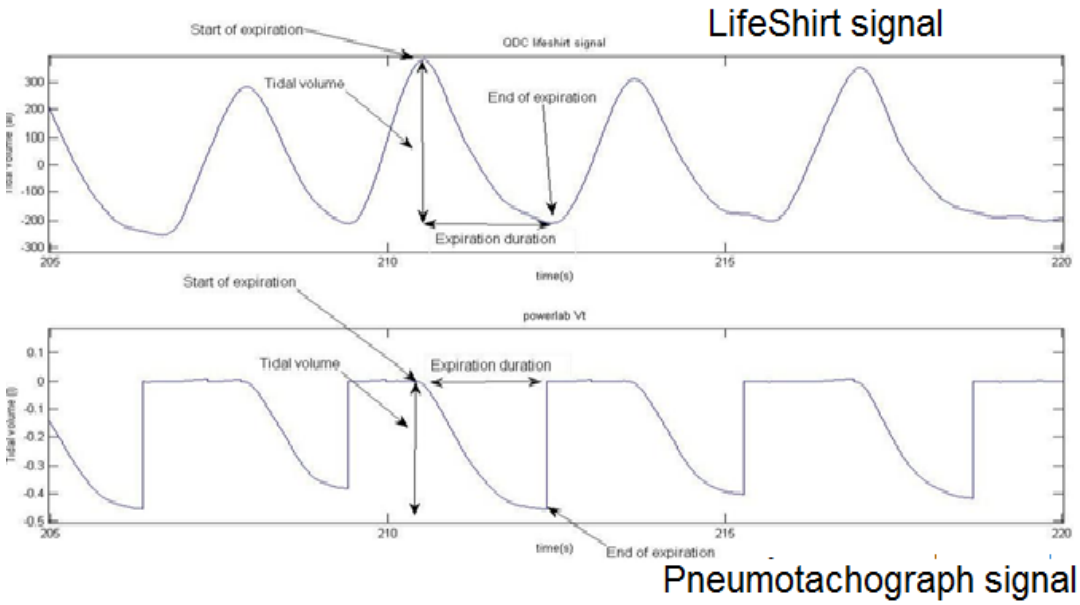


Figure 10: Diagram to show how the parameters were calculated from the signals

Figure 10 shows a graphical representation of how the parameters were calculated from the signals. For the LifeShirt® signals, the algorithm identified the peak (the start of expiration) and the trough (the end of expiration) for each cycle. The amplitude of the cycle was the tidal volume, and the time in between the peak and the trough was the expiration duration. Breaths that were shorter than one third of the mean expiration duration were rejected and were not recorded as a breath. For the pneumotachograph signal, since it measures only expiration tidal volume, the signals stays at zero during the inspiration phase. The algorithm identified the point where the signal became negative as the start of expiration. The end of expiration is the minimum point of the cycle. The amplitude (measured from zero to the minimum point) was the tidal volume, and the time taken from zero to the minimum point was the expiration duration.

There were several steps to prepare the signals for analysis. The steps are outlined below, and graphical representations of the steps are shown in Figures 11 to 13.

Steps for preparing recorded respiratory signals for analysis:

Step 1: The signals were exported in a MatLab format from the associated software in each device

Step 2: The signals were loaded into MatLab, using the algorithms provided by ISVR. In MatLab, the two ten-second breath holds were visually identified (see Figure 11)

Step 3: The second ten-second breath hold marked the beginning of recording for the data used in the analysis. The beginning of the first expiration after the second breath hold from each device was manually selected as the beginning of the signal for analysis (see Figure 12). To clearly identify the start point, the signals were manually zoomed in to identify the moment when the signal first began to move towards negative.

Step 4: After selecting the start points of both signals, the algorithm then calibrated the signals and aligned them so that respiratory cycles from both devices were in step (see Figure 13). With the aligned signals, another piece of MatLab code was used to measure the tidal volume and expiration for each respiratory cycle. The algorithm also plotted the start time for each respiratory cycle and the expiration duration. Visual identification was used to locate any un-matched breaths (refer to Section 3.8.1.1 for the definition of an un-matched breath)

Step 5: The measurements of tidal volume and expiration duration calculated from the signals were then exported into a spreadsheet (.csv file)

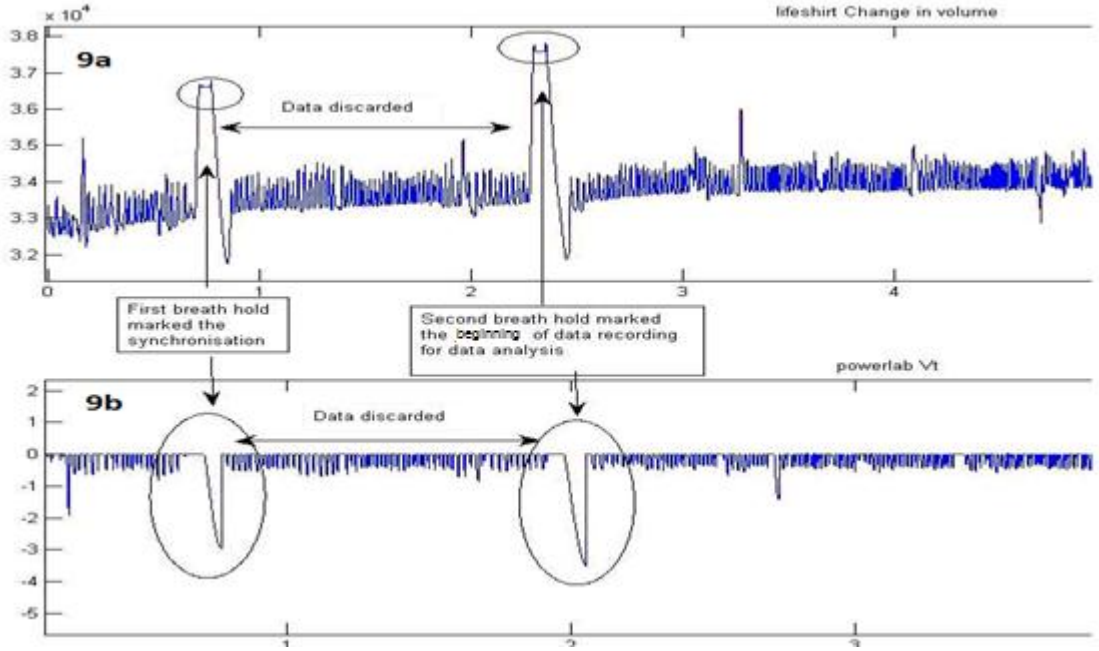


Figure 11: Diagram to illustrate step 2, where the signals were imported into MatLab. The algorithm was developed to allow both signals to be displayed and to identify the breath hold marker. 9a is the signal from the LifeShirt® and 9b is the signal from the pneumotachograph.

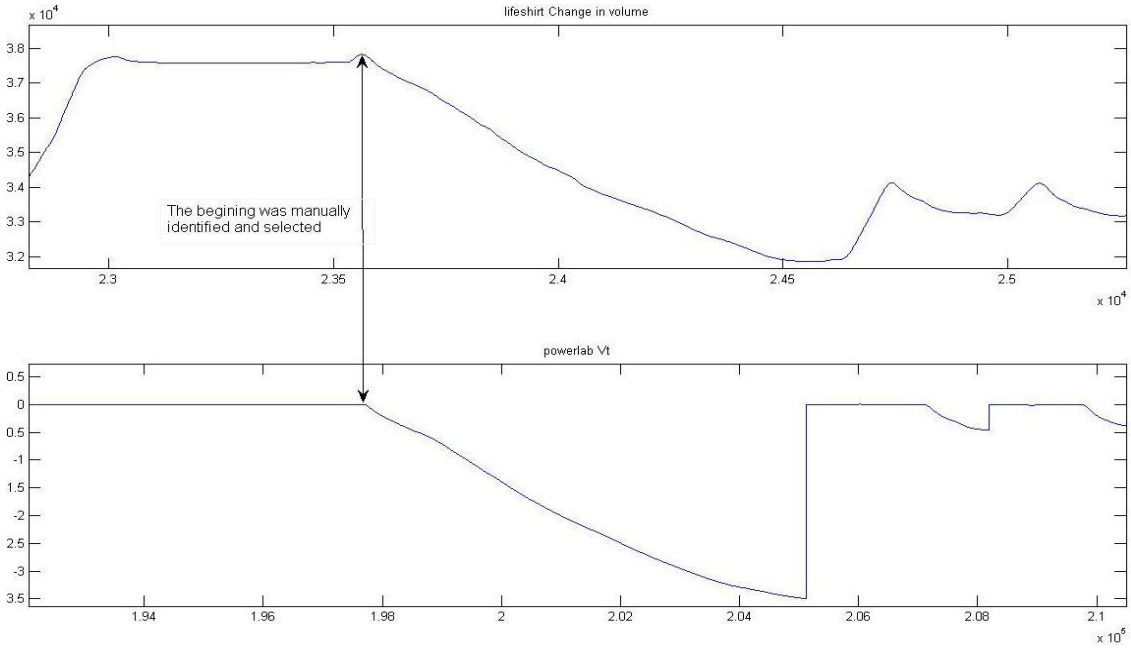


Figure 12: Diagram to illustrate step 3, where the second ten-second breath hold marked the beginning of recording for the data used for analysis. The beginning of the first expiration after the breath hold from each device was manually selected as the beginning of the signal.

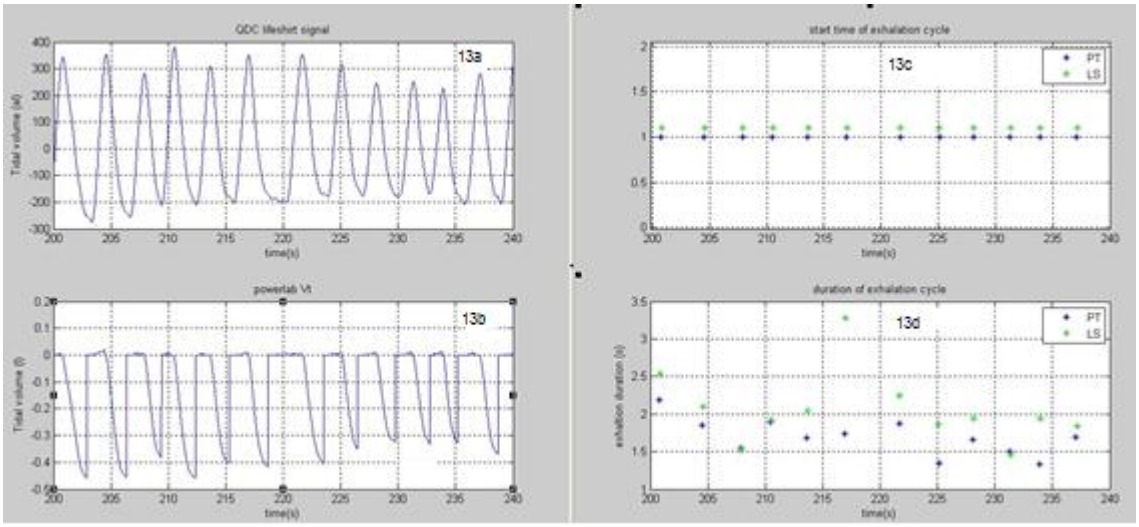


Figure 13: Diagram to illustrate step 4, where the algorithm calibrated the signal and aligned the signals so that each respiratory cycle corresponded to each other on the same time scale.

13a and 13b show the aligned signals of the two devices. The algorithm also plots the start time for each respiratory cycle and its expiration duration, to allow visual identification of any un-matched breaths (13c and 13d). 13a shows the signal of the LifeShirt® and 13b shows the signal of the pneumotachograph. 13c shows the start time of each respiratory cycle and 13d shows the expiration duration of each respiratory cycle.

4.8.1.1 Un-matched breath identification and removal

For the majority of the signal traces, any deviation from zero in the signal for one device was reflected in a similar deviation in signal from the other device. However, this was not always the case, and these non-matching deviations were called un-matched breaths. Un-matched breaths were identified by a visual inspection of all breath cycles for each participant. An un-matched breath was defined as:

- Algorithm recorded a deviation in signal as a breath, using pneumotachograph as reference
- Pneumotachograph recorded a breath and LifeShirt® did not
- Pneumotachograph recorded two breaths and LifeShirt® recorded one
- Pneumotachograph recorded one breath and LifeShirt® recorded two

Examples of un-matched breath cycles are illustrated in Figure 14.

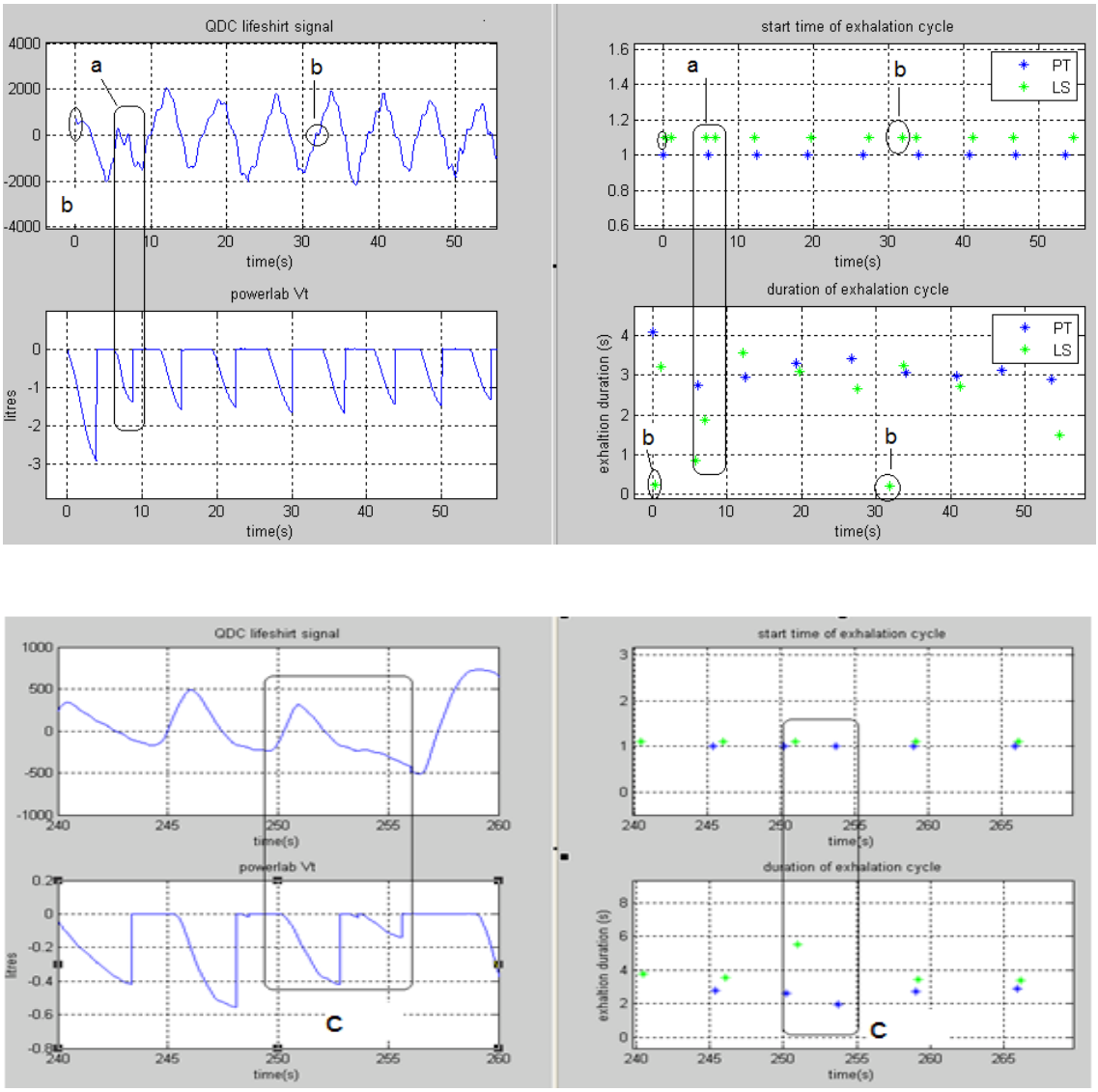


Figure 14: Examples of un-matched respiratory cycles.

Two types of error are shown in this diagram. One was where the algorithm detected a small deviation in the signals (b) and recorded it as a breath when it was not a breath. The other was the when the LifeShirt® recorded two breaths and the pneumotachograph recorded one (a) or vice versa (c). These were not comparable respiratory cycles, and they were removed from the output file in the spreadsheet.

Once the un-matched breath cycles had been visually identified in MatLab, the breaths were then identified in the spreadsheet output file by the start time of the respiratory cycle. The un-matched breaths were removed from the main data set and compiled for future analysis.

After all the respiratory data set from all participants had been processed by the same method, the obtained measurements for tidal volume and expiration duration (without the un-matched breaths) were imported into SPSS (version 17) for statistical analysis.

4.8.2 Statistical analysis

This section discusses the statistical analyses used to test the hypotheses described previously.

4.8.2.1 Data normality

Respiratory parameters of tidal volume and expiration duration were checked for normality using visual estimation of histograms and the Kolmogorov-Smirnov test. This determined whether parametric or non-parametric tests should be used.

4.8.2.2 Analysis of demographic data

Date of birth, gender, height and weight were entered into SPSS for each participant. Body mass index was also calculated from height and weight using the formula:

$$\text{BMI} = \text{weight in kilograms} / \text{height in metres}^2$$

Descriptive statistics were used to describe the participants and the sample characteristics.

4.8.2.3 Analysis of linear association of tidal volume between devices

Association of the tidal volume data between the pneumotachograph and the LifeShirt® was assessed by correlation coefficients. Pearson's correlation was used on data sets that were normally distributed. The Spearman's rank correlation test is appropriate when: 1) the data set is non-normally distributed;

2) there are outliers within the dataset. Spearman's rank correlation test was originally designed to test for non-linear relation, whereas Pearson's correlation is more suited to test for linear relationships (Portney & Watkins, 2000). Although Spearman's correlation test may not be as robust as Pearson's test in determining a linear relationship, the relation between two variables may be made clearer by converting variables to ranks when variables are skewed (Kowalwski, 1975). Kowalwski (1975) examined the issue of both Pearson's and Spearman's rank correlation test and argued that a violation in the distribution assumption of the data set would mean the Pearson's correlation test is unsuitable when the data is not normally distributed. Kowalwski concluded that Spearman's rank correlation should be used if the dataset is not at least close to normal distribution. The key reason leading to the use of Spearman's rank correlation coefficient test in this study was the existence of outliers. It is known that the Pearson test is susceptible to outliers, which are likely to affect the results of the test (Portney & Watkins, 2000). By using Spearman's ranked correlation coefficient, data points were converted to ranks which should minimise the effect of outliers.

In Chapter 3.3.1 the limitations of correlation coefficients for assessing differences in measurement methods were discussed. However, the test was suitable in this case because it gave an indication of how strongly the tidal volume measured by the LifeShirt® was associated with the tidal volume measured by pneumotachograph – i.e. how reliably one could state that when the pneumotachograph recorded an increase in tidal volume, the LifeShirt® also recorded a proportionate increase. In addition, as the tidal volumes measured by the two devices are in different units due to their calibration, correlation coefficient tests are appropriate because they are capable of working on ratio data and are not affected by the difference in units.

4.8.2.4 Analysis of agreement of expiration duration between devices

The analysis of the agreement of expiration duration data between devices was different to the analysis of the agreement of tidal volume data. This was

because expiration duration was measured at its absolute value and in the same unit. Hence direct comparisons could be made between the devices. Correlation coefficients are therefore not sufficient to assessing the agreement of expiration duration data between devices, because they can only demonstrate the linear association between devices.

Bland and Altman analysis

A Bland and Altman plot was used to assess the agreement of the expiration duration data between devices. The extent of the agreement was examined by plotting the difference in expiration duration for each respiratory cycle between the LifeShirt® and the pneumotachograph against the mean expiration duration of each respiratory cycle from the LifeShirt® and the pneumotachograph. A figure showing the Bland and Altman plot can be found in Chapter 4: Results (Figure 19).

For the graph shown in Figure 19, the x-axis represents the mean of the two methods of measurement, whereas and the y-axis represents the difference between the measures. A solid line is used to define the mean difference between the two measures, and two dotted lines represent the 95% limits of agreement. These define the range within which 95% of the differences between measurements would lie (Bland & Altman, 1999). The 95% limits of agreement are calculated as the mean difference plus or minus two standard deviations. The wider the 95% limits of agreement, the wider the range of differences between devices. If the repeated measurements completely agree with each other, then all the points would lie along the solid line. Systematic bias can also be identified from the graph. Bias refers to a situation in which a measure is consistently higher or lower than the reference values. In a Bland and Altman plot, if the majority of the data points are above the mean difference (represented by the solid line) then the device has a bias towards overestimating. If the majority of the data points are below the mean difference, then the device has a bias towards underestimating.

Intra-Class Correlation Coefficient (ICC)

The intra-class correlation (ICC) was also used to analyse the agreement between expiration duration data recorded by the LifeShirt® and the pneumotachograph. The ICC value was also used in equation 7 for the calculation of the standard error of measurement. The intra-class correlation coefficient of expiration duration between the LifeShirt® and the pneumotachograph was calculated from the pooled data for each respiratory cycle included in the analysis.

The details of all ICC models were previously described in Chapter 3.3.3. The (1,1) model is based on the design of different raters rating different people. In this case it would mean comparing a group of people measured using the pneumotachograph with another group of people who were measured using LifeShirt®; this is not the case in this study. The remaining two models (2,1) and (3,1) are for study designs where the same subjects were measured by two (or more) raters (or devices in this study). The difference between these two models is whether one can assume the results are generalisable, and whether the raters (or devices) can be considered random or fixed effects. In this study it is not suitable to suggest that results obtained are generalisable as the results for this study cannot be applied to other types of devices measuring the same respiratory parameters, e.g. in the way that the spirometer was comparable to the pneumotachograph for measuring tidal volume. Therefore, the devices are fixed effects and the (3,1) ICC model was adopted.

Standard Error of Measurement (SEM)

The SEM is based on the principle that if two devices were to record a score an infinite number of times, it may be assumed that the responses would vary from measurement to measurement. These differences would be a function of measurement error. Theoretically, if the scores from these trials were to be plotted, the distribution would resemble a normal distribution curve and the mean would equal the true score, with errors falling above and below the mean.

This theoretical distribution represents the population of all possible measurement errors that could occur. SEM also takes into account the intraclass correlation coefficient. This allows SEM to indicate the error range (precision) of the measurement, which neither the ICC nor error variance can provide. If the two devices completely agree with each other on every measurement, the mean difference would be zero, the standard deviation would be zero and ICC would be one. The SEM will then be zero, suggesting that there is no difference between the devices. The higher the agreement between devices the smaller the difference will be, and consequently the distribution will be less variable. Therefore the SEM reflects the agreement of the devices. SEM can be interpreted according to the properties of the normal distribution curve. For example, suppose a series of breaths were recorded by the LifeShirt® and the pneumotachograph. The mean expiration duration of the two devices is 5 seconds and the SEM is 1.5 seconds. Therefore, there is a 68% chance that each score will be within ± 1 SEM of the mean (between 3.5 and 6.5 seconds) or a 95% change that it falls within ± 2 SEM of the mean (between 2 seconds and 8 seconds).

Thus, a small standard error of measurement reflects a small difference between the devices, and therefore a higher agreement between devices.

Paired t-test

Paired t-tests can be used to test the significance of differences between the means of two sets of scores that are related, such as when the same participant is measured on two occasions or when a parameter is measured by two different devices (Thomas *et al*., 2005a). The significance of the difference between the means for expiration duration and tidal volume recorded by both devices were therefore tested by paired t-tests. The confidence level was set at 0.05, as is standard for this test (Diamond & Jefferies, 2008).

4.8.2.5 Analysis of breath by breath variability in tidal volume

Although many people assume quiet breathing to be regular in both volume and timing, there is evidence of within-subject variability (Wuyts *et al*., 2011;

Kuratomi *et al*., 1985; Fiamma *et al*., 2007a). Breath by breath variability of tidal volume was quantified by the coefficient of variability (CV). This was calculated from the mean and standard deviation to assess the spread of the data. Since the LifeShirt® and the pneumotachograph were not calibrated to the same units, the method of assessing variability by using raw values of the mean and standard deviation was not possible. The CV values are directly comparable since it is the ratio of the standard deviation to the mean expressed as a percentage. It is calculated with the following formula:

$$\text{Coefficient of variation} = (\text{standard deviation} / \text{mean}) \times 100 \qquad (8)$$

4.8.2.6 Analysis of difference in tidal volume and expiration duration between quiet and exercise breathing

The mean tidal volume and expiration duration collected during quiet breathing from the two devices were compared to test: 1) if there was a difference in the tidal volumes and expiration durations between quiet breathing and exercise, using the pneumotachograph as a reference; and 2) whether the LifeShirt® also recorded a difference in tidal volume and expiration duration if the pneumotachograph recorded a difference.

1. Paired t-tests were conducted on i) the mean tidal volume and ii) the mean expiration duration of all breaths included in the analysis during normal breathing and exercise breathing, recorded on the pneumotachograph. The aim was to test whether there was a statistically significant difference in the mean values of either parameter between the two experimental conditions.
2. A paired t-test was then applied to the data recorded by the LifeShirt® for i) mean tidal volume and ii) mean expiration duration of all breaths included in the analysis during normal breathing and exercise breathing, to test whether the LifeShirt® recorded a statistically significant difference between the two experimental conditions. Differences observed in the LifeShirt® were compared to those found for the pneumotachograph to establish whether the LifeShirt®

also recorded significant differences between the two experimental conditions when significant differences were recorded by the pneumotachograph.

The reason that the mean values of tidal volume and expiration duration were used for comparison is that the natural breath-to-breath variability expected in breathing pattern (Benchetrit, 2000) means that breaths from quiet breathing and exercise breathing cannot be paired in a reasonable and systematic way.

4.9 Summary

This chapter has described the methodology of the validation study and the analysis methods used to: 1) test if the LifeShirt® can estimate relative linear changes in tidal volume in relation to the pneumotachograph during quiet breathing and exercise; 2) test if the LifeShirt® measurements of expiration duration correlate and agree with the pneumotachograph; 3) test whether the LifeShirt® records significant changes in tidal volume and expiration duration if significant changes are also recorded by the pneumotachograph; and 4) test if the LifeShirt® measures the same amount of breath by breath variability as the pneumotachograph during quiet breathing and exercise. The next chapter will present the results of the analysis.

Chapter 5: Results of the LifeShirt® validation study

5.0 Introduction

This chapter presents the results of the data analysis for the LifeShirt® validation study. Demographic data for the sample population will be presented first, followed by the respiratory data collected during quiet breathing, and then the respiratory data collected during exercise. In each section, the tidal volume data are presented first, followed by the expiration duration data and finally the breath by breath variability data.

The order of results presented is outlined below:

5.1 Demographic data

Data related to gender, age, height, weight and body mass index (BMI) were collected to characterise the sample. The following table presents the results of the demographic data analysis.

Participant	Gender	Age	Weight (Kg)	Height (m)	BMI (Kg/m^2)	LS Size
1	M	24	65	1.71	22	Small
2	M	28	56	1.75	18	Small
3	M	26	69	1.71	24	Medium
4	F	33	81	1.68	22	Medium
5	M	27	86	1.64	32	Large
6	F	31	71	1.65	26	Medium
7	M	32	58	1.68	21	Small
8	F	26	55	1.53	24	Small
9	M	29	74	1.75	24	Medium
10	M	23	72	1.83	22	Medium
11	M	26	56	1.58	22	Small
Mean (stdv)		28 (3)	67.55 (10.58)	1.68 (0.08)	23.25 (3.55)	

Table 2: Demographic data for the sample population.

Key: LS – LifeShirt®, stdv – standard deviation

As shown in Table 2, eleven healthy individuals (eight males and three female), with ages ranging from 24 to 32 years old, were recruited for the study. Their BMI values ranged from 18 to 32; nine participants had normal weights, one had a low BMI and one was defined as clinically obese (NHS, 2009).

5.2 Quiet breathing

This section presents the respiratory data collected during quiet breathing. The data for tidal volume will be presented first, then the data for expiration duration, and finally the data for breath by breath variability of tidal volume.

5.2.1 Tidal volume during quiet breathing

The following figures give examples of the time series for tidal volume recorded from the LifeShirt and the pneumotachograph recorded during quiet breathing.

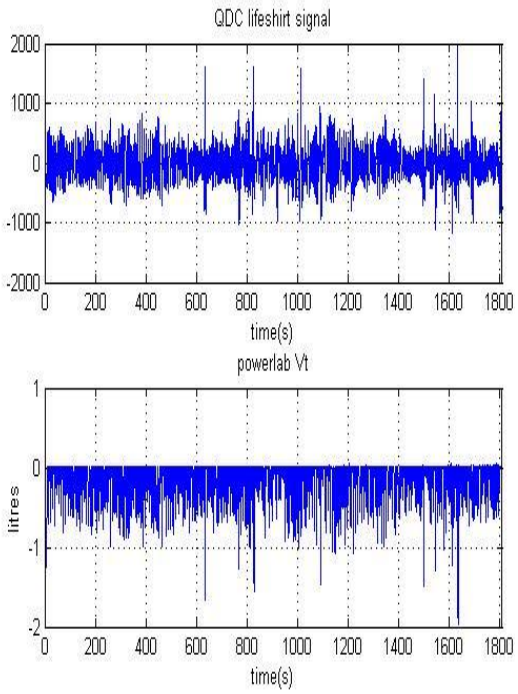


Figure 15: Time series of tidal volume recorded from LifeShirt® and PT for participant 2 during quiet breathing.

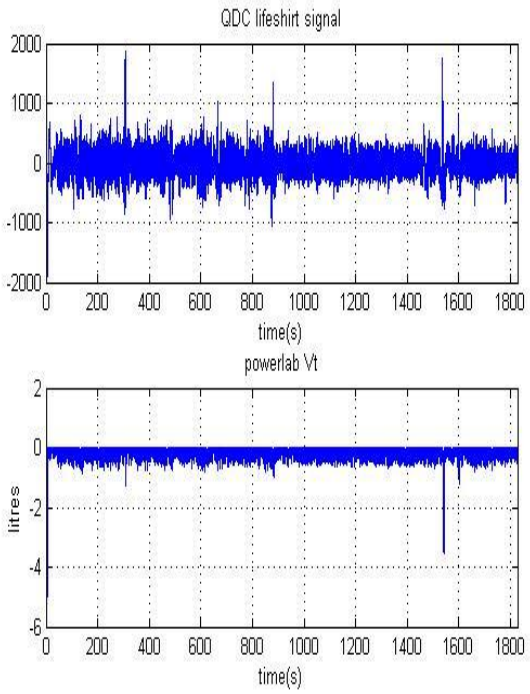


Figure 16: Time series of tidal volume recorded from LifeShirt® and PT for participant 4 during quiet breathing.

The following table provides the descriptive analysis of the tidal volume data recorded simultaneously by the LifeShirt® and the pneumotachograph.

Participant	N	Mean PT Vt (l)	Stdv	Mean LS Vt (al)	Stdv	PT CV (%)	LS CV (%)
1	392	0.46	0.22	0.68	0.4	48	58
2	251	0.63	0.27	0.77	0.37	44	48
3	403	0.5	0.22	0.62	0.25	44	40
4	473	0.52	0.24	0.7	0.26	46	37
5	241	0.67	0.57	0.93	0.68	85	73
6	415	0.20	0.10	0.32	0.13	50	41
7	182	1.16	0.51	0.85	0.42	44	49
8	198	1.15	0.51	0.76	0.36	42	45
9	578	0.47	0.10	0.54	0.12	21	20
10	576	0.60	0.18	0.4	0.11	33	30
11	292	0.72	0.18	0.89	0.16	24	18
Group Mean (stdv)	363 (142)	0.61 (0.32)		0.67 (0.20)		44 (17)	42 (16)

Table 3: Descriptive statistics for tidal volume during quiet breathing

Key: N – number of breaths, al – assumed litre, l – litre, PT – pneumotachograph, LS – LifeShirt®, CV – coefficient of variability, stdv – standard deviation

Table 3 shows the number of breaths available for analysis for each participant (ranging from 182 to 578, group total 4001), along with the group and individual means and standard deviations of the quiet breathing tidal volume data measured by each device. The tidal volume measurements of the two devices are not directly comparable because the LifeShirt® cannot be calibrated to give an absolute value for tidal volume. The unit of measurement for the LifeShirt® is an ‘assumed litre’. This terminology is adopted from the manufacturer, VivoMetrics, who termed the uncalibrated output an ‘assumed millilitre’ (VivoMetrics user manual). The output was converted to an ‘assumed litre’ by dividing the LifeShirt® output by one thousand. The quiet breathing tidal volume coefficient of variability (CV) was calculated from the mean and standard deviation in order to assess the spread of the data. The CV is the ratio of the standard deviation to the mean, which is expressed as a percentage. The CV values show that there is variability in quiet breathing tidal volume both within and between individuals. Both the LifeShirt® and the pneumotachograph

recorded a similar mean level of variability (44% in the pneumotachograph, 42% in the LifeShirt®) when averaging across all subjects.

The following box and whisker plot (Figure 17) gives a graphical representation of the distribution of the quiet breathing tidal volume data from both devices. In these plots, the box shows the distance between upper and lower quartiles, and the line across it represents the median. If the data distribution is roughly symmetrical then both whiskers will be about the same length. If the data points are sparse on one side then one whisker may be considerably shorter than other. A ‘step’ represents 1.5 times the box length. If a datum point falls there, then the whiskers are drawn to one step beyond the quartiles. If no datum point falls there, the whiskers are drawn to the largest observed value that is still one step away from the quartile. Any data points that fall beyond one step are called outliers (presented as an open circle ‘o’ on the graph), with any falling more than two steps away being called extreme outliers (presented as a star * on the graph). Outliers were observed in all participants, showing the variability in tidal volume within individuals.

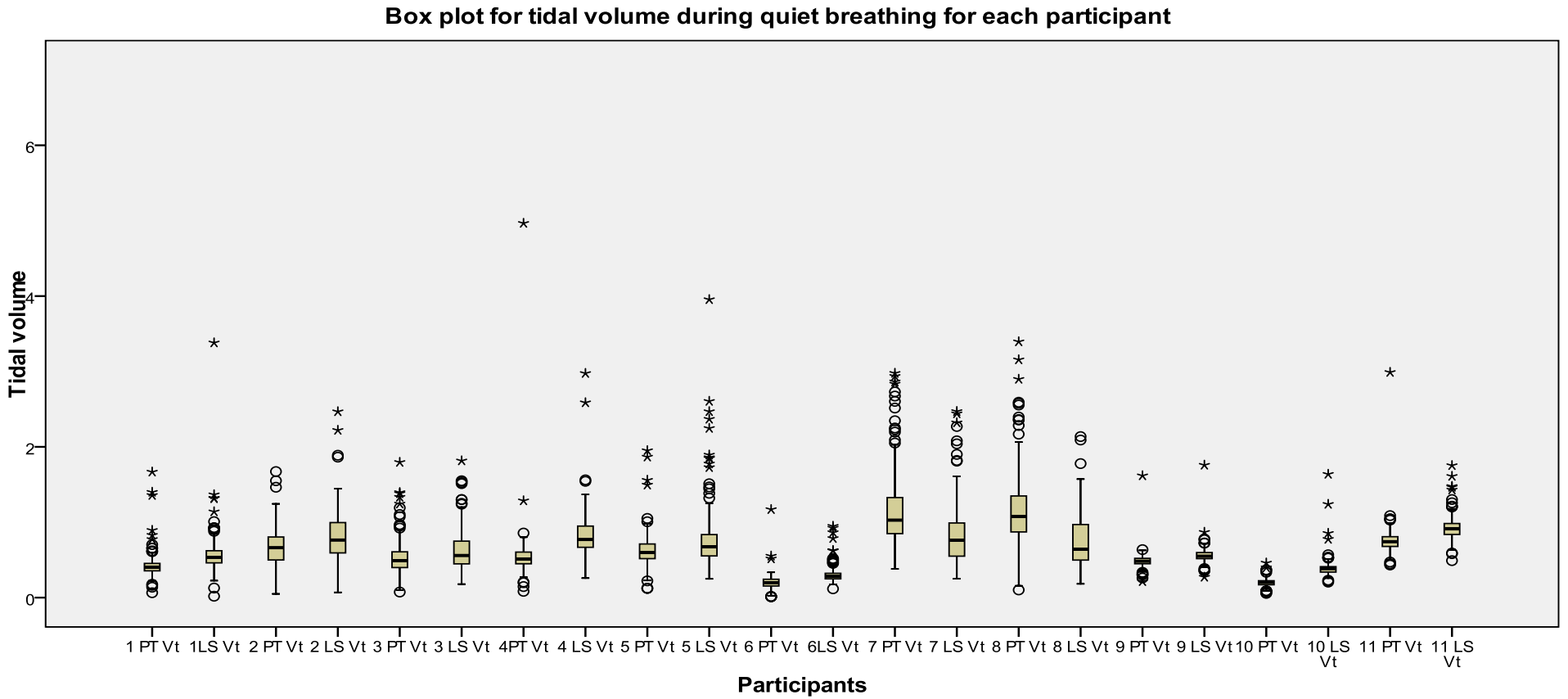


Figure 17: Box plot for tidal volume during quiet breathing for each participant. ***Key: PT – pneumotachograph, LS – LifeShirt®, Vt – tidal volume***

5.2.1.1 Correlation of tidal volume between devices during quiet breathing

Intra-participant correlation of breath by breath tidal volume during quiet breathing

The breath by breath quiet breathing tidal volume data from all participants were checked for normality using histogram and the Kolmogorov-Smirnov test. The results showed that the data sets were unlikely to be not normally distributed ($p < 0.05$). A histogram also demonstrated a skewed distribution, and therefore the data were analysed using non-parametric tests.

Participant	Vt correlation
1	0.8 *
2	0.8 *
3	0.7 *
4	0.8 *
5	0.7 *
6	0.5 *
7	0.6 *
8	0.7 *
9	0.7 *
10	0.7 *
11	0.9 *
Mean (stdv)	0.7 (0.11)

* Correlation is significant at the 0.01 level (two-tailed)

Table 4: Spearman's rank correlation between the LifeShirt® and pneumotachograph data for quiet breathing tidal volume from each participant on a breath by breath basis

Table 4 shows the results of the Spearman's rank correlation test between the quiet breathing tidal volume recorded by the LifeShirt® and the data recorded by the pneumotachograph on a breath by breath basis. This test was performed to see if the LifeShirt® could detect linear relative changes of tidal volume in relation to the pneumotachograph on a breath by breath basis when calibrated by the method proposed by Millard (2002). On average, the quiet breathing tidal volume correlation coefficient is 0.7 between the LifeShirt® and the

pneumotachograph. All participants had statistically significant correlations at the 0.01 level. However, not all participants showed ‘high’ correlation, as the correlation coefficients ranged from 0.5 to 0.9. The variation in correlation between subjects is not associated with BMI (Spearman’s rank correlation 0.4). There are insufficient data to demonstrate whether the variation is associated with gender.

Inter-participant correlation of mean tidal volume during quiet breathing

The correlation of the mean tidal volume between the LifeShirt® and the pneumotachograph was tested across all participants. The set of mean tidal volumes from all participants (shown in Table 1) was checked for normality using histogram and Kolmogorov-Smirnov Test. The results showed that the data were likely to be normally distributed, and therefore Pearson’s correlation coefficient test was used. The mean tidal volume measured by the LifeShirt® and the pneumotachograph were significantly correlated with a correlation coefficient of 0.8 (at the 0.01 level).

These results demonstrate that the linear tidal volume changes detected by the pneumotachograph and the LifeShirt® are linearly related during quiet breathing, both on a breath by breath basis and on the interval averaged basis.

5.2.2 Expiration duration during quiet breathing

This section presents the results of the analysis of expiration duration during quiet breathing. A table of descriptive statistics is presented below, followed by the results of correlation coefficient, paired t-test, Bland and Altman analysis and standard error of measurement.

Table 5 below shows the results of the descriptive analysis of quiet breathing expiration duration recorded simultaneously by the LifeShirt® and the pneumotachograph. Table 5 shows the number of breaths available for analysis, the minimum and maximum expiration durations, and the mean and standard deviation expiration durations recorded simultaneously by the LifeShirt® and the pneumotachograph during quiet breathing. This analysis was performed on a breath by breath basis, i.e. on the corresponding breaths recorded by both the LifeShirt® and the pneumotachograph. The LifeShirt® samples at 50Hz and therefore does not have the precision to sample changes in hundredths of a second. Expiration duration is therefore shown to two decimal places to reflect the sampling frequency.

Participant	Device	N	Te Min (s)	Te Max (s)	Te Mean (s)	Te Stdv
1	PT	392	0.32	3.78	1.61	0.35
	LS	392	0.40	8.70	2.47	0.82
2	PT	251	0.18	8.49	3.41	1.33
	LS	251	0.44	8.60	4.09	1.46
3	PT	403	0.37	9.89	2.12	0.68
	LS	403	0.80	9.36	2.48	0.81
4	PT	473	0.61	5.58	2.04	0.49
	LS	473	0.60	4.72	2.08	0.52
5	PT	234	1.47	8.84	3.85	0.83
	LS	234	1.28	8.84	4.48	1.05
6	PT	415	1.60	2.72	1.33	0.36
	LS	415	0.32	3.60	1.72	0.35
7	PT	182	1.94	8.61	4.80	1.21
	LS	182	1.72	10.40	5.51	1.43
8	PT	198	0.65	9.16	3.90	1.23
	LS	198	0.40	9.26	4.81	1.57
9	PT	578	0.21	3.14	1.38	0.26
	LS	578	0.62	4.9	1.47	0.31
10	PT	576	0.55	2.52	1.22	0.20
	LS	576	1.06	4.06	1.70	0.31
11	PT	292	1.54	5.11	2.99	0.48
	LS	292	2.10	5.28	3.28	0.52
Group mean (Stdv)	PT	363 (142)	0.86 (0.64)	6.16 (2.87)	2.60 (1.24)	
	LS	363 (142)	0.89 (0.59)	7.06 (2.52)	3.09 (1.41)	

Table 5: Descriptive statistics for expiration duration during quiet breathing on a breath by breath basis.

Key: N – number of breaths, Te – expiration duration (s), Min – minimum expiration duration, Max – maximum expiration duration, stdv – standard deviation

The mean and standard deviations of the expiration duration from both devices are directly comparable because both measure the absolute value in seconds. The results show that on average, the LifeShirt® overestimated the quiet breathing expiration duration by 0.51 seconds. The standard deviations are

similar with a difference of 0.71 seconds, suggesting a similar amount of variance in both data sets.

A box plot is shown in Figure 18 to give a graphical representation of the distribution of expiration duration between the two devices during quiet breathing. Figure 18 shows the spread of quiet breathing expiration duration recorded by both devices. Outliers were observed in all participants, reflecting the variability of expiration duration during quiet breathing.

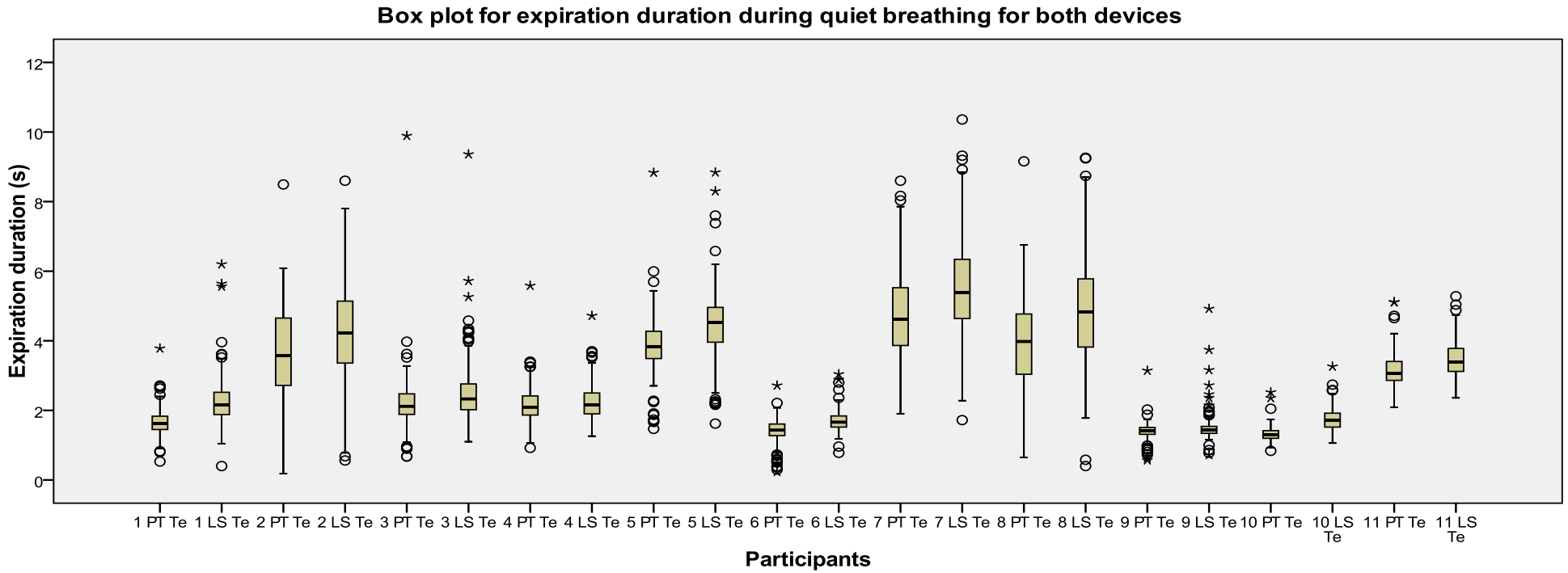


Figure 18: Box plot for expiration duration during quiet breathing for both devices from each participant.

Key: Te – expiration duration (s), LS – LifeShirt®, PT – pneumotachograph

5.2.2.1 Correlation of quiet breathing expiration duration between devices

Intra-participant correlation coefficient of breath by breath expiration duration during quiet breathing

The breath by breath expiration duration data during quiet breathing from all participants were checked for normality using histogram and Kolmogorov-Smirnov test. The results showed that the data sets of breath by breath expiration duration for all participants were unlikely to be normally distributed ($p < 0.05$). A histogram demonstrated a negative skew of the distribution. Therefore the data were analysed using non-parametric tests.

Participant	Te correlation
1	0.2 *
2	0.7 *
3	0.6 *
4	0.9 *
5	0.5 *
6	0.2 *
7	0.8 *
8	0.5 *
9	0.7 *
10	0.4 *
11	0.9 *
Mean (Stdv)	0.6 (0.25)

Table 6: Spearman's rank correlation coefficient between the LifeShirt® and pneumotachograph data for quiet breathing expiration duration from each participant on a breath by breath basis.
** Significant at p = 0.01 level*

Table 6 shows the results of a Spearman's rank correlation test between the quiet breathing expiration duration recorded by the LifeShirt® and the pneumotachograph on a breath by breath basis. This test was performed to see if there was a linear relationship between expiration duration measured on a breath by breath basis by the LifeShirt® and the pneumotachograph when calibrated by the method proposed by Millard (2002). All corresponding breaths

from the LifeShirt® and the pneumotachograph were included in the analysis. On average, the quiet breathing expiration duration correlation coefficient between the LifeShirt® and the pneumotachograph was 0.6. All participants had statistically significant correlations at the 0.01 level. However, the correlation varied between participants, ranging from 0.2 to 0.9.

Inter-participant correlation of quiet breathing mean expiration duration

The correlation of the mean expiration duration between the LifeShirt® and the pneumotachograph was tested across all participants. The quiet breathing mean expiration duration data from all participants (shown in Table 4) were checked for normality using histograms and the Kolmogorov-Smirnov test. The results showed that the data were normally distributed, and therefore the Pearson's correlation coefficient test was used. The mean expiration duration during quiet breathing was significantly correlated at 0.9 (at the 0.01 level)

5.2.2.2 Difference in breath by breath expiration duration between devices during quiet breathing

This section shows the results of the analysis of the paired t-test on quiet breathing expiration duration. This was carried out to test if the difference in expiration duration during quiet breathing measured by the two devices is statistically significant or not. An analysis of the difference within individuals is presented first, followed by an analysis of the mean difference between individuals.

Intra-participant breath by breath differences in expiration duration between the LifeShirt® and the pneumotachograph during quiet breathing

Paired t-tests were performed on the breath by breath expiration duration data recorded during quiet breathing by the LifeShirt® and the pneumotachograph from each participant. Table 7 shows the results of the paired t-test between the quiet breathing expiration duration recorded by the LifeShirt and the pneumotachograph on a breath by breath basis. Results show a statistically significant difference (at $p = 0.05$ level) between the LifeShirt and pneumotachograph recorded expiration duration on a breath by breath basis for each participant.

Pair	Mean difference	Std. Deviation	t	df	Sig. (2-tailed)
1 PT Te - 1 LS Te	-0.9	0.8	-20.1	391	0.00*
2 PT Te - 2 LS Te	-0.7	1.1	-10.3	250	0.00*
3 PT Te - 3 LS Te	-0.4	0.7	-10.7	402	0.00*
4 PT Te - 4 LS Te	0.0	0.2	-4.1	472	0.00*
5 PT Te - 5 LS Te	-0.6	1.0	-9.8	233	0.00*
6 PT Te - 6 LS Te	-0.4	0.5	-16.2	414	0.00*
7 PT Te - 7 LS Te	-0.7	0.9	-11.0	181	0.00*
8 PT Te - 8 LS Te	-0.9	1.5	-8.5	197	0.00*
9 PT Te - 9 LS Te	-0.1	0.3	-7.3	577	0.00*
10 PT Te - 10 LS Te	-0.5	0.3	-38.7	575	0.00*
11 PT Te - 11 LS Te	-0.3	0.3	-20.3	291	0.00*

Table 7: Paired t-test of expiration duration between LS and PT recorded breath by breath expiration duration during quiet breathing from each participant
*Key: PT – pneumotachograph, LS – LifeShirt®, Te – expiration duration (s), * – significant at 0.05 level*

Inter-participant mean difference in expiration duration between LifeShirt® and pneumotachograph during quiet breathing

The difference in the mean expiration duration for each participant during quiet breathing was tested across all participants. The mean expiration duration for each participant from both devices is shown in Table 8.

	Mean difference	Std. Deviation	T	df	Sig. (2-tailed)
Mean PT Te – Mean LS Te	-0.51	0.28	-6.01	10.00	0.00*

Table 8: Paired t-test of the mean expiration duration of the sample group between LS and PT during quiet breathing.
The difference is significant at p = 0.05 Key: PT – pneumotachograph, LS – LifeShirt®, Te – expiration duration (s), – significant at 0.05 level*

The results of the paired t-test (shown in Table 8) show that the group mean difference in expiration duration between the LifeShirt® and the pneumotachograph during quiet breathing is statistically significant at the 0.05 level.

5.2.2.3 Bland and Altman analysis of expiration duration during quiet breathing

The Bland and Altman analysis was used to assess the agreement between methods of measurements. The extent of agreement was examined by plotting the difference between the expiration duration data measured by the LifeShirt® and the pneumotachograph against the mean of the LifeShirt® and pneumotachograph data. A detailed description of the Bland and Altman analysis procedure can be found in Section 4.8.2.4. The Bland and Altman test and the 95% limits of agreement are based on the normal distribution of the data. As the expiration duration data was not normally distributed, the expiration duration was log transformed to work out the 95% limits of agreement. The values were then anti-logged to obtain the values measured in seconds.

The following section shows the results of the Bland and Altman analysis of the agreement between the expiration duration data measured by the LifeShirt® and the pneumotachograph during quiet breathing.

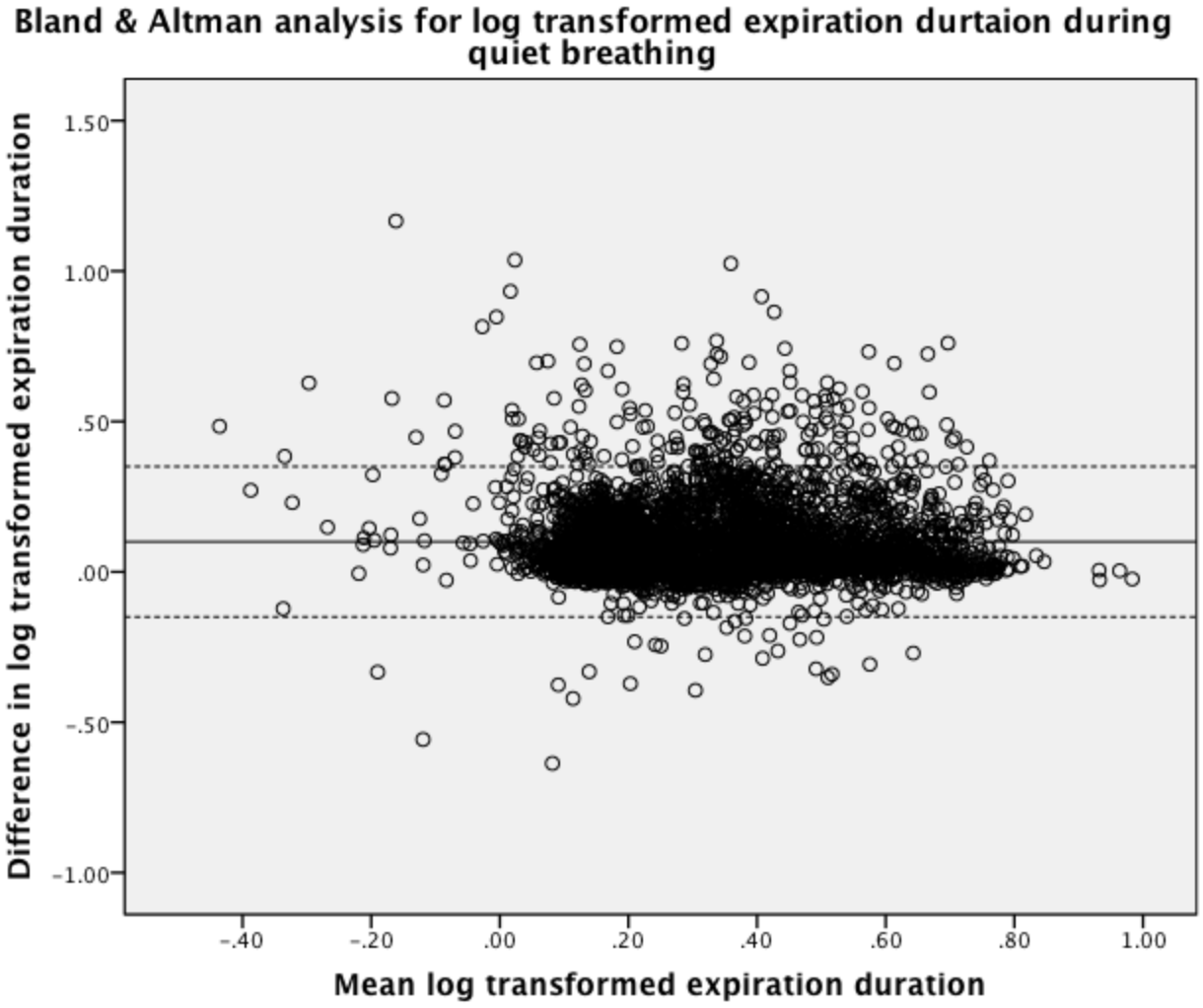


Figure 19: Bland and Altman plot for log transformed expiration duration during quiet breathing.

The Bland and Altman plot (shown in Figure 19) shows that the LifeShirt® has a bias towards overestimating the expiration duration because there are data points outside the upper limits of agreement. The 95% limits of agreement are log -1.8 to +3.8, showing that 95% of all the recorded expiration durations during quiet breathing are within log 5.6 seconds of each other. After taking the anti-log of 5.6 seconds, expiration durations during quiet breathing are within 1.72 seconds of each other.

5.2.2.4 Standard error measurement of expiration time during quiet breathing

The details of the calculation of SEM can be found in Section 4.8.2.4.

The intra-class correlation between the expiration durations measured by the LifeShirt® and the pneumotachograph was calculated with the ICC (1, 1).

The ICC for the expiration duration recorded by the LifeShirt and the pneumotachograph during quiet breathing was 0.7. The standard deviation of the mean expiration duration of the LifeShirt® and the pneumotachograph for all recorded breaths from all participants during quiet breathing was 1.3s. The SEM was calculated as:

$$SEM = 1.3 \times \sqrt{1-0.7}$$

which gives a standard error measurement of 0.71s.

5.2.3 Breath by breath variability in tidal volume during quiet breathing.

Breath by breath variability in the tidal volumes recorded by each device during quiet breathing was assessed by the coefficient of variation (CV). The following section shows the results of the analysis of the CV of tidal volume measurements from each device during quiet breathing.

	PT (%)	LS (%)
1	49	58
2	44	48
3	44	40
4	46	37
5	45	64
6	50	41
7	44	49
8	42	45
9	21	20
10	33	30
11	24	18
Mean CV	41	39

Table 9: Breath by breath variability in tidal volume recorded by pneumotachograph (PT) and LifeShirt® (LS) during quiet breathing, expressed as the coefficient of variation (CV)

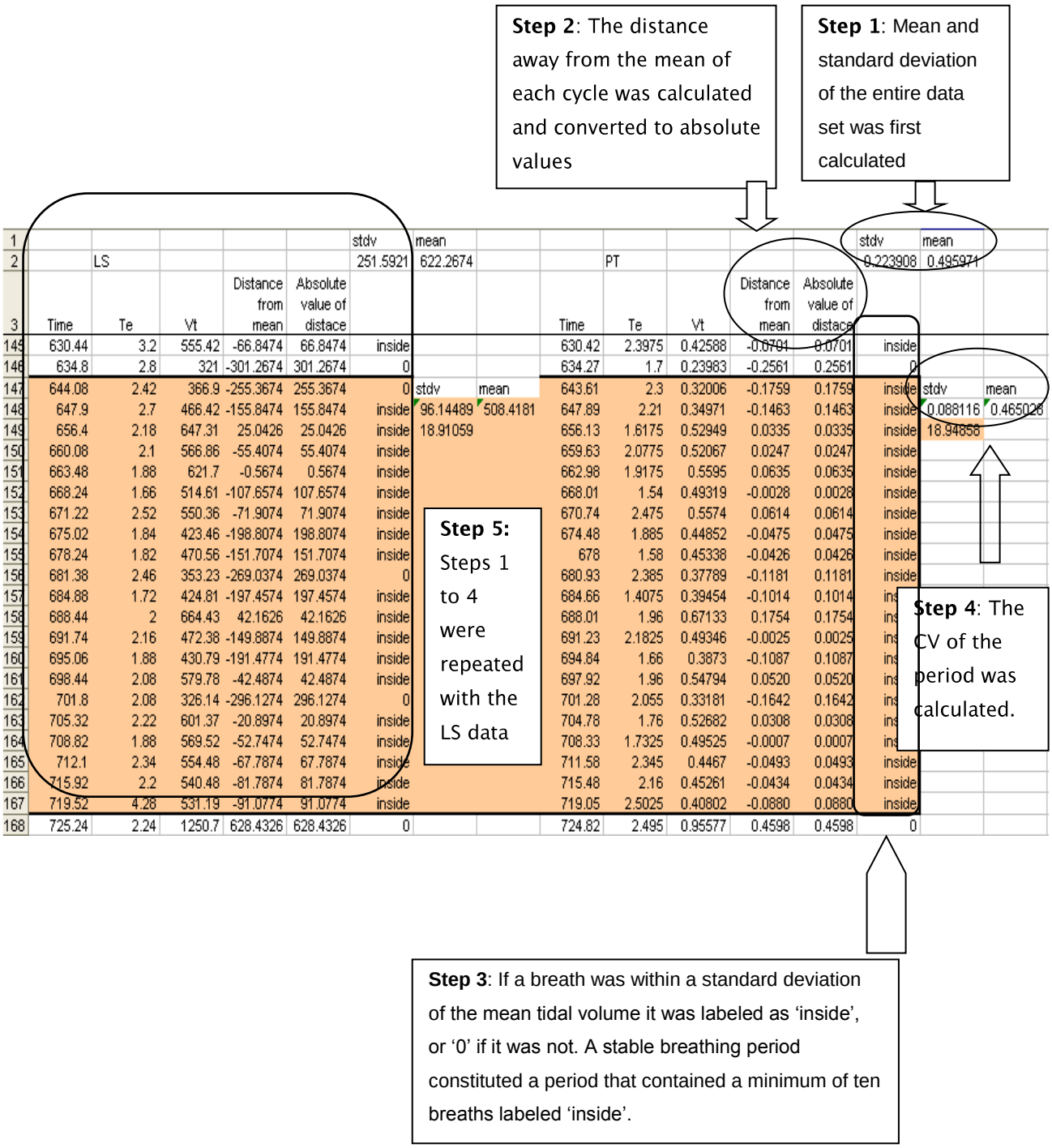
A paired t-test was performed on the mean CVs of the tidal volume data during quiet breathing measured by the LifeShirt® and the pneumotachograph. There was no significant difference ($p=0.78$ at the 0.05 level) between the mean CV for the LifeShirt® and that for the pneumotachograph. A Kolmogorov-Smirnov test showed that the coefficients of variation for both the LifeShirt® and the pneumotachograph were normally distributed, and therefore a Pearson correlation coefficient was used to test for correlation between devices. The Pearson correlation coefficient was 0.8 (significant at the 0.01 level).

5.2.3.1 Breath by breath variability of stable tidal volume period during quiet breathing

Since the tidal volume data set during quiet breathing was not normally distributed, the reported CV figures might have been affected by the skewing of the data. The CV values for the tidal volume from each participant indicate that the LifeShirt® and the pneumotachograph recorded similar overall variability for each participant during a thirty-minute period of quiet breathing. It was deemed useful to establish whether the LifeShirt® is able to record similar variability in tidal volume to the pneumotachograph during periods of 'stable' breathing, as identified from the pneumotachograph signal. To explore this, breath by breath variability was analysed in a different way. Periods of 'stable' breathing were identified from the pneumotachograph data for analysis. There is little information in the literature to define what constitutes a stable breathing period, even though the term is frequently used in other studies. In this study, a stable breathing period was defined as a period during which all respiratory cycles were within one standard deviation of the mean, using the pneumotachograph data as reference. The coefficients of variation were then calculated for each stable period, which gave the breath by breath variability of the period. The corresponding periods from the LifeShirt® were identified and the coefficients of variation were calculated and compared with the coefficients from the pneumotachograph data for the same period. This analysis aimed to establish whether the LifeShirt® recorded stable tidal volumes when the pneumotachograph was also recording stable tidal volumes. The process is outlined in detail below, and Figures 20 and 21 give a graphical presentation of how the procedure was performed.

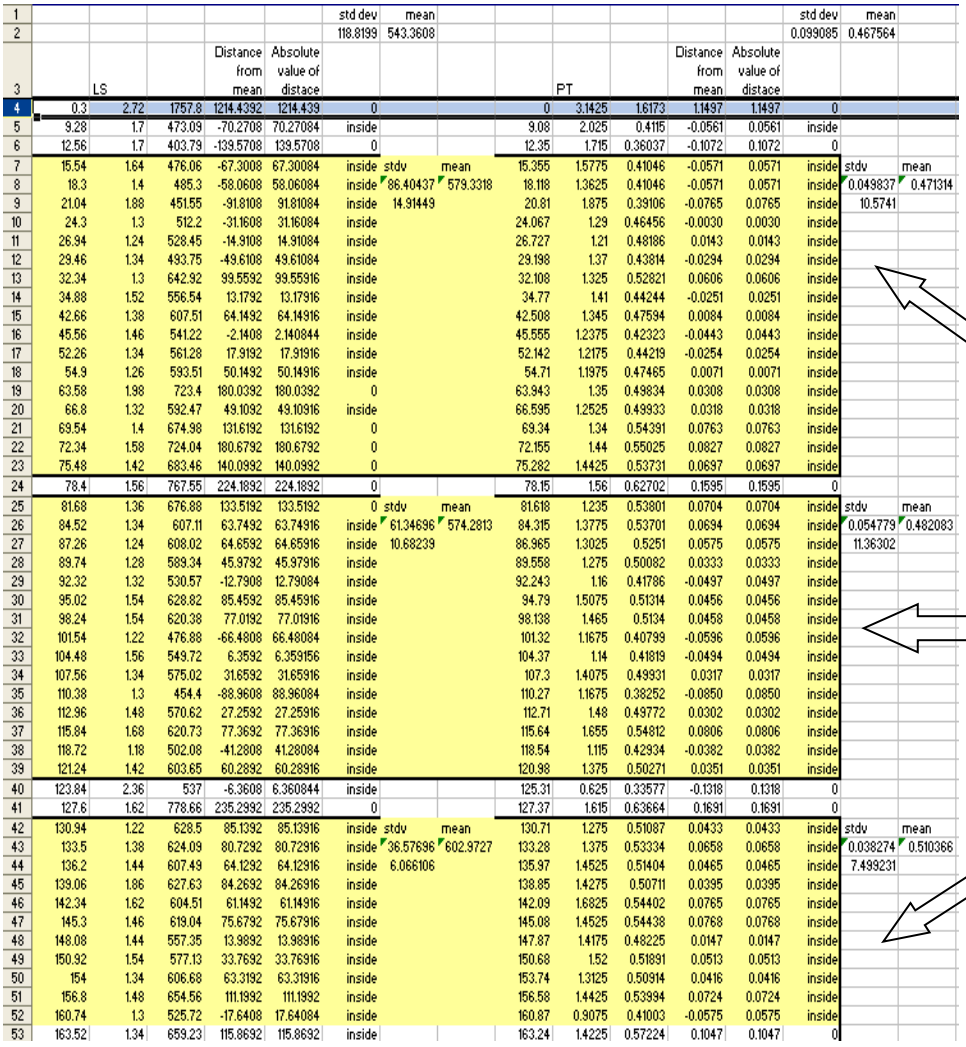
Procedure to identify stable period:

1. Tidal volume mean and standard deviation of the entire pneumotachograph data set were first calculated
2. The distance away from the mean of each breath cycle tidal volume was calculated and converted to positive values
3. If a breath had a tidal volume of one standard deviation away from the mean, then it would be labeled as ‘inside’. If not, it was labeled as ‘0’. Periods with a minimum of ten breaths labeled as ‘inside’ were identified as a stable period
4. The mean, standard deviation and CV of each stable period were then calculated
5. Steps 1 to 4 were repeated for the LifeShirt® data
6. Steps 1 to 5 were repeated for the rest of the data to produce a series of CVs
7. At this stage, each participant had a series of CV values, corresponding to the number of stable breathing periods. The CV values from all stable periods were then used to calculate the mean CV for each participant
8. Paired t-test and correlation coefficient analyses were performed on the mean CV values to test whether the LifeShirt® recorded stable tidal volumes when the pneumotachograph also recorded stable tidal volumes



						stdv	mean								stdv	mean
2		LS				251.5921	622.2674			PT					0.223908	0.495971
3	Time	Te	Vt	Distance from mean	Absolute value of distace				Time	Te	Vt	Distance from mean	Absolute value of distace			
145	630.44	3.2	555.42	-66.8474	66.8474	inside			630.42	2.3975	0.42588	-0.0701	0.0701	inside		
146	634.8	2.8	321	-301.2674	301.2674	0			634.27	1.7	0.23983	-0.2561	0.2561	0		
147	644.08	2.42	366.9	-255.3674	255.3674	0	stdv	mean	643.61	2.3	0.32006	-0.1759	0.1759	inside	stdv	mean
148	647.9	2.7	466.42	-155.8474	155.8474	inside	96.14489	508.4181	647.89	2.21	0.34971	-0.1463	0.1463	inside	0.088116	0.465028
149	656.4	2.18	647.31	25.0426	25.0426	inside	18.91059		656.13	1.6175	0.52949	0.0335	0.0335	inside	18.94858	
150	660.08	2.1	566.86	-55.4074	55.4074	inside			659.63	2.0775	0.52067	0.0247	0.0247	inside		
151	663.48	1.88	621.7	-0.5674	0.5674	inside			662.98	1.9175	0.5595	0.0635	0.0635	inside		
152	668.24	1.66	514.61	-107.6574	107.6574	inside			668.01	1.54	0.49319	-0.0028	0.0028	inside		
153	671.22	2.52	550.36	-71.9074	71.9074	inside			670.74	2.475	0.5574	0.0614	0.0614	inside		
154	675.02	1.84	423.46	-198.8074	198.8074	inside			674.48	1.885	0.44852	-0.0475	0.0475	inside		
155	678.24	1.82	470.56	-151.7074	151.7074	inside			678	1.58	0.45338	-0.0426	0.0426	inside		
156	681.38	2.46	353.23	-269.0374	269.0374	0			680.93	2.385	0.37789	-0.1181	0.1181	inside		
157	684.88	1.72	424.81	-197.4574	197.4574	inside			684.66	1.4075	0.39454	-0.1014	0.1014	ins		
158	688.44	2	664.43	42.1626	42.1626	inside			688.01	1.96	0.67133	0.1754	0.1754	ins		
159	691.74	2.16	472.38	-149.8874	149.8874	inside			691.23	2.1825	0.49346	-0.0025	0.0025	ins		
160	695.06	1.88	430.79	-191.4774	191.4774	inside			694.84	1.66	0.3873	-0.1087	0.1087	ins		
161	698.44	2.08	579.78	-42.4874	42.4874	inside			697.92	1.96	0.54794	0.0520	0.0520	ins		
162	701.8	2.08	326.14	-296.1274	296.1274	0			701.28	2.055	0.33181	-0.1642	0.1642	ins		
163	705.32	2.22	601.37	-20.8974	20.8974	inside			704.78	1.76	0.52682	0.0308	0.0308	ins		
164	708.82	1.88	569.52	-52.7474	52.7474	inside			708.33	1.7325	0.49525	-0.0007	0.0007	ins		
165	712.1	2.34	554.48	-67.7874	67.7874	inside			711.58	2.345	0.4467	-0.0493	0.0493	inside		
166	715.92	2.2	540.48	-81.7874	81.7874	inside			715.48	2.16	0.45261	-0.0434	0.0434	inside		
167	719.52	4.28	531.19	-91.0774	91.0774	inside			719.05	2.5025	0.40802	-0.0880	0.0880	inside		
168	725.24	2.24	1250.7	628.4326	628.4326	0			724.82	2.495	0.95577	0.4598	0.4598	0		

Figure 20: Diagram for steps 1 to 5 of the process of identifying stable breathing periods. It also shows how the CV of stable breathing period was obtained.



						std dev	mean							std dev	mean	
2						118.8199	543.3608							0.099085	0.467564	
3		LS		Distance from mean	Absolute value of distace					PT		Distance from mean	Absolute value of distace			
4	0.3	2.72	1757.8	1214.4392	1214.439	0			0	3.1425	1.6173	1.1497	1.1497	0		
5	9.28	1.7	473.09	-70.2708	70.27084	inside			9.08	2.025	0.4115	-0.0561	0.0561	inside		
6	12.56	1.7	403.79	-139.5708	139.5708	0			12.35	1.715	0.36037	-0.1072	0.1072	0		
7	15.54	1.64	476.06	-67.3008	67.30084	inside	stdv	mean	15.355	1.5775	0.41046	-0.0571	0.0571	inside	stdv	mean
8	18.3	1.4	485.3	-58.0608	58.06084	inside	86.40437	579.3318	18.118	1.3625	0.41046	-0.0571	0.0571	inside	0.049837	0.471314
9	21.04	1.88	451.55	-91.8108	91.81084	inside	14.91449		20.81	1.875	0.39106	-0.0765	0.0765	inside	10.5741	
10	24.3	1.3	512.2	-31.1608	31.16084	inside			24.067	1.29	0.46456	-0.0030	0.0030	inside		
11	26.94	1.24	528.45	-14.9108	14.91084	inside			26.727	1.21	0.48186	0.0143	0.0143	inside		
12	29.46	1.34	493.75	-49.6108	49.61084	inside			29.198	1.37	0.43814	-0.0294	0.0294	inside		
13	32.34	1.3	642.92	99.5592	99.55916	inside			32.108	1.325	0.52821	0.0606	0.0606	inside		
14	34.88	1.52	556.54	13.1792	13.17916	inside			34.77	1.41	0.44244	-0.0251	0.0251	inside		
15	42.66	1.38	607.51	64.1492	64.14916	inside			42.508	1.345	0.47594	0.0084	0.0084	inside		
16	45.56	1.46	541.22	-2.1408	2.140844	inside			45.555	1.2375	0.42323	-0.0443	0.0443	inside		
17	52.26	1.34	561.28	17.9192	17.91916	inside			52.142	1.2175	0.44219	-0.0254	0.0254	inside		
18	54.9	1.26	593.51	50.1492	50.14916	inside			54.71	1.1975	0.47465	0.0071	0.0071	inside		
19	63.58	1.98	723.4	180.0392	180.0392	0			63.943	1.35	0.49834	0.0308	0.0308	inside		
20	66.8	1.32	592.47	49.1092	49.10916	inside			66.595	1.2525	0.49933	0.0318	0.0318	inside		
21	69.54	1.4	674.98	131.6192	131.6192	0			69.34	1.34	0.54391	0.0763	0.0763	inside		
22	72.34	1.58	724.04	180.6792	180.6792	0			72.155	1.44	0.55025	0.0827	0.0827	inside		
23	75.48	1.42	683.46	140.0992	140.0992	0			75.282	1.4425	0.53731	0.0697	0.0697	inside		
24	78.4	1.56	767.55	224.1892	224.1892	0			78.15	1.56	0.62702	0.1595	0.1595	0		
25	81.68	1.36	676.88	133.5192	133.5192	0	stdv	mean	81.618	1.235	0.53801	0.0704	0.0704	inside	stdv	mean
26	84.52	1.34	607.11	63.7492	63.74916	inside	61.34696	574.2813	84.315	1.3775	0.53701	0.0694	0.0694	inside	0.054779	0.482083
27	87.26	1.24	608.02	64.6592	64.65916	inside	10.68239		86.965	1.3025	0.5251	0.0575	0.0575	inside	11.36302	
28	89.74	1.28	589.34	45.9792	45.97916	inside			89.558	1.275	0.50082	0.0333	0.0333	inside		
29	92.32	1.32	530.57	-12.7908	12.79084	inside			92.243	1.16	0.41786	-0.0497	0.0497	inside		
30	95.02	1.54	628.82	85.4592	85.45916	inside			94.79	1.5075	0.51314	0.0456	0.0456	inside		
31	98.24	1.54	620.38	77.0192	77.01916	inside			98.138	1.465	0.5134	0.0458	0.0458	inside		
32	101.54	1.22	476.88	-66.4808	66.48084	inside			101.32	1.1675	0.40799	-0.0596	0.0596	inside		
33	104.48	1.56	549.72	6.3592	6.359156	inside			104.37	1.14	0.41819	-0.0494	0.0494	inside		
34	107.56	1.34	575.02	31.6592	31.65916	inside			107.3	1.4075	0.49931	0.0317	0.0317	inside		
35	110.38	1.3	454.4	-88.9608	88.96084	inside			110.27	1.1675	0.38252	-0.0850	0.0850	inside		
36	112.96	1.48	570.62	27.2592	27.25916	inside			112.71	1.48	0.49772	0.0302	0.0302	inside		
37	115.84	1.68	620.73	77.3692	77.36916	inside			115.64	1.655	0.54812	0.0806	0.0806	inside		
38	118.72	1.18	502.08	-41.2808	41.28084	inside			118.54	1.115	0.42934	-0.0382	0.0382	inside		
39	121.24	1.42	603.65	60.2892	60.28916	inside			120.98	1.375	0.50271	0.0351	0.0351	inside		
40	123.84	2.36	537	-6.3608	6.360844	inside			125.31	0.625	0.33577	-0.1318	0.1318	0		
41	127.6	1.62	778.66	235.2992	235.2992	0			127.37	1.615	0.63664	0.1691	0.1691	0		
42	130.94	1.22	628.5	85.1392	85.13916	inside	stdv	mean	130.71	1.275	0.51087	0.0433	0.0433	inside	stdv	mean
43	133.5	1.38	624.09	80.7292	80.72916	inside	36.57696	602.9727	133.28	1.375	0.53334	0.0658	0.0658	inside	0.038274	0.510368
44	136.2	1.44	607.49	64.1292	64.12916	inside	6.066106		135.97	1.4525	0.51404	0.0465	0.0465	inside	7.499231	
45	139.06	1.86	627.63	84.2692	84.26916	inside			138.85	1.4275	0.50711	0.0395	0.0395	inside		
46	142.34	1.62	604.51	61.1492	61.14916	inside			142.09	1.6825	0.54402	0.0765	0.0765	inside		
47	145.3	1.46	619.04	75.6792	75.67916	inside			145.08	1.4525	0.54438	0.0768	0.0768	inside		
48	148.08	1.44	557.35	13.9892	13.98916	inside			147.87	1.4175	0.48225	0.0147	0.0147	inside		
49	150.92	1.54	577.13	33.7692	33.76916	inside			150.68	1.52	0.51891	0.0513	0.0513	inside		
50	154	1.34	606.68	63.3192	63.31916	inside			153.74	1.3125	0.50914	0.0416	0.0416	inside		
51	156.8	1.48	654.56	111.1992	111.1992	inside			156.58	1.4425	0.53994	0.0724	0.0724	inside		
52	160.74	1.3	525.72	-17.6408	17.64084	inside			160.87	0.9075	0.41003	-0.0575	0.0575	inside		
53	163.52	1.34	659.23	115.8692	115.8692	inside			163.24	1.4225	0.57224	0.1047	0.1047	0		

Step 6: By repeating step 1 to 5 to the rest of the data, a series of CVs was obtained for each person

Figure 21: A diagram to show step 5, describing how a series of CV values was obtained. This diagram shows three periods of stability in tidal volume.
Mean standard deviation and CV were calculated for each period.

The results for the breath by breath variability of stable periods of breathing are presented below. The descriptive statistics are presented first, followed by paired t-tests and correlation coefficients for the mean tidal volume CV.

		LS		PT	
Participant	**Number of stable breathing periods**	**Mean Vt CV (%)**	**Std. Deviation**	**Mean Vt CV (%)**	**Std. Deviation**
1	10	22	7.5	18	3.67
2	6	22	3.51	21	4.16
3	10	25	5.83	20	4.99
4	6	23	4.02	20	3.86
5	7	48	18.73	17	3.38
6	4	18	7.83	18	4.78
7	12	26	3.34	29	8.80
8	6	27	6.86	20	10.54
9	4	11	10.16	8	1.7
10	12	10	2.89	14	3.93
11	5	11	3.09	10	1.23
Group Mean (stdv)	*8 (3)*	*22 (10.62)*		*18 (5.67)*	

Table 10: Descriptive statistics for the CV of stable breathing periods of tidal volume for each participant during quiet breathing.

Table 10 shows that the number of stable breathing periods available for analysis from each person during quiet breathing ranged from four to twelve. The difference in the group mean of the individual mean CV of tidal volume between the LifeShirt® and the pneumotachograph is 4 %.

A Kolmogorov-Smirnov test showed that the mean CV of the tidal volume measurements during the stable breathing period from each participant measured by both devices are likely to be normally distributed, and a Pearson's correlation coefficient was therefore used to test the correlation of the mean CV of tidal volumes during stable breathing periods between the LifeShirt® and the pneumotachograph for all participants. The Pearson's correlation coefficient of the mean tidal volume CV during stable breathing periods was 0.5 (not significant at the 0.01 level), so there was no significant difference between the LifeShirt® and the pneumotachograph. A paired t-test showed that the difference of mean tidal volume CV for all participants during stable breathing

measured by the LifeShirt® and the pneumotachograph was not significant ($p = 0.15$).

The results of the two breath by breath variability analyses (overall variability and variability during stable breathing periods) show that over a longer period of time (the full duration of the recording period), the LifeShirt® recorded a similar of level of variability in relation to the pneumotachograph. However, the amount of variability in the LifeShirt® data does not always correspond to the amount of variability measured by the pneumotachograph during stable periods.

5.3 Exercise breathing

This section presents the results of analysis of the respiratory data collected during exercise. The data related to tidal volume will be presented first, then expiration duration, followed by breath by breath variability of tidal volume.

The following figures give examples of the time series for tidal volume recorded from the LifeShirt® and the pneumotachograph recorded during exercise.

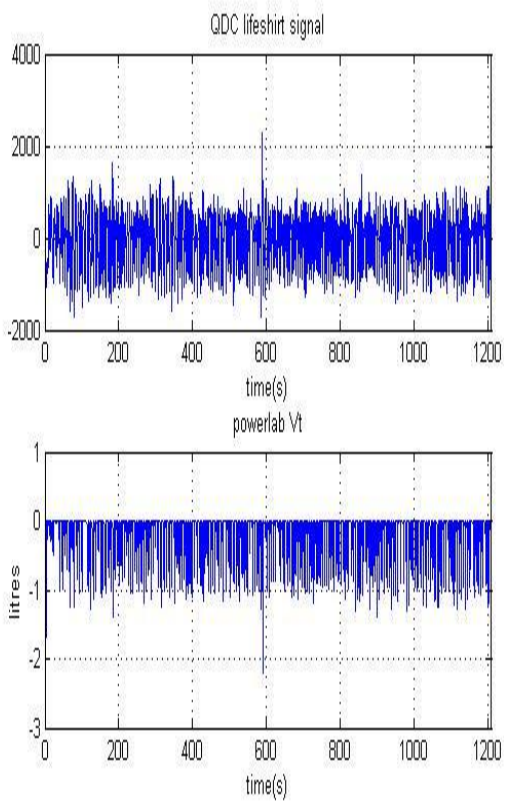


Figure 22: Time series for tidal volume recorded from the LifeShirt ® and PT for participant 2 during exercise.

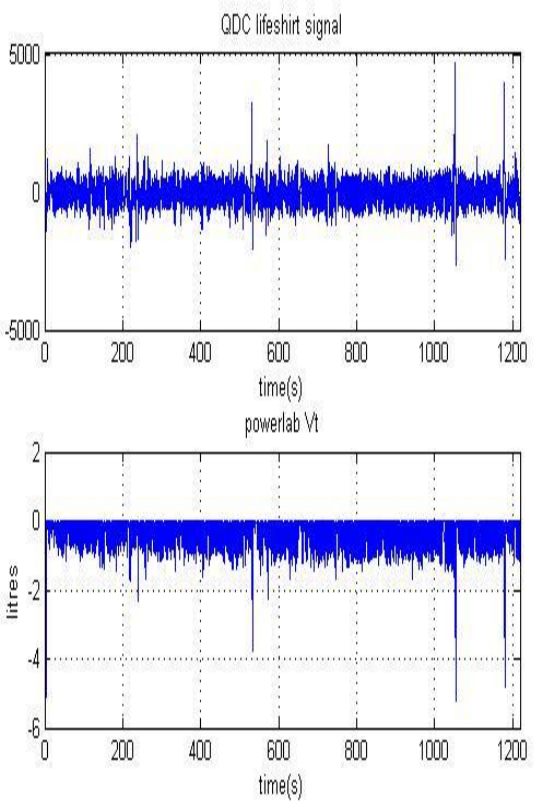


Figure 23: Time series for tidal volume recorded from the LifeShirt® and PT for participant 4 during exercise.

5.3.1 Tidal volume during exercise breathing

The following table provides the descriptive analysis of tidal volume recorded by the LifeShirt® and the pneumotachograph during exercise.

Participant	N	PT Mean Vt (l)	PT Vt stdv	LS Mean Vt (al)	LS Vt stdv	PT CV (%)	LS CV (%)
1	259	0.68	0.31	1.64	0.7	46	43
2	194	0.91	0.26	1.54	0.43	29	29
3	389	0.53	0.16	1.42	0.4	30	29
4	150	0.98	0.48	1.25	0.5	49	42
5	217	1.37	0.35	1.62	0.49	27	30
6	220	0.45	0.25	1.24	0.43	53	35
7	164	2.51	0.96	2.34	0.81	38	35
8	187	2.05	0.66	1.47	0.53	32	36
9	454	0.48	0.1	0.95	0.23	19	24
10	523	0.47	0.11	0.67	0.21	23	31
11	274	2.75	0.7	5.07	1.23	25	25
Mean (stdv)	281 (124)	1.2 (0.86)		1.75 (1.18)		35	32

Table 11: Descriptive statistics for tidal volume during exercise.
Key: N – number of breathes, al – assumed litre, l – litre, PT- pneumotachograph, LS – LifeShirt®, CV – coefficient of variability, stdv – standard deviation

Table 11 shows the number of breaths available for analysis per participant (ranging from 150 to 523, total n = 3031), and the mean and standard deviations of the tidal volumes measured by each device. The tidal volume measurements from the two devices are again not directly comparable because the LifeShirt® cannot be calibrated to give the absolute value. The coefficient of variability (CV) was therefore calculated from the mean and standard deviation to assess the spread of the data. The CV values show that there is variability in tidal volume during mild to moderate exercise both within and between individuals (ranging from 19% to 46% among the participants). Both the LifeShirt® and the pneumotachograph recorded similar variability in tidal volume during exercise (32% and 35% respectively).

The following box plot (Figure 24) gives a graphical representation of the distribution of the exercise tidal volume from both devices

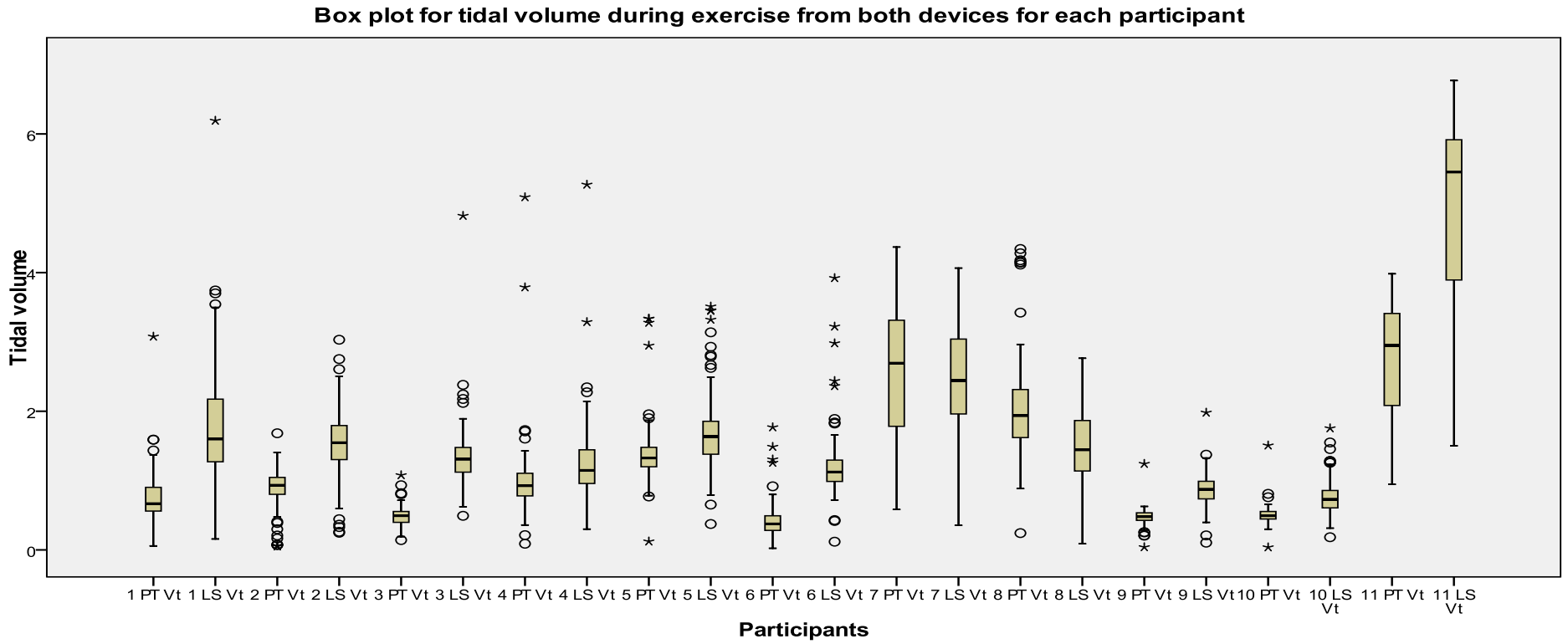


Figure 24: Box plots of tidal volume for all eleven participants from both devices.
Key: PT – pneumotachograph, LS – LifeShirt®, Vt – tidal volume

This box and whisker plot gives a graphical representation of the spread of tidal volume recorded by both devices. Outliers were observed in all participants, showing the variability in tidal volume within individuals.

5.3.2 Correlation between devices in tidal volume during exercise

Intra-participant correlation of breath by breath tidal volume during exercise

The breath by breath tidal volume data from all participants during exercise were checked for normality using histograms and Kolmogorov-Smirnov test. The results showed that the data set was unlikely to be normally distributed ($p<0.05$). Therefore the data were analysed using non-parametric tests.

Participant	Vt correlation
1	0.8 *
2	0.7 *
3	0.6 *
4	0.6 *
5	0.5 *
6	0.6 *
7	0.8 *
8	0.5 *
9	0.5 *
10	0.6 *
11	0.9 *
Mean (stdv)	0.7 (0.14)

* Correlation is significant at the 0.01 level

Table 12: Spearman's rank correlation between the LifeShirt® and pneumotachograph data for exercise breathing tidal volume of each participant on a breath by breath basis.
Key: Vt – tidal volume, stdv – standard deviation

Table 12 shows the results of the Spearman's rank correlation test between the exercise tidal volume recorded by the LifeShirt® and the pneumotachograph. On average, the exercise tidal volume correlation coefficient between the LifeShirt® and the pneumotachograph on a breath by breath basis was 0.7. The correlation coefficients were statistically significant for all participants (at the 0.01 level). However, as with quiet breathing, not all participants showed a 'high' correlation; the values ranged from 0.5 to 0.9, which was the same range as during quiet breathing.

Inter-participant correlation of mean tidal volume during exercise

The inter-participant correlation of the mean tidal volume between the LifeShirt® and the pneumotachograph was tested across all subjects. The mean tidal volumes from all participants were checked for normality using histogram and Kolmogorov-Smirnov test. The results showed that the data set is likely to be normally distributed. Therefore the Pearson's correlation coefficient test was used. The mean tidal volume measured by the LifeShirt® and the pneumotachograph during exercise showed a significant correlation of 0.8 (at the 0.01 level).

These results demonstrate that the tidal volume changes detected by both the pneumotachograph and the LifeShirt® during exercise are comparable on both a breath by breath basis and on the interval averaged basis.

5.3.3 Expiration duration during exercise

This section presents the results of the analysis of expiration duration during exercise. A table of descriptive statistics is presented first, followed by analyses of correlation coefficient, a paired t-test, Bland and Altman analysis and standard error of measurement of expiration duration during exercise.

Table 13 shows the results of the descriptive analysis of the exercise expiration duration data recorded simultaneously by the LifeShirt® and the pneumotachograph.

Participant	Device	N	Min (s)	Max (s)	Mean (s)	Std. Deviation
1	PT	259	0.47	7.71	1.72	0.61
	LS	259	0.50	6.38	2.06	0.65
2	PT	194	0.41	5.17	2.19	0.73
	LS	194	0.54	5.26	2.31	0.76
3	PT	389	0.32	4.04	1.53	0.23
	LS	389	0.52	4.20	1.55	0.36
4	PT	150	0.35	4.18	1.60	0.43
	LS	150	0.42	3.78	1.50	0.43
5	PT	217	0.55	4.46	2.55	0.54
	LS	217	1.00	4.48	2.44	0.62
6	PT	220	0.16	4.3	1.41	0.61
	LS	220	0.42	5.9	1.72	0.70
7	PT	617	0.76	5.64	3.18	0.91
	LS	617	0.46	6.20	3.10	1.09
8	PT	187	0.77	4.08	2.32	0.57
	LS	187	0.38	4.36	2.22	0.72
9	PT	454	0.26	2.94	1.17	0.21
	LS	454	0.56	3.74	1.26	0.25
10	PT	523	0.14	1.99	1.12	0.25
	LS	523	0.38	2.02	1.11	0.26
11	PT	274	0.74	4.09	1.97	0.55
	LS	274	0.76	3.56	1.96	0.54
Group Mean(stdv)	PT	281 (130)	0.45 (0.23)	4.42 (1.46)	1.89 (0.63)	
	LS	281 (130)	0.54 (0.19)	4.53 (1.31)	1.93 (0.58)	

Table 13: Descriptive statistics for expiration duration during exercise on a breath by breath basis.
Key: N – number of breaths, min – minimum expiration duration, max – maximum expiration duration, stdv – standard deviation

Table 13 shows the number of breaths available for analysis, the minimum and maximum expiration durations, the group and individual means, and the standard deviation of the expiration duration data recorded simultaneously by the LifeShirt® and the pneumotachograph during exercise. This analysis was performed on a breath by breath basis, i.e. on the corresponding breaths recorded by both devices. The means and standard deviations of expiration

duration during exercise are directly comparable between the devices since expiration duration was measured in seconds. The results show that on average the LifeShirt® overestimated the exercise expiration duration by 0.04 seconds during exercise. The standard deviations from each device are within 0.05 of each other.

A box plot is shown below in Figure 25 to give a graphical representation of the distribution of the expiration duration data collected by the two devices during exercise.

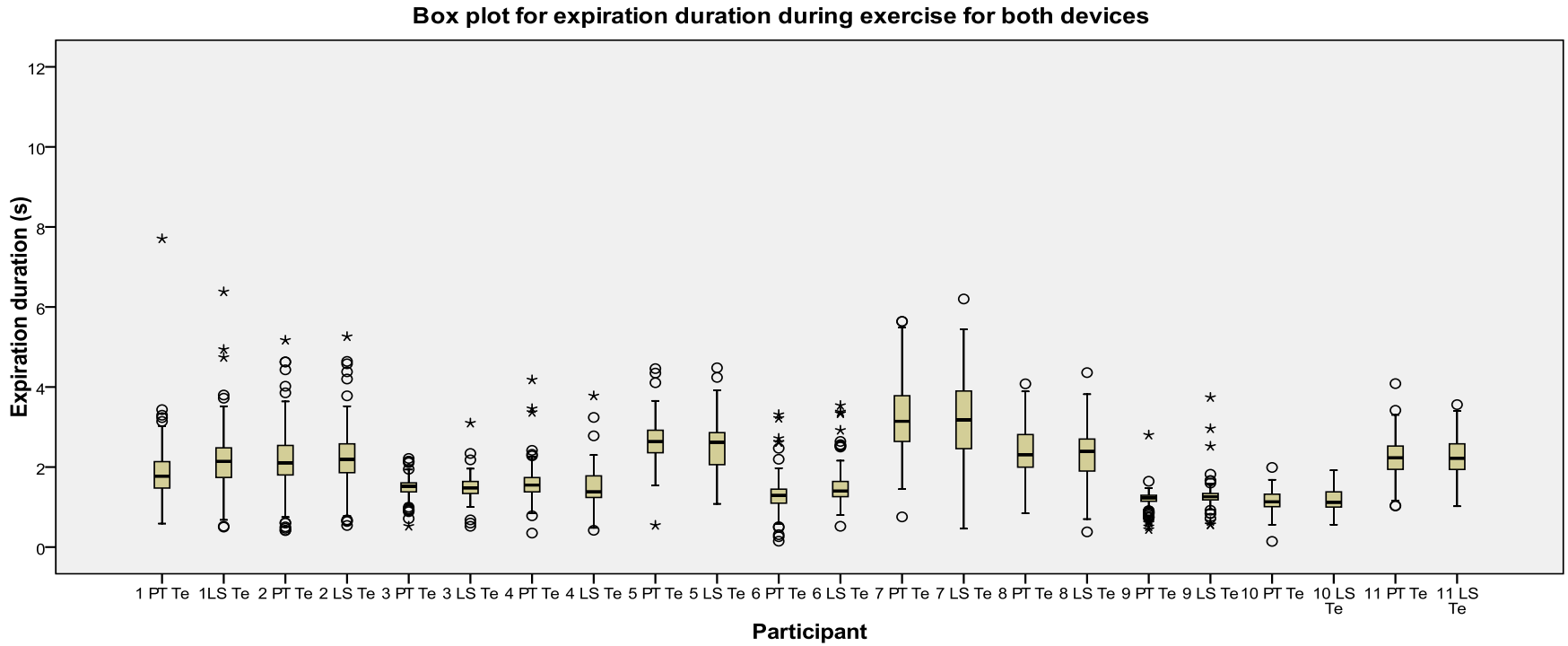


Figure 25: Box plot for expiration duration during exercise for both devices from each participant.
Key: Te – expiration duration (s), LS – LifeShirt®, PT – pneumotachograph

This box plot gives a graphical representation of the spread of the expiration duration data recorded during exercise by both devices. Outliers were observed in all participants, reflecting the variability of expiration duration during exercise.

5.3.4 Correlation of expiration duration during exercise

The breath by breath expiration durations measured during exercise from all participants were checked for normality using histograms and Kolmogorov-Smirnov test. Results showed that the data sets for breath by breath expiration duration from all participants during exercise were unlikely to be normally distributed ($p < 0.05$). Histogram demonstrated skewed distribution. The data were therefore analysed using non-parametric tests.

Intra-participant correlation coefficient of breath by breath expiration duration during exercise

Participant	Te correlation
1	0.5 *
2	0.7 *
3	0.7 *
4	07 *
5	0.6 *
6	0.6 *
7	0.7 *
8	0.7 *
9	0.5 *
10	0.7 *
11	0.9 *
Mean (stdv)	0.7 (0.13)

Table 14: Spearman's rank correlation coefficient between the LifeShirt® and pneumotachograph data of expiration duration between PT and LS during exercise on a breath by breath basis.* Indicates statistically significant ($p<0.01$)

Table 14 shows the results of a Spearman's rank correlation test between the expiration duration data recorded on a breath by breath basis by the LifeShirt® and the pneumotachograph during exercise. All the corresponding breaths from the LifeShirt® and the pneumotachograph were included in the analysis. On average, the correlation coefficient for exercise expiration duration between the LifeShirt® and the pneumotachograph was 0.7. All participants had a statistically significant

correlation at the 0.01 level. However, as with tidal volume, the correlation coefficients ranged from 0.5 to 0.9.

Inter-participant correlation of mean expiration duration during exercise

The means of the expiration duration data measured during exercise from all participants were normally distributed (tested by a Kolmogorov-Smirnov test). The correlation of the mean expiration duration between the LifeShirt® and the pneumotachograph during exercise was tested across all participants using Pearson's correlation coefficient. The results showed that the correlation of mean expiration duration during exercise between the LifeShirt® and the pneumotachograph was 0.9, which was statistically significantly correlated at 0.01 level.

5.3.5 Difference in breath by breath expiration duration between devices during exercise

This section shows the results of the analysis of the paired t-test on exercise expiration duration. This was carried out to test whether the difference in mean expiration duration measured by the two devices during exercise was statistically significant or not. The analysis of the mean difference within individuals is presented first, followed by the analysis of the mean difference between individuals.

Intra-participant breath by breath differences in expiration duration between the LifeShirt® and the pneumotachograph during exercise

A paired t-test was performed on the breath by breath expiration duration data from each participant recorded by the LifeShirt® and the pneumotachograph during exercise. Table 15 shows the results of this analysis.

Pair	Mean difference	Std. deviation	t	df	Sig. (2-tailed)
1 PT Te - 1LS Te	0.34	0.54	-10.081	258	0.00*
2 PT Te - 2 LS Te	0.12	0.53	-3.092	193	0.02*
3 PT Te - 3 LS Te	0.02	0.29	-1.360	388	0.18
4 PT Te - 4 LS Te	0.10	0.25	5.011	149	0.00*
5 PT Te - 5 LS Te	0.12	0.51	3.334	216	0.00*
6 PT Te - 6 LS Te	0.12	0.37	-9.156	219	0.00*
7 PT Te - 7 LS Te	0.09	0.77	1.527	163	0.13
8 PT Te - 8 LS Te	0.08	0.57	2.488	186	0.01*
9 PT Te - 9 LS Te	0.10	0.26	-7.096	453	0.00*
10 PT Te - 10 LS Te	0.00	0.24	0.739	522	0.46
11 PT Te - 11 LS Te	0.00	0.23	0.448	273	0.65

Table 15: Paired sample t-test of expiration duration from LifeShirt® and pneumotachograph during exercise on breath by breath basis.
*Key: PT – pneumotachograph, LS® – LifeShirt, Te – expiration duration (s), * significant at 0.05 level*

Table 15 shows the results of the paired t-test between the expiration duration data recorded by the LifeShirt® and the pneumotachograph on a breath by breath basis during exercise. The results show a statistically significant difference in expiration

duration between the LifeShirt® and the pneumotachograph in seven participants ($p<0.05$). No statistically significant difference in expiration duration between the LifeShirt and the pneumotachograph was observed in four participants ($p>0.05$).

	Mean difference	Std. Deviation	t	df	Sig. (2-tailed)
Mean PT Te - Mean LS Te	0.05	0.1900	-0.832	9	0.427

Table 16: Paired sample t-test of the mean expiration duration between LifeShirt® and pneumotachograph during exercise.
Key: PT – pneumotachograph, LS – LifeShirt®, Te – expiration duration (s)

The results of the paired t-test show that the group mean difference in expiration duration between the LifeShirt® and the pneumotachograph during exercise was not statistically significant at the 0.05 level.

5.3.6 Bland and Altman analysis of expiration duration during exercise

This section shows the results of the Bland and Altman analysis of the agreement in the expiration duration data measured by the LifeShirt® and the pneumotachograph during exercise.

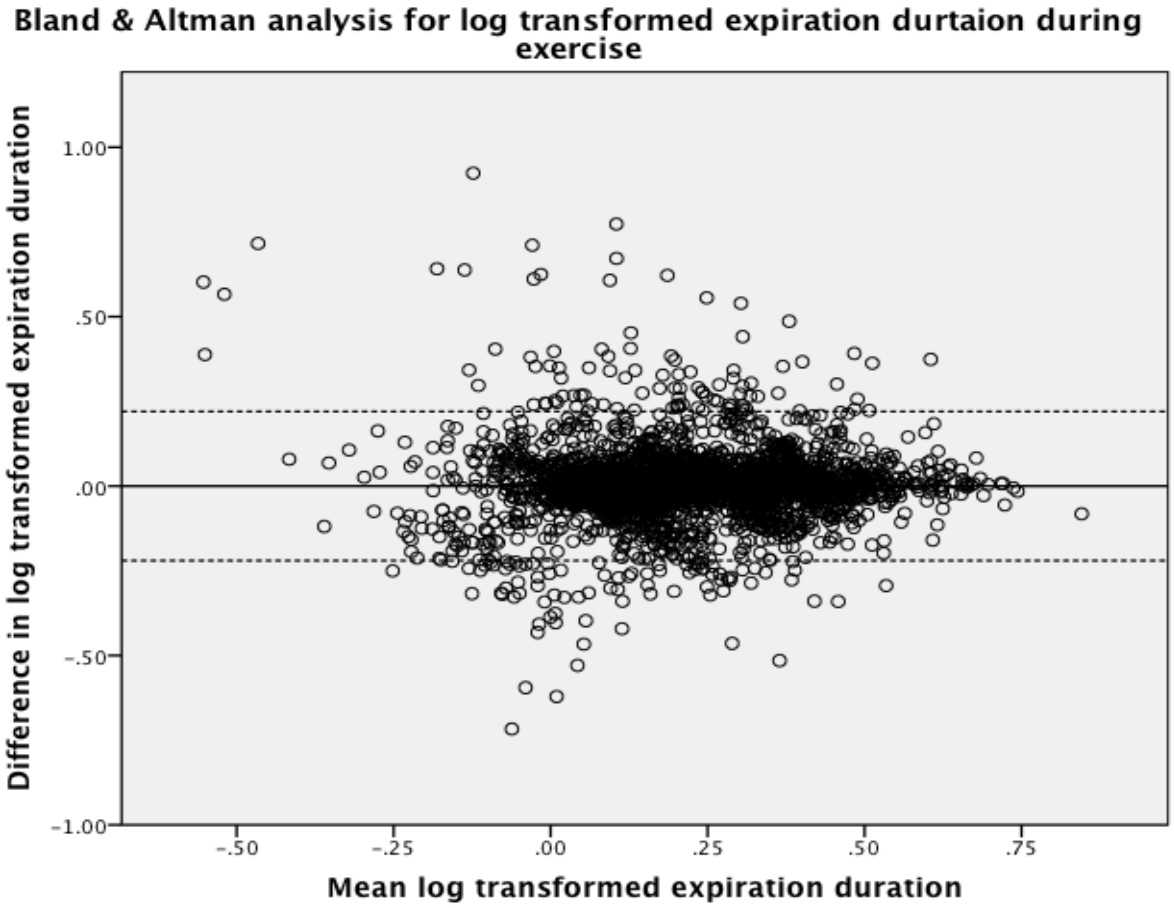


Figure 26: Bland and Altman plot for expiration duration during exercise

The Bland and Altman (Figure 26) shows that the LifeShirt® has a bias in over-estimating the expiration duration. The log transformed 95% limits of agreement are -0.22 to 0.22, showing that 95% of all the recorded breaths during exercise were within log 0.44 seconds. Taking the antilog of 0.44 shows that 95% of all the recorded breaths during exercise were within 0.82 seconds.

5.3.7 Standard error of measurement of expiration duration during exercise

The standard error of measurement was calculated with the formula described in Section 2.8.2.4. The intra-class correlation between the LifeShirt® and the pneumotachograph was calculated with the ICC (1, 1).

The ICC between the LifeShirt® and the pneumotachograph during exercise was 0.85. The standard deviation of the mean expiration duration of all breaths was 0.7s. The standard error of measurement during exercise was 0.3s.

5.3.8 Breath by breath variability of tidal volume during exercise

The following section shows the results of the calculation of the coefficients of variation (CV) in tidal volume from each device during exercise.

Participant	PT CV(%)	LS CV (%)
1	46	43
2	29	29
3	30	29
4	49	42
5	27	30
6	53	35
7	38	35
8	32	36
9	19	24
10	23	31
11	25	25
Mean (stdv)	35 (11)	32 (5)

Table 17: Breath by breath variability of PT and LS recorded tidal volume during exercise expressed as a coefficient of variation (CV)

Paired t-tests were performed on the CV of tidal volume during exercise recorded by the LifeShirt® and the pneumotachograph. Table 17 shows that there is no statistical significant difference between the tidal volume of CV measured by the pneumotachograph and that measured by the LifeShirt® ($p=0.675$). A Kolmogorov-Smirnov test showed that the coefficients of variation for both the LifeShirt® and the pneumotachograph were likely to be normally distributed, and therefore the Pearson's correlation coefficient was used to test for the correlation between devices. The Pearson's correlation coefficient was 0.8, and the correlation is significant at the 0.01 level.

5.3.9 Breath by breath variability of stable tidal volume period during exercise

As described in Section 3.2.3.4, breath by breath variability was analysed in a different way to find out whether the LifeShirt® was able to record the same amount of variability as the pneumotachograph during the periods of stable breathing that were defined by the pneumotachograph. The steps to obtain the stable breathing period were the same as previously described (Section 3.2.3.4).

The results for breath by breath variability of stable breathing are presented below. The descriptive statistics are presented first, followed by the results of paired t-test and correlation coefficient analyses of the mean tidal volume CV.

Participant	Numb of blocks	LS Mean CV (%)	LS Std. Deviation	PT Mean CV (%)	PT Std. Deviation
1	10	25	6.19	20	3.73
2	6	20	9.08	14	1.70
3	10	18	4.38	15	2.28
4	6	24	1.77	20	2.46
5	7	26	6.47	15	9.34
6	4	21	8.85	24	5.04
7	3	22	3.94	19	3.34
8	6	34	11.71	14	2.51
9	16	15	4.45	9	2.03
10	12	27	7.26	9	2.56
11	5	12	2.16	11	2.54
Group Mean (stdv)	*8 (4)*	*21*	*(6.66)*	*15*	*(4.74)*

Table 18: Descriptive statistics of CV tidal volume for stable breathing periods for each participant during exercise.

Table 18 shows that the number of stable breathing periods available from each participant during exercise for analysis ranged from 3 to 16. The mean CVs for tidal volume of all participants from both devices were within 6% of each other.

A Kolmogorov-Smirnov test shows that the mean CV of tidal volume is normally distributed, and therefore the Pearson's correlation coefficient was used. The Pearson's correlation coefficient of the mean CV of the stable periods was 0.3 and the correlation was not significant at the 0.01 level. A paired t-test shows that the difference in mean tidal volume CVs between the LifeShirt® and the pneumotachograph for all participants during stable breathing was significant ($p<0.05$).

The results of the two breath by breath variability analyses (overall variability and variability during stable breathing periods) are similar to the results obtained during quiet breathing. This shows that over a longer period of time (the full duration of the recording period), the LifeShirt® recorded similar levels of variability in relation to the pneumotachograph. However, the amount of variability in the LifeShirt® measurements does not always correspond to the amount of variability measured by the pneumotachograph during the stable periods.

5.4 Difference in tidal volume and expiration duration between quiet breathing and exercise

This section shows the results of the analysis of the difference in tidal volume and expiration duration between quiet breathing and exercise. This analysis was performed to test: 1) if there was any difference in the two parameters between quiet breathing and exercise, using the pneumotachograph as the reference; and 2) if there was a difference shown by the pneumotachograph data, did the LifeShirt® also record a difference. The difference in tidal volume and expiration duration between quiet breathing and exercise was assessed using the mean of each parameter. This is because the analysis of the quiet breathing and exercise data has revealed a large amount of variability in both tidal volume and expiration duration. It was impossible to do breath by breath comparisons as breaths did not correspond in a pair-wise manner. The results for tidal volume are presented first, followed by those for expiration duration.

5.4.1 Difference in tidal volume between quiet breathing and exercise breathing

The following table presents the mean tidal volume data collected by the pneumotachograph and the LifeShirt® during quiet breathing and exercise for each participant.

	Pneumotachograph		LifeShirt®	
Participant	Mean Vt during quiet breathing (l)	Mean Vt during exercise (l)	Mean Vt during quiet breathing (al)	Mean Vt during exercise (al)
1	0.46	0.68	0.68	1.64
2	0.63	0.91	0.77	1.54
3	0.50	0.53	0.62	1.42
4	0.52	0.98	0.70	1.25
5	0.67	1.37	0.84	1.62
6	0.20	0.45	0.32	1.24
7	1.16	2.51	0.85	2.34
8	1.15	2.05	0.76	1.47
9	0.47	0.48	0.54	0.95
10	0.18	0.47	0.40	0.67
11	0.72	2.75	0.89	5.07
Group Mean (stdv)	0.31 (0.32)	1.04 (0.74	0.67 (0.19)	1.67 (1.17)

Table 19: Mean tidal volume recorded by pneumotachograph and LifeShirt® during quiet breathing and exercise.
Key: Vt – tidal volume, al – assumed litres

Table 19 shows the mean values of tidal volume during quiet breathing and exercise breathing for each participant, recorded by the LifeShirt® and the pneumotachograph. The pneumotachograph recorded an increase in tidal volume from quiet breathing to exercise in all participants. The LifeShirt® also recorded an increase in tidal volume from quiet breathing to exercise in all participants. The pneumotachograph recorded a mean tidal volume increase from 0.61 to 1.04, whereas the LifeShirt® recorded a mean tidal volume increase from 0.67 to 1.67. (Note that it is not possible to compare values between devices due to the LifeShirt® not being calibrated in absolute values).

A paired t-test was used to see if the difference in tidal volume between quiet breathing and exercise for each device was statistically significant. The results showed that the difference in tidal volume between quiet breathing and exercise recorded by the pneumotachograph was statistically significant (t (10) = 0.10, $p < 0.05$). The difference in tidal volume between quiet breathing and exercise recorded by the LifeShirt® was also statistically significant (t (9) = 0.00, $p < 0.05$).

5.4.2 Difference in expiration duration between quiet breathing and exercise

The following table presents the mean expiration duration recorded from each participant by the pneumotachograph and the LifeShirt® during quiet breathing and exercise.

	Pneumotachograph		**LifeShirt**	
Participant	Mean Te during quiet breathing (s)	Mean Te during exercise (s)	Mean Te during quiet breathing (s)	Mean Te during exercise (s)
1	1.6	1.7	2.5	2.1
2	3.4	2.2	4.1	2.3
3	2.1	1.5	2.5	1.6
4	2.0	1.6	2.1	1.5
5	3.9	2.6	4.5	2.4
6	1.3	1.4	1.7	1.7
7	4.8	3.2	5.5	3.1
8	3.9	2.3	4.8	2.2
9	1.4	1.2	1.5	1.3
10	1.2	1.1	1.7	1.1
11	3.0	2	3.3	2.0
Group Mean (stdv)	2.6 (1.25)	1.7 (0.55)	3.1 (1.41)	1.7 (0.50)

Table 20: Mean expiration duration recorded by pneumotachograph and LifeShirt® during quiet breathing and exercise.

Table 20 shows the mean expiration duration during quiet breathing and exercise recorded by the LifeShirt® and the pneumotachograph. A decrease in mean expiration duration during exercise was recorded for all participants by both the LifeShirt® and the pneumotachograph. The pneumotachograph recorded a group mean decrease in expiration duration from 2.6 seconds to 1.7 seconds, whereas the LifeShirt® recorded a larger group mean decrease of expiration duration from 3.1 seconds to 1.7 seconds. A paired t-test was used to see if the difference in expiration duration between quiet breathing and exercise was significant for each device. The mean differences in expiration duration recorded by both the pneumotachograph and the LifeShirt® between quiet breathing and exercise were significant ($p<0.05$).

5.5 Matched breaths vs. all breaths

5.5.1 Matched breaths vs. all breaths during quiet breathing

The above analysis was performed on the respiratory cycles that were recorded by both devices after the removal of respiratory cycles that were not ‘matched’ across both devices. This section presents the results of comparing the data from the matched breaths with all breaths that were recorded by both devices during quiet breathing and exercise. Descriptive statistics were used to describe the results of the matched breaths and all recorded breaths for each device. Pair-sampled t-tests were used to test if the between-device and within-device differences in matched and all recorded breaths were statistically significant.

Due to the LifeShirt® not being calibrated to the same measurement unit as the pneumotachograph, the difference in tidal volume between devices was not assessed.

Because direct breath by breath comparison within and between devices was not possible due to the presence of unmatched breaths, a Bland and Altman analysis and a standard error of measurement analysis were not performed.

5.5.1.2 Tidal volume

	Quiet breathing			
	LS		PT	
Participant	Matched (Vt)	All (Vt)	Matched (Vt)	All (Vt)
1	0.68 (0.40)	0.69 (0.43)	0.46 (0.22)	0.45 (0.24)
2	0.77 (0.37)	0.76 (0.37)	0.63 (0.27)	0.54 (0.29)
3	0.62 (0.25)	0.63 (0.27)	0.50 (0.22)	0.47 (0.22)
4	0.70 (0.26)	0.70 (0.25)	0.52 (0.24)	0.52 (0.21)
5	0.84 (0.68)	0.82(0.55)	0.67 (0.57)	0.65 (0.31)
6	0.32 (0.13)	0.31 (0.14)	0.20 (0.10)	0.19 (0.11)
7	0.85 (0.42)	0.82 (0.44)	1.16 (0.51)	1.01 (0.52)
8	0.76 (0.36)	0.77 (0.44)	1.15 (0.51)	1.00 (0.56)
9	0.54 (0.13)	0.54 (0.11)	0.47 (0.10)	0.44 (0.14)
10	0.40 (0.11)	0.40 (0.11)	0.18 (0.60)	0.18 (0.06)
11	0.89 (0.16)	0.89 (0.17)	0.72 (0.18)	0.71 (0.12)
Mean	0.69	0.66	0.61	0.56
stdv	(0.20)	(0.19)	(0.32)	(0.27)

Table 21: Comparison of tidal volume in matched respiratory cycles and all respiratory cycles recorded by both devices during quiet breathing.

The above table demonstrates that there is very little difference in the mean tidal volume values between matched and all recorded breaths, with a mean within-device difference of 0.03 in the LifeShirt® and 0.05 in the pneumotachograph.

Quiet breathing	Mean difference	Std. Deviation	t	df	Sig. (2-tailed)
Mean Matched LS Vt – Mean All LS Vt	0.01	0.19	0.34	10	0.86
Mean Matched PT Vt – Mean All PT Vt	0.05	.06	1.78	10	0.25

Table 22: Paired t-test of the mean tidal volume of the sample group between matched breaths and all recorded breaths by LS and PT during quiet breathing.
The difference is significant at p = 0.05 Key: PT – pneumotachograph, Vt – tidal volume, LS-LifeShirt® (s), significant at 0.05 level*

The paired sample t-test results show that the difference in mean tidal volume between matched and all recorded breaths is not statistically significant.

5.5.1.3 Expiration duration

Quiet breathing

	LS		PT	
Participant	Matched (Te)	All (Te)	Matched (Te)	All (Te)
1	2.47 (0.82)	2.49 (0.88)	1.61(0.35)	1.59 (0.36)
2	4.09 (1.46)	4.17 (1.52)	3.41(1.33)	3.09 (1.36)
3	2.48 (0.81)	2.50 (0.88)	2.12(0.68)	2.04 (0.62)
4	2.08 (0.52)	2.11 (0.60)	2.04(0.49)	2.03 (0.48)
5	4.48 (1.05)	4.27 (1.38)	3.85(0.83)	3.74 (1.04)
6	1.72 (0.25)	1.73 (0.44)	1.33(0.36)	1.38 (0.40)
7	5.51 (1.43)	5.61 (1.61)	4.80(1.21)	4.57 (1.4)
8	4.81 (1.57)	4.92 (1.73)	3.90(1.23)	3.55 (1.37)
9	1.47 (0.31)	1.54 (0.49)	1.38(0.26)	1.30 (0.36)
10	1.70 (0.31)	1.72 (0.49)	1.22(0.20)	1.24 (0.25)
11	3.28 (0.52)	3.31 (0.17)	2.99(0.48)	2.94 (0.12)
Mean	3.10	3.12	2.60	2.50
(Stdv)	(1.41)	(1.42)	(1.24)	(1.14)

Table 23: Comparison of expiration duration in matched respiratory cycles and all respiratory cycles recorded by both devices during quiet breathing.

The above table demonstrates that there is very little difference in the mean expiration duration between matched and all recorded breathes during quiet breathing. The mean within-device difference between matched breaths and all recorded breaths is 0.02 seconds in the LifeShirt® and 0.1 seconds in the pneumotachograph. The between-device differences are also very similar for both matched and all recorded breaths. The mean difference between LS and PT for matched breaths only is 0.5 seconds, whereas the mean difference between devices for all recorded breaths is 0.62 seconds.

Quiet breathing	Mean difference	Std. Deviation	t	df	Sig. (2-tailed)
Mean Matched LS Te – Mean All LS Te	-0.17	2.22	-0.26	10	0.80
Mean Matched PT Te – Mean All PT Te	0.13	0.14	2.09	10	0.19
Mean All LS Te – Mean All PT Te	0.73	0.42	5.82	10	0.00

Table 24: Paired t-test of the mean tidal volume of the sample group between matched breaths and all recorded breaths by LS and PT during quiet breathing.
The difference is significant at p = 0.05 Key: PT – pneumotachograph, Vt – tidal volume LS — LifeShirt® (s),* significant at 0.05 level

The paired sample t-tests show that the difference in expiration duration between matched and all recorded breaths within each device was not statistically significant. However, the difference between LS and PT for all recorded breaths is statistically significant; this is also the case for matched breaths.

5.5.1.4 Tidal volume variability

Quiet breathing

	LS		PT	
Participant	Matched Vt CV (%)	All Vt CV (%)	Matched Vt CV (%)	All Vt CV (%)
1	58	62	48	53
2	48	49	44	53
3	40	44	44	47
4	37	36	46	36
5	64	66	45	48
6	50	74	41	86
7	49	51	44	48
8	45	56	42	57
9	20	21	21	31
10	30	30	33	33
11	18	19	24	18
Mean	41	46	39	46
(Stdv)	(14.60)	(18.13)	(9.16)	(17.62)

Table 25: Comparison of tidal volume variability in matched respiratory cycles and all respiratory cycles recorded by both devices during quiet breathing.

The tidal volume variability is higher in all recorded breaths than in matched breaths for both devices. There is a 5% mean difference between matched and all recorded breaths for the LifeShirt®, and a 7% mean difference for the pneumotachograph.

Quiet breathing	Mean difference	Std. Deviation	t	df	Sig. (2-tailed)
Mean Matched LS Vt CV – Mean All LS Vt CV	-2.36	3.23	-0.24	10	0.04
Mean Matched PT Vt CV – Mean All PT Vt CV	-3.23	7.01	1.44	10	0.15
Mean All LS Vt CV – Mean All PT Vt CV	-0.18	8.2	5.41	10	0.95

Table 26: Paired t-test of the mean tidal volume variability of the sample group between matched breaths and all recorded breaths by LS and PT during quiet breathing.
The difference is significant at p = 0.05 Key: PT – pneumotachograph, Vt – tidal volume LS-LifeShirt (s), significant at 0.05 level*

The paired sample t-tests show that the difference in mean tidal volume variability between matched and all recorded breaths within each device was statistically significant for the LifeShirt® but not for the pneumotachograph. The difference in mean tidal volume variability between the LS and the PT with all recorded breaths is not statistically significant; this is also the case for matched breaths.

5.5.2 Matched breaths vs. all breaths during exercise

5.5.2.1 Tidal volume

This section presents the analysis to compare the data from matched breaths and all breaths that were recorded by both devices during exercise.

Exercise

	LS		PT	
Participant	Matched Vt (aml)	All Vt (aml)	Matched Vt (ml)	All Vt (ml)
1	1.64 (0.7)	1.53 (0.71)	0.68 (0.31)	0.60 (0.33)
2	1.54 (0.43)	1.45 (0.51)	0.91 (0.26)	0.87 (0.31)
3	1.42 (0.40)	1.39 (0.45)	0.53 (0.16)	0.51 (0.18)
4	1.25 (0.50)	0.76 (0.28)	0.98 (0.48)	1.00 (0.38)
5	1.62 (0.49)	1.47 (0.65)	1.37 (0.35)	1.31 (0.48)
6	1.24 (0.43)	1.80 (0.99)	0.45 (0.25)	0.35 (0.25)
7	2.34 (0.81)	2.03 (0.97)	2.51 (0.96)	2.38 (1.08)
8	1.47 (0.53)	1.22 (0.66)	2.05 (0.66)	1.85 (0.83)
9	0.95 (0.23)	0.88 (0.30)	0.48 (0.10)	0.45 (0.13)
10	0.67 (0.21)	0.63 (0.25)	0.47 (0.11)	0.46 (0.11)
11	5.07 (1.23)	4.89 (1.50)	2.75 (0.70)	2.73 (0.71)
Mean	1.79	1.64	1.30	1.13
(Stdv)	(1.17)	(1.16)	(0.82)	(0.79)

Table 27: Comparison of tidal volume in matched respiratory cycles and all respiratory cycles recorded by both devices during quiet breathing.

The above table shows that on average, all recorded breaths had lower values than the matched breaths in both devices. The within-device differences between matched and all recorded breaths were small. The LS had a difference of 0.15ml between matched and all recorded breaths, whereas the PT has a difference of 0.17ml.

Exercises	Mean difference	Std. Deviation	t	df	Sig. (2-tailed)
Mean Matched LS Vt – Mean All LS Vt	0.18	0.13	4.53	10	0.00
Mean Matched PT Vt – Mean All PT Vt	0.06	0.07	3.11	10	000

Table 28: Paired t-test of the mean tidal volume of the sample group between matched breaths and all recorded breaths by LS and PT during quiet breathing.
The difference is significant at p = 0.05 Key: PT – pneumotachograph, Vt – tidal volume LS-LifeShirt (s),* significant at 0.05 level

The paired sample t-tests show that the difference in tidal volume during exercise between matched and all recorded breaths within each device was statistically significant for both the LifeShirt® and the pneumotachograph.

5.5.2.2 Expiration duration

	Exercise			
	LS		PT	
Participant	Matched Te	All Te	Matched Te	All Te
1	2.06 (0.65)	1.01 (0.78)	1.72 (0.61)	1.52 (0.64)
2	2.31 (0.76)	2.37 (0.95)	2.19 (0.73)	2.27 (0.82)
3	1.55 (0.36)	1.54 (0.43)	1.53 (0.23)	1.48 (0.40)
4	1.50 (0.43)	0.85 (0.56)	1.60 (0.43)	1.67 (0.45)
5	2.44 (0.62)	2.25 (0.88)	2.55 (0.54)	2.46 (0.77)
6	1.72 (0.70)	1.83 (0.99)	1.41 (0.61)	1.37 (0.63)
7	3.10 (1.09)	2.78 (1.32)	3.18 (0.91)	3.07 (1.10)
8	2.22 (0.72)	1.80 (1.12)	2.32 (0.57)	2.11 (0.73)
9	1.26 (0.25)	1.26 (0.45)	1.17 (0.21)	1.01 (0.30)
10	1.11 (0.26)	1.08 (0.39)	1.12 (0.25)	1.11 (0.28)
11	1.96 (0.54)	1.92 (0.62)	1.97 (0.55)	1.97 (0.55)
Mean	1.93	1.69	1.89	1.82
Stdv	(0.58)	(0.62)	(0.63)	(0.60)

Table 29: Comparison of expiration duration in matched respiratory cycles and all respiratory cycles recorded by both devices during exercise.

The above table demonstrates that there is very little difference in the mean expiration durations between the matched and all recorded breaths during

exercise. The mean within-device difference between the matched and all recorded breaths during exercise is 0.24 seconds for the LifeShirt® and 0.07 seconds for the pneumotachograph. The mean difference between the LS and the PT for matched breaths is 0.04 seconds, whereas the mean difference between devices for all recorded breaths is 0.13 seconds.

Exercise	Mean difference	Std. Deviation	t	df	Sig. (2-tailed)
Mean Matched LS Te – Mean All LS Te	-0.22	0.35	2.14	10	0.06
Mean Matched LS Te – Mean All PT Te	0.07	0.35	2.19	10	0.06
Mean All LS Te – Mean All PT Te	0.73	0.42	5.82	10	0.28

Table 30: Paired t-test of the mean tidal volume of the sample group between matched breaths and all recorded breaths by LS and PT during exercise.
The difference is significant at p = 0.05 Key: PT – pneumotachograph, Vt – tidal volume LS – LifeShirt® (s)

The paired sample t-tests show that the differences in expiration duration between matched and all recorded breaths within each device were not significant for both the LifeShirt® and the pneumotachograph during exercise. The difference in mean expiration duration between the LS and the PT for all recorded breaths was not statistically significant; this was also the case for matched breaths.

5.5.2.3 Tidal volume variability

Exercise				
	LS		PT	
Participant	Matched CV (%)	All CV (%)	Matched CV (%)	All CV (%)
1	43	47	46	57
2	29	35	29	36
3	29	32	30	36
4	42	70	49	58
5	30	42	27	38
6	32	51	33	32
7	35	48	38	46
8	36	54	32	45
9	24	34	19	31
10	31	35	23	25
11	25	30	25	26
Mean	32	43	32	39
(Stdv)	(6)	(12)	(9)	(11)

Table 31: Comparison of tidal volume variability in matched respiratory cycles and all respiratory cycles recorded by both devices during exercise.

Once again the tidal volume variability was higher for all recorded breaths than for matched breaths for both devices. There is a 9% mean difference between matched and all recorded breaths for the LifeShirt®, and a 7% mean difference for the pneumotachograph. The standard deviation is also higher for all recorded breaths than matched breaths for both devices.

Exercise	Mean difference	Std. Deviation	t	df	Sig. (2-tailed)
Mean Matched LS Vt CV – Mean All LS Vt CV	11.09	7.94	4.63	10	0.01
Mean Matched PT Vt CV – Mean All PT Vt CV	-7.18	4.73	-5.04	10	0.01
Mean All LS Vt CV – Mean All PT Vt CV	4.36	7.99	1.81	10	0.10

Table 32: Paired t-test of the mean tidal volume variability of the sample group between matched breaths and all recorded breaths by LS and PT during exercise.
The difference is significant at p = 0.05 Key: PT – pneumotachograph, Vt – tidal volume LS – LifeShirt® (s)

The paired sample t-tests show that the differences in mean tidal volume variability between matched and all recorded breaths within each device were statistically significant for both the LifeShirt® and the pneumotachograph. The difference in mean tidal volume variability between the LS and the PT for all recorded breaths was not statistically significant; this was also the case for matched breaths.

5.5.3 Correlation analysis

The following section provides a comparison of the correlation analyses between matched and all recorded breaths for tidal volume, expiration duration and tidal volume variability.

	Quiet breathing		Exercise	
Parameters	Matched	All	Matched	All
Vt	0.8	0.8	0.8	0.7
Te	0.9	0.9	0.9	0.8
Vt CV	0.8	0.9	0.8	0.9

Table 33: Comparison of Pearson's correlation analyses between LS and PT for matched breaths and all recorded breaths.

5.6 Summary

This chapter presented the results for the analysis of respiratory parameters recorded by the LifeShirt® and the pneumotachograph. Table 34 below presents a summary of findings the with respect to the hypotheses described in Chapter 4.

The next chapter will discuss the findings of this study and put them into context in relation to previous research.

Hypothesis	Summary of findings	Rejected/Partially Supported/Supported?
H1a: LS Vt correlates with PT Vt during quiet breathing	• Mean intra-subject correlation of matched breaths is 0.7. • Mean inter-subject correlation of matched breaths is 0.8. • Mean inter-subject correlation of all recorded breaths is 0.7.	Supported
H1b: LS Te agrees with the PT Te during quiet breathing	• Mean LS overestimation of Te by 0.49s. • The difference in Te between LS and PT is significant in all participants. • 95% limits of agreement are 1.7s. • SEM is 0.71s.	Supported
H1c: LS Te correlates with PT Te during quiet breathing	• Mean intra-subject correlation of matched breaths is 0.6. • Mean inter-subject correlation of matched breaths is 0.9. • Pooled ICC analysis of matched breaths is 0.7. • Mean inter-subject correlation of all recorded breaths is 0.9.	Supported
H1d: LS Vt correlates with PT Vt during exercise	• Mean intra-subject correlation of matched breaths is 0.7. • Mean inter-subject correlation of matched breaths is 0.8. • Mean inter-subject correlation of all recorded breaths is 0.7.	Supported
H1e: LS Te agrees with the PT Te during exercise	• Mean LS overestimation of 0.04s. • The difference in Te is significant in 7 participants, non-significant in 4 participants. • 95% limits of agreement are 0.82s. • SEM is 0.3s.	Supported
H1f: LS Te correlates with PT Te during quiet breathing	• Mean intra-subject correlation of matched breaths is 0.7*. • Mean inter-subject correlation of matched breaths is 0.9*. • Pooled ICC analysis of matched breaths is 0.85. • Mean inter-subject correlation of all recorded breaths is 0.9*.	Supported
H2a: LS records significant	• Significant difference in mean Vt between quiet breathing and exercise was recorded in both devices.	

difference in Vt if PT records significant difference in Vt between quiet breathing and exercise		Supported
H2b: LS records significant difference in Te if PT records significant difference in Vt between quiet breathing and exercise	• Significant difference in mean Te between quiet breathing and exercise was recorded in both devices.	Supported
H3a: LS records similar breath by breath variability to the PT during quiet breathing	• Mean CV of matched breaths for LS is 39% and for PT is 41%. • Correlation coefficient of matched breaths is 0.8*. • Mean CV of all recorded breaths is 46% for LS and PT. • Correlation coefficient of all recorded breaths is 0.9*. • No significant difference between the mean LS CV and PT CV. • CV was 22% in LS and 18% in PT during stable periods. • Correlation coefficient was 0.5 during stable periods.	Supported
H3b: LS records similar breath by breath variability to the PT during exercise	• Mean CV of LS is 32%, 35% for PT. • Correlation coefficient is 0.8*. • No significant difference between the mean LS CV and PT CV. • CV was 21% in LS and 15% in PT during stable periods. • Correlation coefficient was 0.3 during stable periods.	Supported

Table 34: Summary of research findings. *indicates significantly correlated

Chapter 6: Discussion of validity

6.0 Introduction

This chapter discusses the findings of the analysis of the validity study. The findings for each parameter during quiet breathing and exercise will be discussed separately. This is followed by a description of the limitations of this study, and some conclusions. The content of this chapter is outlined below:

6.1 Original hypothesis

The main aims of this validity study were

- To establish whether the LifeShirt® can estimate realistic and predictable relative changes in tidal volume on a breath by breath basis during quiet breathing and exercise, when calibrated with the method proposed by Millard (2002)
- To establish the level of agreement between the LifeShirt® and the pneumotachograph in measuring expiration duration during quiet breathing and exercise on a breath by breath basis, when calibrated with the method proposed by Millard (2002)
- To measure the variability in the tidal volume recorded by the LifeShirt® and pneumotachograph during quiet breathing and exercise
- To determine if the LifeShirt® can detect significant changes in tidal volume and expiration duration (using the pneumotachograph as the reference standard)

Table 34 (in the previous chapter) presents a summary of the findings with respect to the hypotheses for each of the main results. These findings will now be discussed.

6.2 Tidal volume

6.2.1 Tidal volume during quiet breathing

To date, there are few published studies that have i) performed breath by breath analysis, and ii) investigated the ability of the LifeShirt®, or the technology of respiratory inductive plethysmography, to estimate relative changes in tidal volume. The majority of published studies have investigated the differences in absolute tidal volume between a pneumotachograph and RIP/LifeShirt® measurements. Therefore, only small amounts of published data are available for direct comparison.

In this current study, the correlation coefficient for the group mean of tidal volume between the pneumotachograph and the LifeShirt® was 0.8 during quiet breathing. This result is similar to the study by Cantineau (1992), which investigated RIP in patients with a history of symptoms of obstructive sleep apnoea during wakefulness and asleep. In this study, the tidal volumes of thirteen patients were simultaneously monitored on a breath by breath basis by RIP and pneumotachograph during normal breathing in a supine position prior to sleep onset. Cantineau reported a correlation coefficient of 0.9 for mean tidal volume on a breath by breath basis between the pneumotachograph and the RIP during quiet breathing in a supine position.

The intra-participant quiet breathing correlation coefficient for tidal volume measured by pneumotachograph and RIP in this study ranges from 0.5 to 0.9, and the mean intra-participant quiet breathing tidal volume correlation coefficient is 0.7. This shows that the LifeShirt® does not have the same level of correlation with the pneumotachograph for every participant. The range of correlation coefficients is wider than the range reported in the study by Cantineau (1992), which reported a range of 0.8 to 0.9. It is unclear at this stage why certain participants had higher quiet breathing tidal volume correlations than others. Individual results are seldom reported in published studies, and individual values have not been reported in the limited amount of literature available with regard to the LifeShirt®. It is therefore unclear whether the variation in tidal volume

correlation between the LifeShirt® and the pneumotachograph within individuals during quiet breathing is unusual. One of the possible explanations for the difference in the correlation coefficient range was the difference in sample size and recording duration. In Cantineau (1992) the recording time of normal breathing during wakefulness was not documented, but the number of total breath cycles was lower (total n = 1009) than in the current study (total n = 3994).

Another possible reason for the low correlation coefficients in tidal volume in some participants may be related to the small dynamic range of tidal volume being recorded. Correlation analysis is similar to the intraclass correlation coefficient, and is dependent on the range of the data set (Bland & Altman, 1990). If data points were clustered together, it is likely to result in a lower correlation coefficient than from a data set with a wider range. This problem has been previously illustrated by a hypothetical data set of blood pressure measured by two devices in Lee *et al.*'s (1989) study. The example demonstrated an increase in correlation coefficients from 0.17 to 0.99 in two hypothetical data sets that had exactly the same difference, but the range of the scores increased from 5mmHg to 55mmHg. A low correlation coefficient therefore does not necessary truly reflect the association between devices. The presence of outliers in some data sets may also be a contributor to low correlational coefficients. Although Spearman's rank correlation coefficient was used to minimise the effect of outliers, such effects cannot be eliminated.

The third possible explanation of the poor correlation between the two devices in some individuals is that the two devices derive tidal volume by indirect methods. The pneumotachograph derives tidal volume from pressure change across the flow head, whereas the RIP device is based on the assumption that changes in chest wall area equate to changes in tidal volume. While the relationship between pressure change and volume change is well defined, the relationship between changes of chest wall area and tidal volume change might not be as clear-cut. The key question remains as to whether the chest wall will move solely within two degrees of freedom in all individuals. It is acknowledged in the original paper by Konno and Mead (1967) that the chest wall is capable of more than two degrees of freedom, especially when breathing at high volume. If the participant has a

natural apical breathing pattern, this will violate the two degrees of movement assumption, thus affecting the volume estimation which may lead to a low correlation between devices.

The results of the group mean tidal volume for all recorded breaths during quiet breathing were similar to the analysis with matched breaths only. Both devices recorded a lower mean tidal volume for all recorded breaths than for matched breaths only. This is likely to be due to the inclusion of the unmatched breath cycles which were removed during the data-cleaning procedure. Despite the lower group mean, paired t-tests demonstrated that the observed differences between matched breaths and all recorded breaths within each device were not significant. The correlation analysis between the LifeShirt® and the pneumotachograph for the mean tidal volume of all recorded breaths was the same as the group mean tidal volume for matched breaths.

Although the group mean correlation coefficient between the pneumotachograph and the LifeShirt® for tidal volume during quiet breathing is ‘high’ and is consistent with published studies, the individual correlation coefficients indicated that the agreement between the two devices may be reduced in some cases. It can therefore be concluded that the LifeShirt® is able to track relative changes of tidal volume during quiet breathing comparably with the pneumotachograph within a group. However, because there are cases where changes of tidal volume are not comparable when measure by the LifeShire® and the pneumotachograph, the relative changes of tidal volume are not interchangeable with the pneumotachograph. Further research is required to identify the reason for the variation in correlation between different individuals.

6.2.2 Tidal volume during exercise

The average intra-participant correlation coefficient for tidal volume during exercise was 0.7, with values ranging from 0.5 to 0.9. The mean tidal volume correlation coefficient between the pneumotachograph and the LifeShirt® during exercise was 0.8. This finding is consistent with another study (Caretti *et al*., 1994) which employed a similar methodology. In the study by Caretti *et al*. (1994) the tidal volumes of eight healthy individuals were simultaneously monitored by RIP and a flowmeter during incremental exercise during twenty-five minutes on a cycle ergometer. The study reported a mean tidal volume correlation coefficient of 0.7 between RIP and a flowmeter during cycling, measured on a breath by breath basis. Variability in tidal volume correlation coefficients between individuals was also reported in this study, with values ranging from 0.6 to 0.9. This finding seems to suggest that it is not unusual for RIP-based devices to show a wide variation in tidal volume correlations in different individuals in relation to pneumotachograph measurements taken during cycling.

Similar mean correlation coefficients between the LifeShirt® and the pneumotachograph was also reported by Witt (2006). Ten healthy males were recruited to the study, and tidal volumes were simultaneously monitored by both devices during incremental treadmill exercise until exhaustion. The mean tidal volumes of the final minutes of each stage were used for analysis. The study reported that the average coefficient of determination (r^2) of all exercise intensities between the LifeShirt® and the pneumotachograph was 0.87, which is equivalent to a correlation coefficient of 0.93. However, no individual data were given in the study by Witt (2006), and therefore it is unclear whether there was also a wide range in the coefficients of correlation for tidal volume between the pneumotachograph and the LifeShirt®.

Caretti (1994) proposed that the variation in correlation in their study was due to the slippage of the RIP band, which is a common problem with traditional RIP models. This should not be the case for the LifeShirt® because sensors are sewn in place to minimise movement of the bands. It could be argued that poor fitting of the LifeShirt® between different individuals may result in some variation because

the sensors might be wrongly sited. However, the author considers this to be unlikely because the difference in intra-participant correlation coefficients was not consistent between quiet breathing and exercise breathing. If the fitting of the LifeShirt® was not correct, one would expect the participants who had lower correlations during quiet breathing (at rest) to have the same or lower correlations during exercise. This was not the case for the three participants who demonstrated a 'moderate' correlation coefficient (between 0.5 and 0.7) during quiet breathing. Of the two participants who had a correlation coefficient of 0.5 during quiet breathing, one remained at 0.5 during exercise and one increased to 0.6. Another participant had an increased correlation coefficient from quiet breathing to exercise (an increase from 0.6 to 0.8). These data suggest that it is unlikely that the variation in correlation coefficients is due solely to poor fitting of the LifeShirt®.

In the analysis of all recorded breaths, both devices showed a lower mean tidal volume value for all recorded breaths than for matched breaths. Paired t-tests demonstrated that the difference between all recorded breaths and matched breaths is statistically significant for both devices. The statistically significant difference is likely to be due to the inclusion of the un-matched breaths which were not included in the matched breaths analysis. The size of the unmatched breaths tends to be very small, and this undoubtedly contributes to the lower mean tidal volume. The reason for the statistically significant difference observed during exercise but not during quiet breathing is likely to be due to more unmatched respiratory cycles being excluded in the matched analysis during exercise than during quiet breathing. The increased number of smaller breaths in all the recorded breaths led to a statistically significant lower mean tidal volume value. Although a lower mean tidal volume value was recorded in all recorded breaths than in matched breaths only, this did not have any effect on the Pearson's correlation coefficient analysis. The correlation coefficient of the mean of all recorded breaths between the LifeShirt® and the pneumotachograph is slightly lower than the group mean correlation coefficient of matched breaths, but the same as the intra-participant mean correlation coefficient for matched breaths.

6.2.3 Tidal volume changes during quiet breathing and exercise

The correlation coefficients for tidal volume between the LifeShirt® and the pneumotachograph did not differ significantly between quiet breathing and exercise. The range of correlation coefficients, the average intra-participant correlation coefficients and the mean inter-participant correlation coefficients were identical during both quiet breathing and exercise. The LifeShirt® was also able to detect a significant change in mean tidal volume from quiet breathing to exercise. These findings suggest that the LifeShirt® is equally valid in estimating relative changes in tidal volume during both quiet breathing and mild exercise.

6.2.3.4 Conclusion of the relative estimation of tidal volume by the LifeShirt® during quiet breathing and exercise

The findings of this study demonstrate that it is feasible to use the LifeShirt® to estimate relative changes in tidal volume, bypassing the recommended complicated calibration procedure. The ability of the LifeShirt® to record overall changes in tidal volume did not appear to deteriorate during exercise. This type of calibration method can be used to record relative changes in tidal volume if this type of measurement is of interest, rather than absolute changes in tidal volume. This could be beneficial in clinical practice because performing the complicated calibration procedure that is currently recommended is a recognised issue for some patients.

6.2.4 Breath by breath variability in tidal volume

One of the most noticeable findings is that the breath by breath variability in tidal volume, assessed by the coefficient of variation (CV), is considerably higher than the values reported in the published literature. Breathing pattern variability has recently attracted a good deal of research interest, and various authors have investigated the variability in tidal volume. Fiamma *et al*. (2007b) conducted a study in which they measured breathing patterns in eight healthy subjects at rest, breathing through a pneumotachograph under three conditions: normal breathing, induced hypocapnia, and induced hypercapnia. They demonstrated a decrease in tidal volume variability in hypercapnia (13%) and an increase in variability in hypocapnia (35%) when compared to normocapnia (20%). Fiamma *et al*. (2007b) reported the tidal volume CV as approximately 20% ± 5% in healthy individuals breathing through a facemask and sitting quietly for eight to fourteen minutes with a fifteen-minute stabilisation period prior to recording.

Kent *et al*. (2009) reported CV values of 18% from the pneumotachograph and 19% from the LifeShirt® during thirteen minutes completing an incremental exercise protocol while wearing both devices. In this study, the mean CVs of the LifeShirt® and the pneumotachograph were 40% and 41% respectively during quiet breathing. During exercise, the mean CVs of the LifeShirt® and the pneumotachograph were 32% and 35% respectively. A possible explanation for the difference is that the recording period in this study was longer than the recording period in other studies. The recording period for quiet breathing in this study was thirty minutes, whereas the mean recording period in Fiamma (2007b) was eleven minutes. The CV of quiet breathing in this study was 53% higher than the CV reported in Fiamma's study (2007b).

A similar pattern was also seen in another study, where the difference in CV was proportionate to the difference in recording period. The recording period for exercise in the current study was twenty minutes, while the recording period in the study by Kent *et al*. (2009) was thirteen minutes. The mean CV of the tidal volume measurements from the pneumotachograph in this study was 43% higher

than the CV of the pneumotachograph tidal volume data reported in Kent *et al*. (2009). These results suggest that the longer the recording period, the more likely it is that variability is detectable in tidal volume. However, an earlier study which recorded the tidal volumes of healthy individuals for sixty minutes reported a mean CV of 26% ±7% (Kuratomi *et al*., 1985). These authors recorded the breathing patterns of twenty-six healthy individuals using electrical impedance pneumography. This is a similar technology to the electro impedance topomography described in Chapter 3, since both devices measure changes in surface impedance via electrodes. However, instead of estimating tidal volume using the image computed from the impedance change, impedance pneumography estimates tidal volume directly from surface impedance changes.

In Kuratomi *et al*.'s study, the range of CV also varied widely between individuals, ranging from 12% to 37%. Wide variation in CV between individuals was also observed in the current study, with the pneumotachograph tidal volume CV ranging from 21% to 49%. The difference in tidal volume variability between the two studies may be explained by the difference in measuring devices. Since Kuratomi *et al*. (1985) used an old version of impedance measurement that utilised a four-electrode system to record impedance changes, there is some doubt about the accuracy of their device. The four-electrode system has been criticised on the grounds that it primarily measures chest wall geometry changes rather than estimating respiratory volume, because the surface area covered might not be sufficient (Grenvik *et al*., 1972). A more recent study has proposed the use of a six-electrode system and a different placement of the electrodes for improved estimation of tidal volume (Khambete *et al*., 2000).

Both studies have a similar range of CV (25% in the Kuratomi *et al*. study and 28% in this study). This suggests that breathing pattern is a very individual process, and it is very likely to differ widely from individual to individual. This is important when making comparisons with a 'normal' pattern, and it also reinforces the need for robust baseline data before initiating interventions designed to alter breathing pattern.

The mean CVs of tidal volume for each participant measured by the LifeShirt® and the pneumotachograph during quiet breathing and exercise were significantly correlated. Fiamma *et al*. (2007a) also conducted a study in which eight healthy subjects breathed through a pneumotachograph while wearing a sleeveless garment encasing RIP technology (Visuresp®, RBI, Meylan, France). Subjects breathed for ten-minute periods during both simultaneous and consecutive recordings, in both supine and seated positions. Their results are in agreement with the current study since they also demonstrated a significant correlation between the CVs of the RIP garment and a pneumotachograph.

The analysis of breath by breath variability during the stable breathing periods demonstrated lower correlation coefficients than the mean breath by breath variability for the whole recording period. This analysis was performed to test specifically whether the LifeShirt® measure a similar amount of variability as the pneumotachograph during stable periods, using the pneumotachograph as a reference standard. Correlation coefficients were not statistically significant at the 0.01 level during both quiet breathing and exercise periods, suggesting that the correlation could be due to chance. When the pneumotachograph measured a stable breathing period, the LifeShirt® measured a higher variability in tidal volume than the pneumotachograph. This finding suggests that the LifeShirt® is better in recording overall variability over a long period of time than it is at recording shorter periods in breathing.

6.2.4.1 Summary of breath by breath variability in tidal volume

The results provide further evidence that breathing is a very individual process. The findings of this study support the hypothesis that breath by breath tidal volume variability differs widely between individuals. The findings demonstrate that RIP embedded in a garment has the potential to record tidal volume variability over a long period of time, but it may be inadequate for short periods. It remains unclear as to what is the optimum period of recording. The results of this study clearly demonstrated a difference in tidal volume variability when compared with other studies that recorded variability over shorter periods of time. It is therefore proposed that recording time should not be less than thirty minutes if variability in tidal volume is to be investigated.

6.3 Expiration duration

6.3.1 Expiration duration during quiet breathing

The correlation coefficients demonstrate that the LifeShirt® has a 'very high' overall correlation with the pneumotachograph for expiration duration. However, correlation coefficients varied widely between individuals, ranging from 0.2 to 0.9. The difference in breath by breath expiration duration between devices is statistically significant. The variation observed in intra-participant correlation coefficients and the statistically significant differences in expiration duration between devices can be explained by the fact that each device is measuring a different aspect – i.e. the LifeShirt® is measuring chest wall contraction and expansion while the pneumotachograph is measuring expiration airflow. On close examination of the signals collected in this study from the LifeShirt® and the pneumotachograph, it appears that if a person maintains or increases the contraction of chest and abdomen at the end of expiration without expiring air, the algorithm will recognise the long contraction as a long expiration despite the lack of airflow. It is hypothesised that this period is the expiratory pause, is defined as the time from the end of expiratory flow to the start of inspiratory flow (Dorsch & Dorsch, 2008).

Figure 27 provides a graphical explanation of how the LifeShirt® tends to overestimate the expiration duration. Some participants (participant 1 and participant 6 in particular) appear to have a particularly long end-expiratory pause which has resulted in a low correlation between the devices. The existence of end-expiratory pauses can be considered to be normal in breathing (Martin, 2005). The difference in expiration duration between devices incorporated periods of indeterminate length caused by the end-expiratory pause. It is therefore concluded that end-expiratory pause partially accounted for the difference in expiration duration observed between the LifeShirt® and the pneumotachograph.

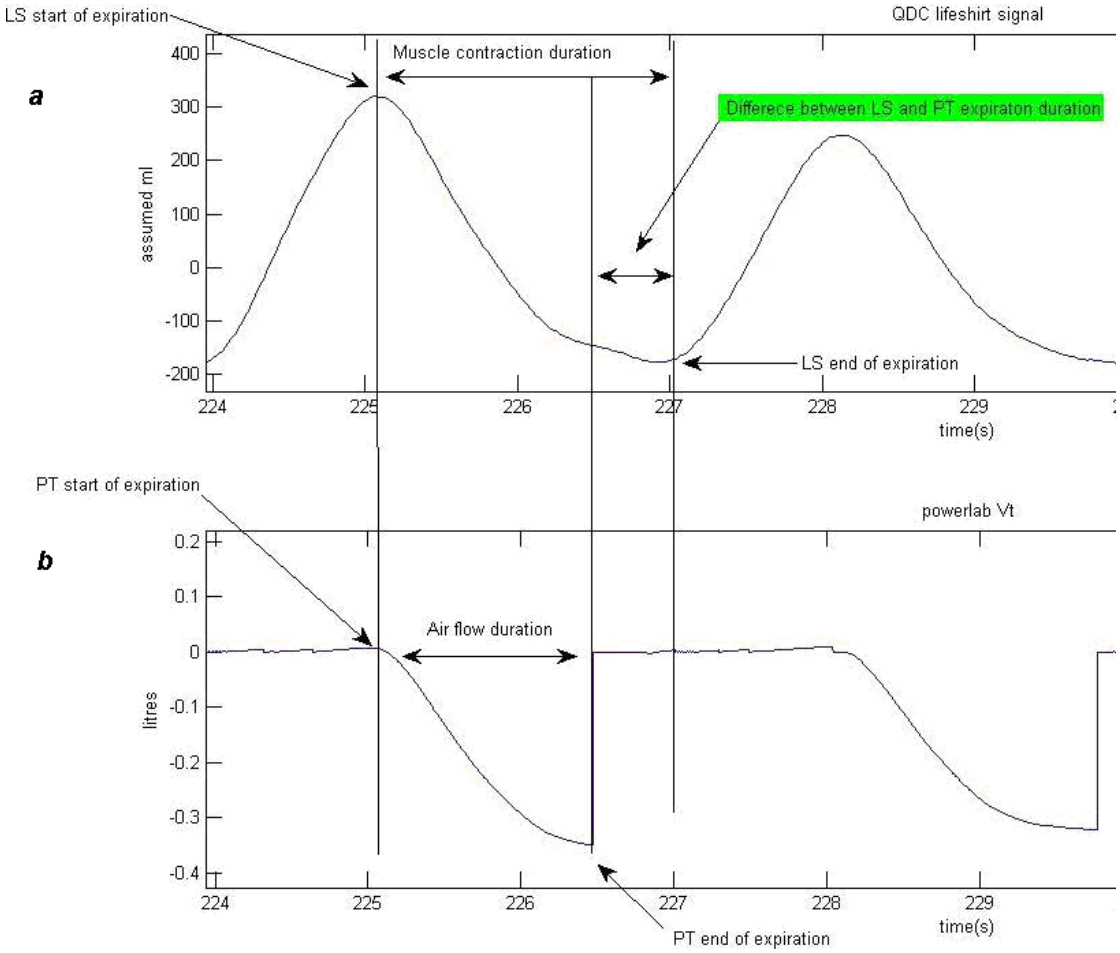


Figure 27: A graphical representation to demonstrate the difference in the LifeShirt® and the pneumotachograph in the measurement of expiration duration.

The signal on the top (a) is from the LifeShirt® and the signal at the bottom (b) is from the pneumotachograph. It shows that in the breaths from Participant 1, who registered some contraction of chest and abdomen at the end of expiration without expiring air, the algorithm will recognise the minimum point as the end of expiration. The long contraction is therefore being interpreted as a long expiration, despite the lack of airflow.

Despite the statistical significance observed, the difference between the expiration durations recorded by the LifeShirt® and the pneumotachograph during quiet breathing can be considered to be very small in clinical terms, since the mean difference between the LifeShirt® and the pneumotachograph was 0.51 seconds, with the LifeShirt® measuring a longer expiration duration than the pneumotachograph in all participants. A Bland and Altman analysis shows that the 95% limit of agreements was 1.72s. This shows that 95% of all the breaths are within approximately 1.72 seconds of each other during quiet breathing. The standard error of measurement also suggests that the difference for each breath

falls within 0.71 seconds of each other. This small amount of difference is unlikely to be of clinical significance because the average of the mean of expiration duration for the LS and the PT is 1.93s. Also, the overestimation includes an indeterminate amount of expiratory pause that happens during natural breathing. If the observed mean overestimation of 0.5 second by the LifeShirt® was accepted to be the length of the expiratory pause that cannot be measured by the pneumotachograph (i.e. every breath cycle recorded by the LifeShirt® is likely to be 0.5 seconds longer than the duration measured by the pneumotachograph), then the SEM of 0.71s includes 0.5 seconds of expiration pause. Therefore the difference between the SEM and the expiration pause should be considered as the discrepancy in expiration duration between the devices. The difference of 0.71 seconds represents 12% of the mean range of expiration duration. It is also shorter than the standard deviation of the mean expiration duration (1.4 seconds). It is unclear that whether the precision of the 1.72-second range is sufficiently small to enable clinical utility. At the moment, there is insufficient data to suggest whether the precision of this range is acceptable.

6.3.2 Expiration duration during exercise

The agreement in expiration duration between the LifeShirt® and the pneumotachograph increased during exercise. The difference in expiration duration between the LifeShirt® and the pneumotachograph was statistically significant in seven participants and not statistically significant in four participants. However, the difference in individual mean expiration duration was not statistically significant between the LifeShirt® and the pneumotachograph.

A similar finding was reported in the study by Kent *et al*. (2007). In their study, tidal volume and expiration duration were simultaneously monitored by the LifeShirt® and the pneumotachograph during progressive and steady treadmill exercise. Statistically significant differences in expiration duration between the LifeShirt® and the pneumotachograph were observed during low exercise load, but the differences became non-significant at the higher exercise load. These findings confirm that the LifeShirt® has a higher agreement with the pneumotachograph during a high exercise load. In Kent *et al*.'s (2009) study no explanation was offered as to why an increase in exercise intensity might result in higher agreement. However, on close inspection of the signals from the LifeShirt® collected in this study it was found that the transition between chest wall contraction and expansion was much more definitive during exercise than during quiet breathing. The end-expiratory pause is shorter during exercise than quiet breathing, which is a normal response to exercise (Martin, 2005). Figure 28 gives a graphical representation of this occurrence.

From Figure 28, it can be seen that participant 1 had a clearer transition between chest wall contraction and expansion during exercise than during quiet breathing. The end-expiratory pause was therefore shorter, thereby reducing the difference in expiration duration between quiet breathing and exercise. This would explain the improvement in agreement during exercise, observed in this study and in Kent *et al*. (2009).

The mean difference in expiration duration between the LifeShirt® and the pneumotachograph is the same as the difference reported in Kent *et al*.'s study (0.04 seconds). However, there is doubt as to whether such a small difference would be detected by the LifeShirt® due to the limitation of the sampling frequency.

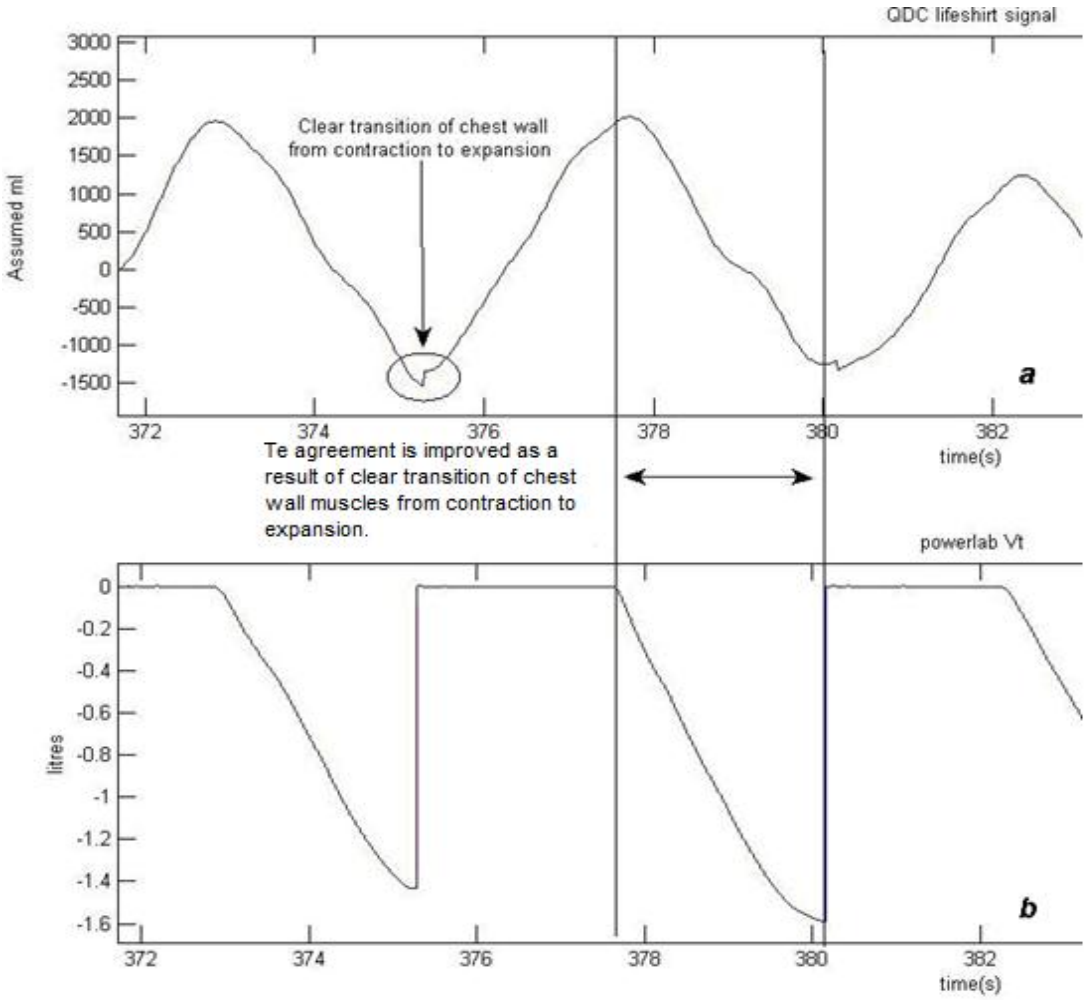


Figure 28: A graphical representation to demonstrate how the LifeShirt® improves agreement with the pneumotachograph in expiration duration during exercise.
The signal on the top (a) is from the LifeShirt® and the signal at the bottom (b) is from the pneumotachograph. The figure shows the breaths from Participant 1, who had shorter end-expiratory pause during exercise than during quiet breathing. It is likely that the shortening of the end-expiratory pause reduced the difference in expiration duration between devices and therefore improved the agreement between devices.

6.3.3 Summary of expiration duration during quiet breathing and exercise

The findings demonstrate that the LifeShirt® slightly overestimated the expiration duration in comparison with the pneumotachograph. At the moment there is insufficient data to facilitate judgment about whether this represents a clinically important difference. The difference appears to be very small (on average 0.51 second longer during quiet breathing and 0.04 second longer during exercise). In the author's opinion, this is unlikely to be clinically significant because of the reason described above. The mean correlation between devices was very high during quiet breathing ($r = 0.9$) and also during exercise ($r = 0.9$), demonstrating an almost perfect linear relationship in the measurement of mean expiration duration between the LifeShirt® and the pneumotachograph.

However, this study highlights a potential issue when interpreting the duration of muscle contraction as the same as the duration of air flow. This study clearly demonstrated that the duration of the contraction of the chest wall muscles does not always reflect the length of airflow. The LifeShirt® might therefore not be suitable to be used in monitoring expiration duration in certain pathologies, such as chronic obstructive pulmonary disease (COPD) where air flow is restricted due to damage to the alveoli and inflammation and narrowing of the bronchioles. A COPD patient could therefore be contracting the chest wall muscles for a longer time than the actual time of expiration air flow. However, the LifeShirt® would still interpret this as a long expiration duration. The LifeShirt®/RIP technology may arguably be more useful in monitoring breathing pattern since it measures the entire expiration phase (see Figure 27) which consists of both the expiration flow and the end-expiration pause (Dorsch & Dorsch, 2008; Martin, 2005). The end-expiratory pause is emphasised in various forms of breathing exercise, e.g. the Buteyko breathing technique (Bruton & Lewith, 2005), and it would therefore be advantageous to record the end-expiratory pause. Further research is needed to investigate the breathing patterns in different pathological populations.

6.4 Breath by breath calibration method

This study investigated the use of a variation of the QDC calibration proposed by Millard (2002). The main advantages of this technique are that it does not require a calibration procedure, and the calibration factor can be updated to suit the latest breathing conditions. This calibration technique had not been tested in subjects in a wakeful state. While this study did not directly compare the respiratory parameters derived from other calibration methods, the validity indices reported in the current study suggest that the method is comparable to other studies which used different calibration methods.

Cantineau *et al*. (1992) and Caretti *et al*. (1994) both utilised the multiple linear regression method to calibrate the RIP. The mean tidal volume correlation coefficients during quiet breathing between the RIP and the pneumotachograph in both studies were in agreement with the current study. Witt *et al*. (2006) utilised the traditional QDC method to calibrate the LifeShirt® and compare the tidal volumes obtained from the LifeShirt® with those obtained from the pneumotachograph during exercise. The correlation coefficient reported in Witt *et al*. (2006) was also in agreement with the correlation coefficient during exercise found in the current study.

The study reported in this thesis also demonstrated that the calibration method proposed by Millard (2002) is capable of estimating variability in tidal volume when compared to the laboratory gold standard (the pneumotachograph). Based on these results, the study would suggest that the calibration method proposed by Millard (2002) is at least as good as other existing methods in relative tidal volume estimation.

The hypothesis that the proposed calibration method allows estimation of relative tidal volume is therefore accepted.

6.5 Limitations

6.5.1 Data cleaning process

This study has a number of limitations. One is that it involved a manual process to remove some respiratory cycles which were not recorded by both devices. This process was considered to be essential to investigate the validity of the LifeShirt® and to test the feasibility of exploring breath by breath variability in tidal volume. However, although this step was considered to be essential for this study, it is unlikely that such a process could be repeated without the reference function of the pneumotachograph. Despite this issue, the analysis between the matched breaths and all recorded breaths demonstrated that the LifeShirt® can estimate relative changes of tidal volume and tidal volume variability that are comparable with the pneumotachograph.

6.5.2 Sample size

This study involved eleven participants, which may be considered a small sample size. However, this sample size is not unusual in previous published studies that have investigated the validity of RIP or the LifeShirt®. Since this study emphasised breath by breath analysis, the number of respiratory cycles analysed in this study was very large. In total, 3994 respiratory cycles were analysed during quiet breathing and 3031 cycles were analysed during exercise. This should be considered sufficient to demonstrate the validity of the LifeShirt®. It is acknowledged that recording a large number of breaths from a small sample group does not have the same power as recording from a large sample population. This might limit the external validity of the results because the results might not be generalisable to a wider population. However, due to time and financial constraints and the slow recruitment process, this limitation could not be addressed.

6.5.3 Number of respiratory cycles

Another limitation of this study is that the numbers of respiratory cycles analysed were not the same for each participant. This may cause an uneven comparison of the mean values between participants, since participants with fewer respiratory

cycles may be affected more by anomalies than those with more respiratory cycles. This situation is again very common in published studies, as shown in Caretti (1994) and Cantineau (1992). The number of breaths recorded in those studies, which both used interval average data, was often not reported. Therefore, it is unlikely that they would have the same numbers of breaths in each interval for every participant. Given the variability in breathing and how individual breathing patterns are, it might not be possible to record the same number of breaths for each participant. One of the options to analyse a specific number of breaths is to analyse the last x breaths in any recording, or the first y breaths recorded after the stabilisation periods. However, this may inevitably leave some selection bias.

6.5.4 Comparison of calibration methods

This study has not attempted to compare the effects of using different calibration methods on the results. This is because the primary goal of the validation study was to assess the agreement between the pneumotachograph and the LifeShirt® when calibrated by the method proposed by Millard (2002) during quiet breathing and exercise, rather than comparing different calibration methods. The lack of comparison with existing calibration methods limits the inferences and conclusions which may be drawn from this study, as it remains unclear whether this novel calibration technique is superior to other calibration methods. Future research is required to investigate whether the calibration method is at least equally as good when compared to other methods, especially when breathing strategy changes.

6.5.5 Insufficient further investigation

There is insufficient further investigation on why some individuals have lower correlation coefficients than others. Several possible reasons have been proposed which might contribute to the observed variation of correlation coefficients between individuals, including the small dynamic range of the data set. Further investigation may help to identify the reason for the observed variation between individuals, and may indicate whether the variation is due to the physics of the measuring device, or the calibration method not being sufficiently robust, the non-linearity of the data set, or the existence of outliers.

This will help to guide future study regarding the use of the calibration method and the RIP device.

6.5.6 Instrument error

Another limitation is the instrument error. All recording instruments are likely to carry some degree of error. Error is often introduced as part of the sample process. In particular, these errors are sampling error, systematic error and random error.

Sampling error:

The sampling processing itself can induce errors into the data. Digital signals are given by a sequence of numbers that represent samples of the signals, as illustrated in Figure 29. Sampling refers to the extraction of signal values at all integer multiples of the sample period. Sampling frequency is defined as the number of samples acquired per unit time, and is usually expressed in Hz. If the sampling rate is sufficiently high, the signals can be reconstructed from the samples with no loss of information.

Error can arise during sampling as the result of insufficient sampling. According to the sampling theorem, a continuous time signal can be exactly reconstructed from samples when the highest frequency present in the signal is lower than half the sampling frequency Simpson *et al.,* (2005). In this study, the sampling frequency of the LifeShirt® was fixed at 50Hz. This is a common sampling frequency for RIP-based devices. The sample frequency of the pneumotachograph was 200Hz, which is sufficient to avoid aliasing effects.

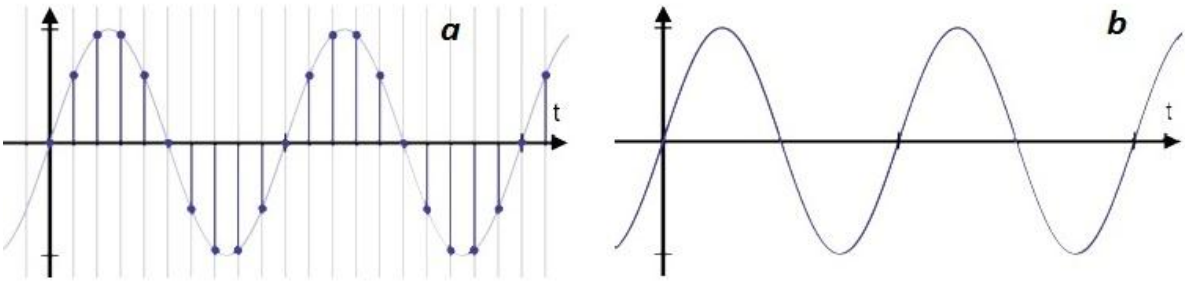


Figure 29: An example to demonstrate signal reconstruction from sample recorded.
Figure 29a represents the sample recorded; Figure 29b represents the reconstructed signal.

Error can also occur when reconstructing the signal. Figure 28 uses a sine wave as an example to illustrate how this error could arise. The data points shown in 29a can be used to reconstruct the sine wave in 29b. From these two figures, it can be seen that although the sine wave plot in 29b has been reconstructed from the data in 29a, some information in between each sample is lost. This leads to uncertainties about the measurement of tidal volume and the start and finish time of inspiration/expiration phases, because the readings at the peaks may or may not be the 'true' reading of the peak value. When the signal is not perfectly sinusoidal, which it is not with respiratory signals, an additional error can be introduced as the reading might be taken at the peak sample, immediately after the peak sample, before the next sample, or at the sample after the next sample (sees Figure 30).

This sampling error would affect the data collected in this study, especially when the signals are not perfect sinusoidal. The identification of the peak is a key aspect in this study because it is used to calculate the peak expiratory volume and the start and finish time for expiration.

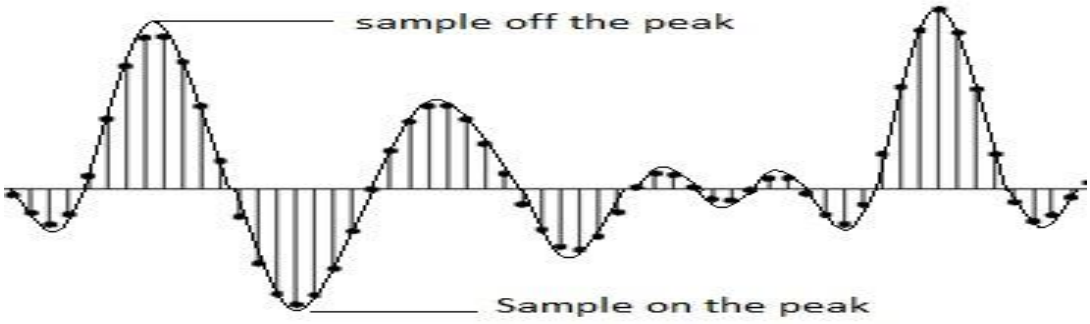


Figure 30: An example of error induced during sample reconstruction.

Systematic error

All measuring devices are subject to some degree of systematic error. Systematic error refers to an instrument giving a consistently lower or higher reading. For example, a bathroom scale may consistently give a reading of 99kg when it should read 100kg. This type of error could be due to zeroing error, which is

when the device has an non-zero initial reading. One solution for minimising systematic error is though calibration. Calibration can zero the instrument so that its initial baseline value is zero. Systematic error may also be offset by adding or deducting the value of the error. However, even with correct calibration, it is difficult to obtain the value for systematic error, because this type of error exists with all measuring devices which makes it difficult to know what the true value is.

With the measurements obtained from the LifeShirt®, the first part of the validation study demonstrated that the device can measure linear relative tidal volume compared to the gold standard. Calibration was also done on a breath by breath basis. These measures help to minimise systematic error in the data collected in this study.

Random error

Random error refers to error that arises randomly; all measurement devices can be affected by this type of error. A typical example of random error is noise. This type of error is difficult to control because it occurs at random.

6.6 Conclusion

This study has provided evidence to support the use of a calibration technique which calibrates RIP signals in order to estimate relative changes in tidal volume. The results of this study have confirmed that the instrumented garment has the potential to monitor tidal volume either during natural breathing or in response to exercise and activity outside of the laboratory, or as an outcome measure in trials of interventions. The overestimation of expiration time by the instrumented garment is unlikely to be clinically significant. It also has an advantage of monitoring the entire expiration phase, including the end-expiratory pause, as opposed to just the expiration airflow as measured by the pneumotachograph. This makes it suitable for monitoring the effects of interventions that emphasise a controlled pause between expiration and inspiration. This study supported the hypothesis that the LifeShirt® is as valid as the pneumotachograph in measuring breathing variability.

Chapter 7 Second Phase – Breathing pattern in a severe asthma population

7.0 Introduction

The first phase of the study confirmed that the instrumented garment has the potential to monitor tidal volume, expiration time and tidal volume variability, both during natural breathing and during exercise. The first part of the study also demonstrated the feasibility of using the calibration method proposed by Millard (2002) to estimate relative tidal volume and absolute expiration duration in healthy individuals. The research findings of the first validation study have been presented in national and international peer-reviewed conferences. The published abstract can be found in Appendices XI and XII. At the time of writing an abstract for the second phase of the study has also been accepted for presentation at an international conference. A copy of the submitted abstract can be found in Appendix XIII.

As the manufacturer of the LifeShirt® ceased business in late 2010, it was felt that further analysis into the reliability and validity of the recorded data would not be beneficial since the equipment was no longer commercially available and therefore further no improvements would be made to the system. However, the technology used in the LifeShirt®, i.e. RIP, is still available and functional. The focus of the research therefore moved away from investigating the LifeShirt® and focused instead on breathing pattern analysis using the LifeShirt® as the measurement tool. Although breathing pattern modification has been trialed in mild and moderate asthma patients, little has yet been done for those suffering from severe asthma. It was therefore decided to focus on patients with severe asthma to determine whether they have any abnormal breathing patterns.

The second phase of this study aimed to explore breathing patterns in a group of patients diagnosed with severe asthma. Breathing pattern assessment of this nature has not yet been conducted in this population. This is likely to be partly due to the difficult of monitoring breathing pattern unobtrusively, and partly due to

the sample pool of patient availability. Existing studies on mild to moderate asthma patients reported symptoms of hyperventilation and reduced $ETCO_2$ levels with little difference in breathing parameters when measured by either RIP (Tobin *et al*., 1983b) or pneumotachograph (Osborne *et al*., 2000). However, there are limited reports available on the $ETCO_2$ level of patients diagnosed with severe asthma.

It is hypothesised that the severe asthma population may show reduced levels of variability in volume and timing components than the normal population. This hypothesis is supported by recent evidence from Fiamma *et al*. (2007b), which shows that the variability in respiratory parameters in induced hypocapnia was higher than in normocapnia. Hypocapnia is a direct result of over-breathing (hyperventilation). It might therefore be expected that patients with hyperventilation symptoms would show increased levels of variability in tidal volume compared with normal individuals. However, in the few other pathologies studied to date, such as chronic lung disease (Brack *et al*., 2002) , variability in tidal volume has been higher in healthy individuals than in those with the pathology. This suggests that there may be an optimal level of variability (as in heart rate variability), and therefore either increased or reduced variability is detrimental to health or an indication of a health problem.

To date, limited published studies have been found which investigate the variability of the timing component. Measuring the variability in tidal volume and timing components in severe asthma patients will give us a better understanding of the breathing pattern in this particular patient group. If the results of the study show that there is either increased or decreased variability of respiratory parameters when compared to healthy individuals, this would support the theory that breathing retraining, which aims to alter breathing pattern, might be of benefit to this patient group. It is therefore proposed to conduct a study involving patients with asthma to determine the level of variability in their breathing pattern.

One hypothesis is that asthma patients have altered levels of carbon dioxide, and this causes the symptoms of hyperventilation and asthma (Buteyko, 1990). Previous authors have supported the first part of the hypothesis by demonstrating that altered levels of end tidal carbon dioxide exist in the mild to moderate asthma population. However, currently there is little data to demonstrate whether this is the case in severe asthma patients. Existing studies have not found obvious clinical signs that would lead to altered carbon dioxide levels, and also there appears to be little difference in the reported physiological respiratory parameters of tidal volume, expiration time, respiratory rate and inspiration drive between healthy volunteers and mild to moderate asthma patients during the stable disease stage.

If the hypothesis that carbon dioxide levels are related to asthma symptoms is true, one would also expect altered levels of carbon dioxide in severe asthma patients. It is currently unclear whether physiological respiratory parameters are different in severe asthma patients in comparison with healthy individuals. Other parameters, such as sigh rate and breathing variability, are commonly believed to be contributors to asthma symptoms but this has yet to be demonstrated in published studies.

7.1 Research Aim

The literature review in Chapter 2 has highlighted that there is limited published evidence to date regarding the breathing pattern of severe asthma patients during the asymptomatic phase of the condition.

The second phase of the study therefore sets out to obtain new knowledge with regard to the breathing pattern of a group of severe asthma patients. The aims of the study are as follow:

- To explore if there are differences in the respiratory parameters of tidal volume, inspiration time, expiration time, respiratory rate, sigh rate, $ETCO_2$ level, tidal volume variability and expiration time variability

between a group of healthy people and a group of patients diagnosed with severe asthma.

- To explore if there is a linear association between: i) hyperventilation symptoms and resting $ETCO_2$ level; ii) hyperventilation symptoms and sigh rate; iii) $ETCO_2$ level and sigh rate; iv) $ETCO_2$ level and tidal volume variability; v) $ETCO_2$ variability and tidal volume variability; and vi) sigh rate and variability.

This study follows the severity guidelines published by the British Thoracic Society (BTS, 2009) for asthma classification.

Chapter 8: Methodology for breathing pattern analysis

8.0 Introduction

This chapter provides a description of the equipment and methodology used in this study. A general overview of the study is presented first, followed by specific details about the procedures. Aspects of the methodology will be described as follows:

8.1 Research hypotheses
8.2 Study design
8.3 Research governance
8.4 Sample population
8.5 Recruitment process and Method
8.6 Equipment
8.7 Experimental procedure
8.8 Signal processing
8.9 Statistical analysis
8.10 Summary

8.1 Research hypothesis

This study was designed to collect breathing pattern data for tidal volume, inspiratory time, expiratory time, respiratory rate, sigh rate, end tidal carbon dioxide level, end tidal carbon dioxide level variability and tidal volume variability in a group of people diagnosed with severe asthma during a stable period of the condition, and to compare their breathing patterns with those of a group of healthy individuals. The specific hypotheses that were explored are as follow:

H1a There is a statistically significant difference in mean relative tidal volume between the healthy and severe asthma populations at rest.

H1b There is a statistically significant difference in mean expiration time between the healthy and severe asthma populations at rest.

H1c There is a statistically significant difference in mean respiratory rate between the healthy and severe asthma populations at rest.

H1d There is a statistically significant difference in mean sigh rate between the healthy and severe asthma populations at rest.

H1e There is a statistically significant difference in mean end tidal carbon dioxide level between the healthy and severe asthma populations at rest.

H1f There is a statistically significant difference in mean tidal volume variability between the healthy and severe asthma populations at rest.

H1g There is a statistically significant difference in mean expiration time variability between the healthy and severe asthma populations at rest.

H1h There is a statistically significant difference in mean ETCO2 variability between the healthy and severe asthma populations at rest.

H1i There is a statistically significant difference in mean NQ score between the healthy and severe asthma populations at rest.

H1j There is a statistically significant difference in mean HADS score between the healthy and severe asthma populations at rest.

H2a There is a significant correlation between end tidal carbon dioxide level and tidal volume variability.

H2b There is a significant correlation between sigh rate and tidal volume variability.

H2c There is a significant correlation between end tidal carbon dioxide levels and sigh rate.

H2d There is a significant correlation between end tidal carbon dioxide variability and tidal volume variability.

H2e There is a significant correlation between NQ score and end tidal carbon dioxide level.

H2f There is a significant correlation between NQ score and sigh rate.

8.2 Study Design

A quantitative observational experimental design was suitable for this study, because it attempted to measure and quantify breathing pattern objectively. This study was exploratory in nature, to explore the breathing pattern of severe asthma patients. Respiratory parameters of tidal volume expiration time and tidal volume variability, end tidal carbon dioxide level, end tidal carbon dioxide level variability, respiratory rate and sigh rate were recorded from a group of severe asthma patients and compared with similar data from a group of healthy participants.

8.3 Research governance

8.3.1 Ethical approval

The breathing pattern recording was added into a larger study, the 'Wessex Severe Asthma Cohort', that aims to phenotype patients with asthma (Ethics No: RHM MED0834). A minor amendment to the existing study was approved by the local ethics committee for the Southampton and South West region, and by Southampton University Hospital Trust. Southampton University Hospital Trust also acted as a sponsor for the study.

The healthy population was recruited as part of the previous validation study for the LifeShirt®. Ethical amendments were sought for the removal of the facemask and the additional use of two questionnaires. The Ethics Committee of the Faculty of Health Sciences, University of Southampton approved the amendments (Appendix VI). The University of Southampton was the sponsor of the study.

The current study was conducted under the Declaration of Helsinki from the World Medical Association (2008)

8.3.2 Ethical consideration

Verbal and written explanations were given to all participants about the aims of the study, and an information sheet regarding the study was given prior to data collection taking place. All participants were informed that the data would be used for the purpose of this study. Voluntary consent was obtained via a written consent form, and participants were reminded that they had the right to withdraw from the study at any time if they wished, without giving any reason. A number was allocated to each participant to ensure anonymity throughout the study. All data were stored in a locked cabinet in accordance with university policy. As the study required people to remove some clothing, a chaperone was offered and provided, if requested by participants. The researcher was a qualified physiotherapist and had received training in operating the equipment to a safe standard. The Code of Conduct of the Chartered Society of Physiotherapy was strictly adhered to at all times. There are no known side effects from the wearing of the instrumented garment or the nasal cannula. This study presented minimum potential harm to participants.

8.3.3 Health and safety

A risk assessment was carried out prior to the study to identify any major hazards. The Southampton University Hospital Trust Hand Hygiene Policy and Infection Control Policy were strictly followed. The LifeShirt® garment used for recording was washed in accordance with the manufacturer's instructions. Disposable single-use nasal cannulas were used for each participant to avoid cross-infection. For the asthma group, medical support was provided during the recording period. A nebuliser was available throughout the data collection period in case it was required by the individual. Southampton University Hospital Trust's emergency policy was in place and was strictly followed. All asthma patients were advised to stop if they experienced chest tightness, shortness of breath or any other form of discomfort during the data collection period.

8.4 Sample population

A power calculation was considered to be unsuitable as this study was exploratory and observational in nature. It aimed to explore the hypotheses listed in Section 8.1. This study included healthy volunteers and severe asthma patients aged 18 and over. The inclusion and exclusion criteria were set to include as many individuals as possible.

8.4.1 Healthy population criteria

Inclusion criteria:

- Aged 18 or over
- Non-smokers (those who have never regularly smoked one or more cigarettes per day)

Exclusion criteria:

- History of serious cardiovascular complication, pulmonary disease, neurological conditions
- History of smoking (one or more cigarettes per day)
- Recent upper respiratory infection (within four weeks)
- Inability to ride an exercise bicycle

The exclusion criteria for healthy individuals were to reduce the likelihood of pathologies and to enhance the internal validity of the results by improving the homogeneity of the sample population. They also ensured the safety of the participants who took part.

8.4.2 Severe asthma population criteria

Inclusion criteria:

- Aged 18 or over
- Non-smokers (have never regularly smoked one or more cigarettes per day)
- Diagnosed with severe asthma by a medical clinician and on step 3 or above of the British Thoracic Society asthma guidelines

Exclusion criteria:

- History of serious cardiovascular complication, pulmonary disease (except asthma) or neurological conditions
- History of smoking
- Recent upper respiratory infection (within four weeks)

The exclusion criteria for severe asthma patients were to reduce the likelihood of breathing patterns being affected by pathologies other than severe asthma. They also ensured the safety of the participants who took part.

8.5 Recruitment process and method

The severe asthma cohort was recruited from an existing volunteer list of asthma patients. All patients on the volunteer list had consented to be contacted for future studies. Patients who met the inclusion criteria were identified by the respiratory nurses and the physicians who were responsible for the recruitment of the original study. Patients' files were reviewed by the physician or the respiratory nurse to confirm suitability. All suitable patients were then contacted and introduced to the breathing pattern study via telephone. An information pack containing an invitation letter (shown in Appendix VIII), a participant information sheet (Appendix IX), a reply slip (Appendix X) and a self-addressed return envelope was sent out to those who expressed interest over the telephone. If individuals decided to participate, they returned the reply slip and the researcher contacted the individual to arrange a visit to the hospital.

The healthy sample population was recruited from the student and staff population at the University of Southampton via posters, word of mouth and announcements at the beginning of selected lectures.

8.6 Equipment

8.6.1 LifeShirt®

Respiratory signals were recorded by the LifeShirt® system (VivoLogic™, VivoMetrics, Ventura, California) which was described in a previous section (see Section 3.1.5). In summary, the LifeShirt® is a non-invasive measurement tool that measures changes in chest wall area. Respiratory parameters such as tidal volume, expiration time and inspiration time can be derived from the signals. Calibration is required for the estimation of tidal volume. The same calibration process discussed in a previous chapter (see Section 3.2.2) was used in this study for tidal volume estimation. As the LifeShirt® was not calibrated to give absolute tidal volume, the unit of measurement was assumed millilitres (aml). The measurement unit was adopted from the manufacturer's instruction book to describe semi-calibrated signals.

8.6.2 Capnograph

End tidal carbon dioxide level was monitored by a standard capnograph (Sleep Capnocheck®, Smiths Medical, London, UK). The company specifications state the response time to be 375 milliseconds (to 90% of value), and the accuracy to be ±0.3kPa in a range from 0kPa to 13.3kPa. Although it is recognised that arterial blood gas analysis is the gold standard for measurement of gas composition in arterial blood, it was felt that this would be too invasive for this study and could itself cause anxiety and hyperventilation. $ETCO_2$ is regarded as equivalent to arterial carbon dioxide in normal resting subjects and mild to moderate asthmatics with no ventilation/perfusion mismatch (Osborne, 2000). A single-use nasal cannula was connected from the participant's nostrils to the capnograph device. During the recording period, $ETCO_2$ was sampled continuously via expired air (using a 4 second epoch). Data were recorded directly onto the computer via associated software (Profox version 2.0).

The output format of the capnograph was fixed on the device and did not allow changes to be made. Because the device was limited to sample using a 4-second epoch, breath by breath comparison of changes in $ETCO_2$ levels to changes in tidal volume was not feasible. The device was calibrated according to the manufacturer's instructions at the beginning of the data collection period and after the recommended interval. This study used the textbook figures of 4.66kPa to 5.99kPa (Esquinas, 2010; Murphy *et al*., 2009) to define the normal level of end tidal carbon dioxide.

8.6.3 Hospital Anxiety and Depression Scale (HADS)

The anxiety and depression state of the severe asthma cohort was assessed using the HADS. The HADS was developed as a reliable instrument for detecting states of depression and anxiety in patients with non-psychiatric conditions (Zigmond & Snaith, 1983). The scale is divided into an anxiety subscale (HADS-A) and a depression subscale (HADS-D). It has been used extensively in research to assess the anxiety and depression states of asthma populations (Deshmukh *et al*., 2007; Cooper *et al*., 2007; Bjelland *et al*., 2002). In a review by Bjelland *et al*., (2002), twenty-four validation studies using HADS in different studies were reviewed, showing a variation in cut-off values in different settings. The most frequently reported cut-off point in the twenty-four studies was 8 for both the HADS-A and HADS-D subscales, giving sensitivity and specificity of approximately 0.8 for both subscales. This result is in agreement with the original study by Zigmond and Snaith (1983), which suggested that a cut-off point of 8 would suggest abnormal levels of anxiety and depression.

The original study by Zigmond and Snaith (1983) also proposed a 'severe disorder' category with a cut-off point of 14 or above. However, no empirical data were presented in the original study to determine the usefulness of the additional category. Also, no studies in Bjelland et al.'s (2002) review reported the use of 14 as a cut-off score. The current study therefore used a cut-off score of 8 or above to indicate abnormal levels of anxiety or depression, since this has the most support from the existing literature.

8.6.4 Nijmegen questionnaire (NQ)

Symptoms of hyperventilation in the severe asthma cohort were assessed by the NQ. The NQ was first developed in 1985 to detect hyperventilation syndrome (van Dixhoorn & Duivenvoorden, 1985). The instrument consists of three domains: shortness of breath, peripheral tetany and central tetany. The original validation study suggested that the cut-off point for positive identification of hyperventilation symptoms is 23 or above. This would give a sensitivity of 91% and a specificity of 95%. This questionnaire has been widely used in published studies to detect the presence of hyperventilation symptoms in asthma populations (Thomas *et al*., 2001; Thomas *et al*., 2003; Thomas *et al*., 2005b) or as an outcome measure too for clinical interventions (Thomas *et al*., 2005a; Holloway & Ram, 2009). The original cut-off value has not been altered or changed since the development of the scale.

8.6.5 Asthma Control Questionnaire (ACQ)

The Asthma Control Questionnaire was developed by Juniper *et al*. (1999) to measure the primary goals of asthma management in accordance with international guidelines. The ACQ contains six questions that are related to asthma symptoms, and an additional question that relates to lung function data prior to the use of a beta2-agonist. Each question has a seven-point scale (where 0 = no impairment and 6 = maximum impairment). FEV1% is also scored on the same seven-point scale. All items are equally weighted, and the ACQ score is the mean of the scores of the seven items, ranging from 0 (totally controlled) to 6 (severely uncontrolled). The items on the ACQ were chosen by ninety-one asthma clinicians who were members of guidelines committees from over eighteen countries. In the original validation study, the ACQ and other measures of asthma health status were assessed at baseline, and then again at one, five and nine weeks later. The results showed that the ACQ was reliable (ICC = 0.9) and was responsive to changes in asthma control ($p>0.0001$).

Since the instrument was developed it has been further validated to be used in both clinical practice and clinical trials. A study in 2006 (Juniper *et al*., 2006) involving1323 patients with asthma aged between 12 and 80 years determined the cut-off points on the ACQ that gave the best definition between adequate and inadequate control. The results of this study suggested that the cross-over point between adequate and inadequate control was close to 1.00, which means that below a score of 1.00 patients are more likely to have well-controlled asthma, while above 1.00 they are more likely to have poorly-controlled asthma. If a person has an ACQ score of 0.75 or less, there is an 85% chance that the asthma symptoms is adequately controlled. If a person has an ACQ score of over 1.50 there is an 88% chance the asthma symptoms are inadequately controlled. Score between 0.75 and 1.49 are less discriminant, and it is therefore less certain whether the person's symptoms are adequately or inadequately controlled.

8.6.6 Spirometry

A laboratory-standard spirometer (Vitalograph® ,APHA, UK) was used to perform standard lung function tests to measure forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and peak expiratory flow (PEF). The spirometer was regularly serviced in accordance with the manufacturer's requirements.

8.6.7 Summary of recorded parameters

A summary of all the parameters that were recorded, and how they were recorded, is provided in the following table.

Parameters	Recorded by	Units
Tidal volume (Vt)	LifeShirt®	aml
Expiration time (Te)	LifeShirt®	s
Inspiration time (Ti)	LifeShirt®	s
Sigh rate (sigh defined as two times the mean tidal volume)	LifeShirt®	per minute
Respiratory rate	LifeShirt®	per minute
End tidal carbon dioxide level	Capnograph	kPa
Depression and anxiety level	HAD scale	N/A
Hyperventilation symptoms	Nijmegen questionnaire	N/A
Asthma control level	Asthma control questionnaire	N/A
Functional vital capacity (FVC)	Spirometry	Litre
Forced expiration volume in 1s	Spirometry	Litre
Forced expiration volume in 1s (% of predicted value)	Spirometry	%
Peak expiratory flow	Spirometry	Litre

Table 35: A summary of all the parameters recorded from all participants.

8.7 Experimental procedure

The healthy cohort and the severe asthma population followed the same experimental procedure. The only difference between the two group was that healthy participants were invited to take part in the study by attending either at a research laboratory at the University of Southampton or at the Gait Laboratory at Southampton General Hospital, whereas the severe asthma participants were only invited to attend the Gait Laboratory at Southampton General Hospital due to the requirement for medical support. This data collection for the study involved a single visit and lasted for approximately one and a half hours. On arrival at the site, participants were greeted by the researcher and the experimental procedure was explained. After confirming the suitability of the participant with the screening questionnaire (shown in Appendix III), written consent (Appendix IV) was then obtained. Demographic data for height, weight, chest and abdomen girdle were measured. The correct size for the garment was chosen based on the chest and abdomen girdle chart supplied by the manufacturer. After a satisfactory snug fit was achieved, the participant was fitted with the nasal cannula and was seated comfortably in a chair. Simultaneous recording of breathing pattern and $ETCO_2$ then began.

8.7.1 Rest period

During the rest period participants were asked to sit quietly for thirty-five minutes, and all participants were asked to fill in the Hospital Anxiety and Depression Scale and the Nijmegen questionnaire during the first five minutes. The severe asthma patients were asked to fill in an additional questionnaire, the Asthma Control Questionnaire. Once the questionnaires were completed the participants were asked to continue reading newspapers and magazines for the rest of the data collection period. This was to minimise the impact of the participant thinking about their breathing pattern (Boiten, 1993).

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The first five minutes of the resting period were not analysed, because this period was used as acclimatisation period and to fill in questionnaires. Therefore the

breathing pattern during this period might not be representative of the participants' natural breathing pattern. However, signals from this period were visually inspected for abnormalities. All participants were instructed to remain still and stay awake during the recording period. Involuntary events such as coughs and sneezes were recorded by the researcher.

8.7.2 Lung function test

After thirty-five minutes, recording ceased and the nasal cannula was removed. Participants were then asked to perform a lung function test using a laboratory standard spirometer (Vitalograph). The lung function test was performed after the breathing pattern recording because the forced expiration maneuver may induce bronchoconstriction, which may have an effect on the breathing pattern. Lung function tests were carried out according to the standard procedure recommended by the European Respiratory Society (Miller *et al*., 2005). The researcher demonstrated the appropriate technique for each participant before they performed the test. The mouthpiece was placed in the mouth and the lips closed around it. The participants were instructed to inhale completely with a pause of less than one second between inhalation and exhalation. Participants were prompted to 'blast' the air out and were encouraged to fully exhale using the phrase 'keep going'. The test was conducted in a sitting position while wearing a nose clip. The whole procedure was repeated three times. After three acceptable spirograms had been obtained, the values of FVC and FEV1 were checked to make sure the two largest measurements were within 0.15L of each other; this was the condition for the test to be concluded. The best measurements of each parameter were taken for analysis.

8.8 Signal processing

8.8.1 LifeShirt®

Once data collection was complete, the respiratory signals collected from the LifeShirt® were entered into MatLab for signal processing. Data were saved into a MatLab format via the associated software. The signals were then imported into MatLab and analysed using the algorithm developed for the previous validation study, with alterations to analyse only the signal from the LifeShirt®. The algorithm detects the first expiration cycle and all the subsequent peaks and troughs of the respiratory signal.

The same calibration method for the LifeShirt® as that used in the validation study was implemented in this part of the study. Once the calibration was complete, the respiratory parameters of relative expiration tidal volume (aml), inspiration time (s) and expiration time (s) were exported in Excel format. Plots that show the data points for each respiratory cycle were available for visual inspection (see Figure 31 below). All data point plots were visually inspected to check for errors and anomalies.

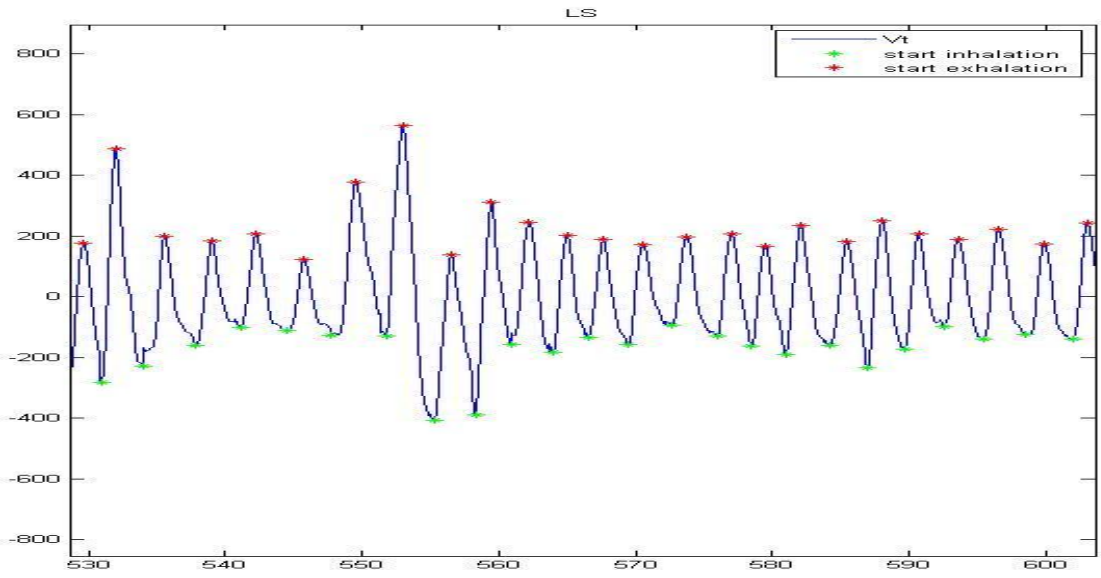

Figure 31: Algorithm picks up the peak and the trough in the signal.

8.8.2 Data cleaning

During the visual inspection it was found that the algorithm was picking up cycles that were of very small size, e.g. 20aml (see Figure 32 below). It was unlikely that a genuine breath cycle would be less than 100ml in volume due to physiological dead space. Physiological dead space is the gas in the conducting areas of the respiratory system such as the mouth and trachea. It is acknowledged that 100aml measured by the LifeShirt® does not equate to 100ml volume. To confirm whether breaths of such a small size are unlikely to be a genuine breath, the data obtained from the pneumotachograph from the previous study was checked. The breath by breath cycle from the whole data set recorded by both the LifeShirt® and the pneumotachograph in the previous study were inspected. Breath cycles from the LifeShirt® of less than 100aml were identified from all the signals recorded previously. The identified cycles were then cross-checked with the pneumotachograph signals to see if these breath cycles were also recorded by the pneumotachograph. It was found that 0.3% of the breaths of less than 100aml detected by LifeShirt® were also detected by the pneumotachograph. Based on this analysis, all the respiratory cycles recorded by the LifeShirt® with an expiration tidal volume of less than 100aml were manually removed from the data set and were not included in the final analysis.

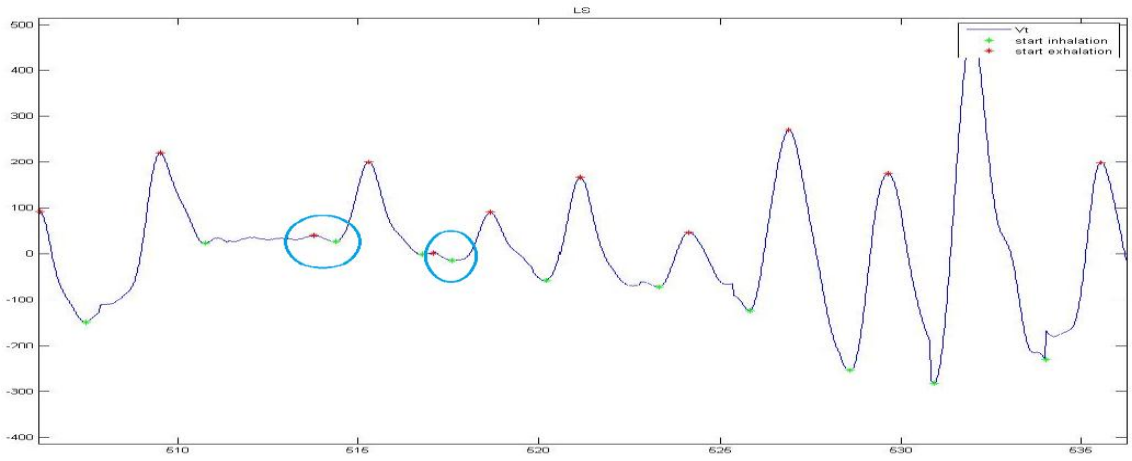

Figure 32: Small breath size picked up by the algorithm.

8.8.3 Capnograph

The data from the capnograph was analysed using the manufacturer's software (Profox version 2.0). This was due to Profox not supporting raw data output in any other format. This has limited the scope of the comparison of $ETCO_2$ level and breath by breath tidal volume data. The capnograph measured $ETCO_2$ level by infrared over a four-second epoch. The same type of device has been used in published studies that investigate changes in $ETCO_2$ level (Bruton, 2007; Bishop *et al*., 2007).

8.9 Statistical analysis

The following section discusses the statistical tests used to test the hypotheses described in a previous section (see Section 8.1).

8.9.1 Data normality

The data for the respiratory parameters of tidal volume, inspiration time, expiration time and end tidal carbon dioxide level were checked for normality using visual estimation of histograms and the Kolmogorov-Smirnov test. This determined whether parametric or non-parametric tests should be used.

8.9.2 Analysis of demographic data

Data for birth, gender, height and weight were entered into SPSS. Body mass index was also calculated from the height and weight. Descriptive statistics were used to describe the participants and the sample characteristics.

8.9.3 Analysis of differences in recorded parameters between the healthy and severe asthma populations

Hypotheses H1a to H1j concern the between-cohort mean differences in the respiratory parameters of tidal volume, inspiration time, expiration time, $ETCO_2$ level, expiration time variability, tidal volume variability, $ETCO_2$ level variability, NQ score and HADS score. The differences in the means between the healthy cohort and the severe asthma cohort were analysed using one-way ANOVA tests. This is a statistical technique that is frequently used to test equality between two or more means by comparing the variance among groups relative to the variance within groups (Larson, 2008). The null hypothesis (H0) is that the means are all equal, and the alternative hypothesis (H1) is that the means are different. The statistical significance level in this study was set at 95%. If the statistical significance level is above 95%, then the null hypothesis is accepted. If the statistical significance level is level is below 95%, then the alternative hypothesis is accepted. The ANOVA test relies on the assumptions that individual observations are mutually independent; and the data are normally distributed.

8.9.4 Analysis of questionnaire scores between healthy and severe asthma population

Although the HADS and NQ questionnaires were originally designed as dichotomous scales to give positive and negative answers, it is common in published studies for both scales to be treated as continuous variables (Herrmann, 1997; van den Bergh *et al*., 1995; Humphriss *et al*., 2004). Studies have seldom documented the reason for treating the scales as continuous variables. To date, there is little published evidence that states that the two scales should not be treated as continuous variables. This study considers HADS and NQ to be continuous variables, because it is the frequency of symptoms that is of interest in this study, rather than their use as a diagnostic tool to determine if the group has negative or positive symptoms. For the purpose of this study, both the HADS and NQ were treated as continuous variables.

8.9.5 Analysis of breath by breath variability

Breath by breath variability of tidal volume and expiration time was assessed by calculating coefficients of variation. The coefficient of variation is independent of

the units of measurement and of the magnitude of the data. It is useful for comparing the variability of two or more samples of data from different variables, or from the same variables when the means might be different (Lovie, 2005). .

8.9.6 Correlation analysis

Hypotheses H2a to H2f concern the linear association between the recorded parameters. The correlation between parameters was analysed using Pearson's correlation coefficient. A detail description of correlation coefficients can be found in Section 2.3.
The questionnaire scores were again treated as continuous in order to test the linear correlation between symptoms and physiological parameters.

8.10 Summary

This chapter described the methodology employed to test the research aims. It has also described the statistical tests that were used to test the research hypotheses. The next chapter presents the results.

Chapter 9: Results of breathing pattern analysis

9.0 Introduction

This chapter presents the results obtained from the analysis of the data collected in this study. Results are presented in the following order:

9.1 Recruitment

The data collection period for this study lasted for approximately four months due to a drying up of the sample pool and a lack of further suitable participants. Overall, 273 asthma patients of all severities were on the research volunteer list. Suitable patients were identified by the respiratory nurses who were responsible for the recruitment of the asthma cohort. Twenty-two patients were initially identified as suitable participants since they met the inclusion criteria. All patients who met the inclusion criteria were invited via telephone to take part in this current study. Twelve patients agreed to take part, of whom two were later found to be unsuitable due to the discovery of a potentially serious pathology. Ten participants were therefore recruited in the severe asthma population. A further ten participants were recruited as healthy controls from the university student population. The following tables present the demographic and anthropometric data from the healthy cohort and the severe asthma cohort.

9.2 Demographic data

Participant	Gender	Age	Height	Weight	BMI
1	f	28	155	64	26.7
2	f	20	164	49	18.1
3	m	29	166	90	32.8
4	f	32	170	99	34.4
5	f	30	171	60	20.7
6	m	33	169	59	20.8
7	f	28	167	60	21.5
8	f	36	159	54	21.4
9	f	27	164	49	18.2
10	f	38	164	67	24.9
Mean	(8F, 2M)	30	164.9	65.1	24.0
Stdv		(5)	(5.0)	(16.7)	(5.7)

Table 36: Demographic data for the healthy population

Participant	Gender	Age	Height (cm)	Weight (kg)	BMI
1	f	52	166	95	34.5
2	m	32	177	74	23.6
3	m	57	181	78	24.5
4	f	57	154	64	27.0
5	f	26	175	79	25.8
6	f	64	160	102	39.9
7	m	53	178	91	28.7
8	m	66	178	72	22.7
9	m	56	162	84	32.5
10	m	42	176	92	29.8
Mean	(4F, 6M)	50	170.7	83.1	28.85
(stdv)		(13)	(9.4)	(11.8)	(5.4)

Table 37: Demographic data for the severe asthma population.

As shown in Tables 36 and 37, the healthy cohort has eight females and two males aged between 20 and 28 years old. The severe asthma cohort has four females and six males aged between 26 and 56 years old. On average, the BMI of the healthy cohort was normal, whereas the severe asthma cohort was overweight.

An ANOVA test confirmed that the age group of the healthy population was statistically significantly lower than the severe asthma cohort ($p = 0.00$). No statistical significance was found between the BMI of the healthy and severe asthma cohorts ($p=0.07$).

9.3 Lung function fest

The following tables present the data from lung function tests of the healthy cohort and the severe asthma cohort. This data was recorded at the end of the recording period.

Participant	FVC (l)	FEV1 (l)	FEV1 (% Predicted Value)	PEF (l)
1	3.75	3.34	117	372
2	2.95	2.64	93	414
3	4.01	3.39	104	642
4	4.01	3.39	101	492
5	3.62	3.23	94	462
6	3.68	2.86	87	522
7	4.06	3.64	109	414
8	2.62	2.61	93	408
9	3.22	3.11	96	450
10	2.56	2.19	87	318
Mean	3.45	3.04	98	449
(Stdv)	(0.57)	(0.45)	(10)	(89)

Table 38: Lung function data from the healthy population.

The above table shows that all participants in the healthy population have normal lung function, and have greater than 80% of the predicted FEV1 value.

Participant	FVC (l)	FEV1(l)	FEV1 (% Predicted Value)	PEF (l)
1	4.15	1.96	60	312
2	4.73	3.1	73	438
3	3.92	1.96	53	342
4	1.78	1.01	49	204
5	2.41	2.27	61	330
6	2.16	1.49	69	252
7	4.54	2.89	80	564
8	4.15	1.96	60	312
9	3.10	2.03	57	498
10	4.44	3.31	85	462
Mean	3.54	2.20	65	371
(Stdv)	(1.08)	(0.72)	(12)	(114)

Table 39: Lung function data for the severe asthma population.

The above table shows that eight participants from the severe asthma population had airflow restriction with FEV1 less than 80% of predicted. An ANOVA test showed that the FEV1 (% Predicted Value) for the severe asthma population was statistically significantly lower than the healthy cohort (p=0.00). This suggests the presence of airway obstructions in the severe asthma population.

9.4 Questionnaire Results

The follow tables present the results of the questionnaires: the Nijmegen questionnaire (NQ), the Hospital and Anxiety Depression Scale (HADS) and the Asthma Control Questionnaire (ACQ; severe asthma population only).

Participant	NQ	HADS-A	HADS-D
1	10	10	1
2	10	7	4
3	4	4	3
4	4	5	2
5	4	1	0
6	2	4	1
7	20	9	6
8	5	1	0
9	15	12	2
10	6	8	2
Mean	8	6	2
(Stdv)	(6)	(5)	(2)

Table 40: Questionnaire scores for the healthy population.

Key: NQ – Nijmegen Questionnaire, HADS-A – Anxiety level of Hospital Anxiety and Depression Scale, HADS-D – Depression level of Hospital Anxiety and Depression Scale.

The above table shows that no participant in the healthy population reported symptoms of hyperventilation. On average, the healthy participants were negative for depression and anxiety. However, within the healthy population, three participants were positive for anxiety (HAD-D > 8). All participants had normal levels of depression.

Participant	NQ	HADS-A	HADS-D	ACQ
1	24	11	5	3.14
2	17	6	3	1.71
3	40	11	6	4.57
4	18	0	1	2.71
5	21	6	2	2.57
6	32	6	8	4.00
7	26	8	5	3.43
8	20	4	2	2.43
9	14	0	1	1.86
10	23	5	2	1.43
Mean	24	6	4	2.79
(Stdv)	(8)	(4)	(2)	(1.01)

Table 41: Questionnaire scores for the severe asthma population.

Key: NQ – Nijemegan Questionnaire, HADS-A – Anxiety level of Hospital Anxiety and Depression Scale, HADS-D – Depression level of Hospital Anxiety and Depression Scale, ACQ – Asthma Control Questionnaire.

The above table shows that on average the severe asthma population had symptoms of hyperventilation (NQ = 23 or above). Five participants within the severe asthma population had symptoms of hyperventilation. On average, the severe asthma population was negative for anxiety and depression. Three participants in the severe asthma population had abnormal levels of anxiety (score > 8), and one participant in the severe asthma population had an abnormal level of depression (score > 8). All the severe asthma participants (except participant 10) had ACQ scores higher than 1.50, which suggests that there is an 88% chance that those nine participants had inadequately controlled asthma symptoms. The ACQ score from participant 10 is above 1 and therefore he can be considered to be very likely to have inadequately controlled asthma symptoms.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1201.25	1	1201.25	26.04	0.00
Within Groups	830.50	18	46.14		
Total	2031.75	19			

Table 42: One-Way ANOVA test of NQ score between the healthy and severe asthma cohorts.

The ANOVA test shows the NQ score to be statically significantly higher in the severe asthma population than in the healthy population ($p<0.05$).

		Sum of Squares	df	Mean Square	F	Sig.
HAD-A	Between Groups	0.80	1	0.80	0.06	0.82
	Within Groups	255.00	18	14.17		
	Total	255.80	19			
HAD-D	Between Groups	9.80	1	9.80	2.17	0.16
	Within Groups	81.40	18	4.52		
	Total	91.20	19			

Table 43: One-Way ANOVA test of HAD-A and HAD-D score between the healthy and severe asthma cohorts.

No statistically significant differences were found in anxiety and depression scores between the severe asthma and healthy cohorts.

9.5 Respiratory parameters

The following tables present the individual means for each respiratory parameter recorded from the healthy and severe asthma cohorts during rest.

Participant	Vt (aml)	$ETCO_2$ (kpa)	Ti (s)	Te (s)	Breathing rate (per min)	Sigh rate (per min)
1	962.82	4.50	2.32	2.56	10	0.60
	(673.27)	(0.34)	(1.42)	(1.47)		
2	631.30	5.00	1.37	2.59	15	0.70
	(328.07)	(0.29)	(0.74)	(0.89)		
3	764.26	5.44	2.15	4.07	10	0.73
	(537.61)	(0.33)	(1.28)	(1.95)		
4	507.35	5.04	1.97	3.03	12	0.83
	(341.10)	(0.18)	(0.71)	(0.88)		
5	408.48	5.30	1.57	2.25	16	0.43
	(208.58)	(0.13)	(0.39)	(0.43)		
6	716.20	5.10	1.38	2.36	16	0.43
	(369.97)	(0.21)	(0.42)	(0.74)		
7	529.57	4.95	1.47	2.15	13	1.33
	(515.48)	(0.28)	(1.17)	(1.20)		
8	657.63	4.99	1.72	2.35	16	1.27
	(503.13)	(0.33)	(1.03)	(1.04)		
9	337.24	5.09	1.06	2.00	21	0.43
	(151.86)	(0.21)	(0.55)	(0.55)		
10	909.69	4.99	1.96	3.15	11	0.8
	(750.76)	(0.32)	(1.24)	(1.81)		
Mean	642.46	5.03	1.70	2.63	14	0.76
(Stdv)	(203.74)	(0.25)	(0.38)	(0.66)	(3)	(0.32)

Table 44: Breathing pattern data for the healthy population.

Key: aml – assumed millilitre, ETCO2 – end-tidal carbon dioxide, Ti – inspiration time, Te – expiration time

Nine participants in the healthy cohort recorded normal levels of $ETCO_{2,}$ with participant 1 below the normal threshold level of $ETCO_2$ ($ETCO_2$ < 4.66kPa). There is a considerable amount of between-subject variation in all respiratory parameters, as might be expected due to the individuality that is known to exist in respiratory parameters.

Participant	Vt (aml)	$ETCO_2$ (kpa)	Ti (s)	Te (s)	Breathing rate (per min)	Sigh rate (per min)
1	503.14	4.53	2.06	2.83	12	1.13
	(427.96)	(0.45)	(1.4)	(1.59)		
2	758.99	5.27	1.62	2.9	13	0.50
	(400.94)	(0.42)	(0.94)	(1.28)		
3	837.14	3.64	1.92	2.08	15	0.06
	(280.68)	(0.27)	(0.34)	(0.47)		
4	304.37	4.97	1.42	1.94	19	0.90
	(144.46)	(0.27)	(0.45)	(0.66)		
5	549.93	5.25	1.68	1.91	15	1.07
	(332.31)	(0.21)	(1.13)	(0.94)		
6	288.45	4.96	1.29	2.2	18	1.07
	(263.22)	(0.56)	(0.68)	(0.71)		
7	474.27	4.28	1.75	2.37	13	0.93
	(417.37)	(0.31)	(1.47)	(1.57)		
8	553.76	4.65	1.37	2.27	16	0.93
	(437.59)	(0.48)	(0.80)	(0.99)		
9	403.45	5.40	1.19	1.66	21	0.20
	(136.61)	(026)	(0.38)	(0.46)		
10	428.22	4.80	1.36	1.79	19	0.97
	(284.61)	(0.04)	(0.75)	(0.59		
Mean	500.74	4.79	1.60	2.19	16	0..77
(Stdv)	(161.45)	(0.53)	(0.33)	(0.62)	(3)	(0.38)

Table 45: Breathing pattern data for the asthma population.
Key: aml – assumed millilitre**,** $ETCO_2$ – end-tidal carbon dioxide, Ti – inspiration time, Te – expiration time

Four participants in the severe asthma population recorded readings below the normal threshold level of $ETCO_2$. As in the healthy population, there is a considerable amount of within-subject difference in all recorded respiratory parameters.

Both the healthy and the severe asthma cohorts yielded mean inspiration time, expiration time and breathing rate that fell within the normal levels previously presented (see Table 1).

The following table presents the results of one-way ANOVA to test if there was a statistically significant difference in the mean of respiratory parameters between the healthy and severe asthma cohorts.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
VT	Between Groups	121882.04	1	121882.04	3.08	0.10
	Within Groups	712300.60	18	39572.26		
	Total	834182.63	19			
Ti	Between Groups	0.02	1	0.02	0.14	0.71
	Within Groups	2.73	18	0.15		
	Total	2.75	19			
Te	Between Groups	0.64	1	0.64	2.17	0.16
	Within Groups	5.29	18	0.29		
	Total	5.93	19			
Respiratory Rate	Between Groups	24.20	1	24.20	2.63	0.12
	Within Groups	165.60	18	9.20		
	Total	189.80	19			
Sigh Rate	Between Groups	0.01	1	0.01	0.07	0.79
	Within Groups	2.37	18	0.13		
	Total	2.38	19			
$ETCO_2$	Between Groups	0.30	1	0.30	1.74	0.20
	Within Groups	3.05	18	0.17		
	Total	3.35	19			

Table 46: One-way ANOVA test for respiratory parameters between the healthy and severe asthma cohorts.

The one-way ANOVA test demonstrates that there is no statistically significant difference in the recorded respiratory parameters between the healthy and severe asthma cohorts. The following table presents a summary of the breathing pattern data and questionnaire scores between the healthy and severe asthma populations.

	Healthy		Severe Asthma		*Mean diff*
	Mean	Stdv	Mean	Stdv	
Tidal Volume (aml)	624.46	203.74	500.74	161.45	123.72
$ETCO_2$	5.03	0.25	4.79	0.53	0.24
Sigh rate (per minute)	0.76	0.32	0.77	0.38	0.01
Breathing rate (per minute)	14	3	16	3	2
Ti (s)	1.70	0.38	1.60	0.33	0.1
Te (s)	2.63	0.66	2.19	0.62	0.44
HADSA	6	2	6	4	0
HADSD	5	2	4	2	1
NQ	8.0*	5.8	24.0*	8.0	16
ACQ	-	-	2.4	1.04	-

Table 47: Group mean data for breathing pattern and questionnaire scores.
(statistically significant, $p < 0.05$)*

The above table shows that the group mean of all the parameters and the between-group mean differences are small in all variables except in NQ score. The ANOVA test shows no statistical significance in any parameters between cohorts except for the NQ score. Both the healthy population and the severe asthma population have 'normal' group mean levels of $ETCO_2$ (normal range: 4.66 – 5.99 kPa) and 'normal' anxiety and depression levels (normal range: 0 – 8).

The analyses suggest that severe asthma cohort has a similar breathing pattern in comparison with the healthy population.

9.6 Analysis of variability

The following table presents the data for tidal volume variability for all participants in the healthy and severe asthma populations.

Healthy				Severe Asthma			
Participant	Vt CV(%)	Te CV (%)	$ETCO_2$ CV (%)	Participant	Vt CV (%)	Te CV (%)	$ETCO_2$ CV (%)
1	70	57	8	1	84	56	8
2	52	34	6	2	53	44	8
3	70	47	6	3	34	22	7
4	67	29	4	4	52	34	5
5	51	19	2	5	60	49	4
6	52	31	4	6	89	32	6
7	90	55	6	7	86	66	7
8	76	44	7	8	79	43	10
9	45	27	4	9	34	28	5
10	82	57	9	10	66	33	4
Mean (stdv)	65.5 (14.99)	40.0 (13.81)	5.6 (2.11)	Mean (stdv)	63.7 (20.60)	40.7 (13.54)	6.4 (1.95)

Table 48: Variability of tidal volume, expiration time and $ETCO_2$ levels for healthy and severe asthma participants

The results show that there is a considerable amount of between-subject variation in tidal volume variability in the healthy cohort (range from 45% to 90%) and also in the severe asthma population (range from 34% to 84%), as might be expected due to the amount of variability observed in the first study. The group mean tidal volume variability is similar between the severe asthma population and the healthy population (difference of 1.8%). The variability of expiration time and $ETCO_2$ level are again similar (differences of 0.7% and 0.8% respectively) between the severe asthma and healthy cohorts.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Vt CV	Between Groups	11.25	1	11.25	0.04	0.85
	Within Groups	5776.50	18	320.91		
	Total	5787.75	19			
Te CV	Between Groups	2.45	1	2.45	0.01	0.91
	Within Groups	3366.10	18	187.01		
	Total	3368.55	19			
$ETCO_2$ CV	Between Groups	6.79	1	6.79	1.62	0.22
	Within Groups	75.21	18	4.18		
	Total	81.99	19			

Table 49: One-Way ANOVA test for respiratory parameters between the healthy and severe asthma cohorts.

The ANOVA test shows no statistical significance between the severe asthma and healthy cohorts in terms of mean tidal volume variability, expiration time variability and $ETCO_2$ variability.

9.7 Non-parametric analysis

While a one-way ANOVA is sufficiently robust to withstand violation of the assumption of normality of the data (Norman & Strenier, 2008), nonetheless skewing of the data may affect the analysis especially if the sample size is small. Non-parametric analyses were performed to compare the results obtained from the parametric tests. This could prevent false conclusions being drawn due to inappropriate use of statistical tests.

The Mann-Whitney U-test is a nonparametric alternative designed to test the null hypothesis in two independent samples (Portney & Watkins, 2000).

The results from nonparametric analyses were the same as the results obtained from the parametric analyses. The severe asthma group again demonstrated a lower median tidal volume, inspiration time, expiration time and $ETCO_2$ levels. A Mann-Whitney U-test revealed no statistically significant difference between the severe asthma cohort and the healthy cohort in all respiratory parameters. No statistically significant differences were found in tidal volume variability, expiration time variability, and end tidal carbon dioxide level variability between the two groups.

The NQ score was found to be statistically significantly higher in the severe asthma cohort than the healthy cohort. The detailed results for the non-parametric analysis and the Mann-Whitney U-test can be found in Appendix XV.

9.8 Correlation coefficient between parameters in the healthy and severe asthma populations

The following table presents the results of the correlation analysis between questionnaire scores and respiratory parameters.

	Group	
Pair	**Healthy**	**Severe asthma**
ACQ vs. HADSA	-	0.60
ACQ vs. HADSD	-	0.69*
ACQ vs. NQ	-	0.77**
NQ vs. HADS-D	0.49	0.84**
NQ vs. HADS-A	0.78**	0.75*

Table 50: Spearman's rank correlation coefficient between questionnaire scores.
($p < 0.05$, ** $p < 0.01$)*

This correlation analysis shows a strong correlation between NQ score and ACQ score, and between NQ score and HADS scores in the severe asthma population. A moderate correlation is observed between ACQ score and HADS scores. A strong correlation is observed between the NQ score and HADS-A in the healthy population.

	Group	
Pair	**Healthy**	**Severe asthma**
NQ vs. $ETCO_2$	-0.34	-0.81**
NQ vs. VtCV	0.24	0.48
NQ vs. sigh	-0.15	0.33
Sigh vs. Vt CV	0.82**	0.83**
Sigh vs. $ETCO_2$	0.22	-0.17
Vt CV vs. $ETCO_2$	-0.04	-0.36
Vt CV vs $ETCO_2$ CV	0.67*	0.37

Table 51: Pearson's correlation coefficient between respiratory parameters and NQ scores.

The correlation analysis shows a significant strong correlation between NQ score and end tidal carbon dioxide level in the severe asthma population. A moderate negative correlation was observed between NQ score and end tidal carbon dioxide level in the healthy population.

A weak positive correlation between NQ score and tidal volume variability was observed in the healthy population, and a moderate positive correlation was observed in the severe asthma population.

A weak negative correlation was observed between tidal volume variability and end tidal carbon dioxide level in the healthy population and in the severe asthma population.

9.9 Individual breathing pattern

The data analyses so far have shown no statistically significant differences between the asthma cohort and the healthy population in any parameters. It would appear to suggest that there might not be any group differences between healthy and severe asthma patients in this sample population. It is possible that difference between individual may be very small, and that the small sample size did not have sufficient power to detect the small differences in this current study. Also, because the mean values were analysed, it is possible that subtle differences are masked in the averaging process. Therefore, an individual analysis of each participant was subsequently carried out. The following section presents the individual patterns of tidal volume and tidal volume variability.

To further explore the pattern of tidal volume in severe asthma subjects and healthy controls, tidal volume pattern analyses were carried out for each individual. This is intended to visually identify tidal volume patterns in order to target more specific statistical tests to investigate relationships with other parameters, if any exist. Tidal volume was chosen to analyse further because previous studies demonstrated differences in tidal volume variability in individuals with restricted lung disease (Brack *et al*., 2002). It has also been shown that there is increased tidal volume complexity (measured by approximate entropy) within the asthma population (Veiga *et al*., 2010).

This section presents the results of this analysis. Six graphs for selected individuals are presented, showing:

1) time series of tidal volume;
2) breath by breath difference away from the mean tidal volume. This is to show the distribution of breaths that are higher or lower than the mean tidal volume, as well as sighs. The dotted line in each graph represents the volume of a sigh (defined by two times the mean tidal volume)
3) frequency distribution of breath by breath tidal volume. This is to see if there is difference in the distribution of breath by breath tidal volume between individuals

4) histogram for tidal volume distribution
5) minute average of tidal volume variability
6) minute average of tidal volume.

The graphs presented below are a selection from the whole sample population, chosen to illustrate differences in breathing pattern between individuals. All the individual graphs are presented in Appendix XIV.

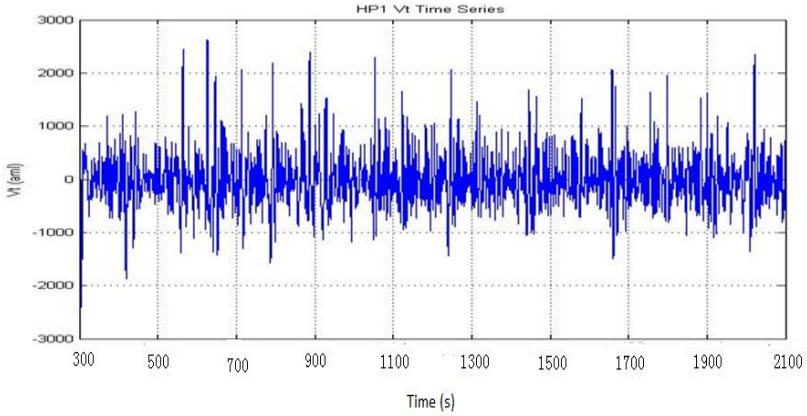

Figure 33: Time series of tidal volume for healthy participant 1.

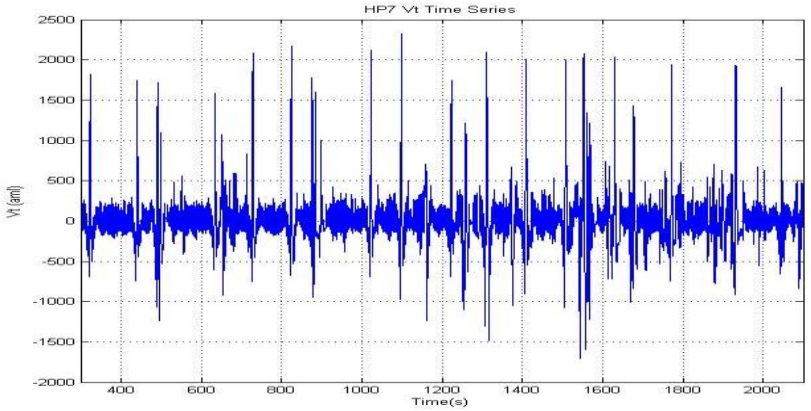

Figure 34: Time series of tidal volume for healthy participant 7.

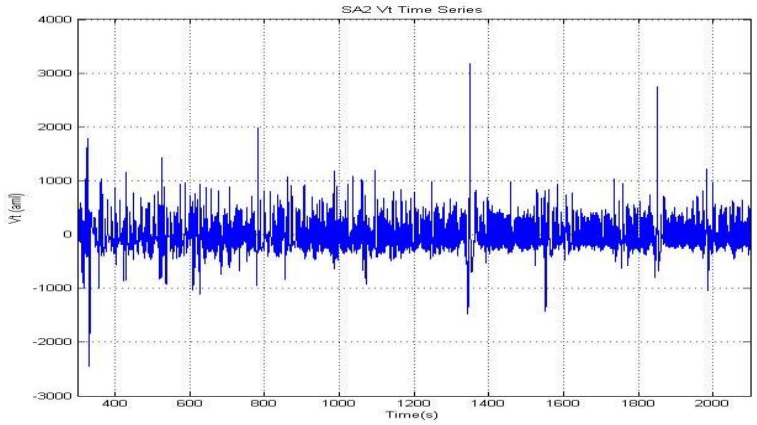

Figure 35: Time series of tidal volume for severe asthma participant 2.

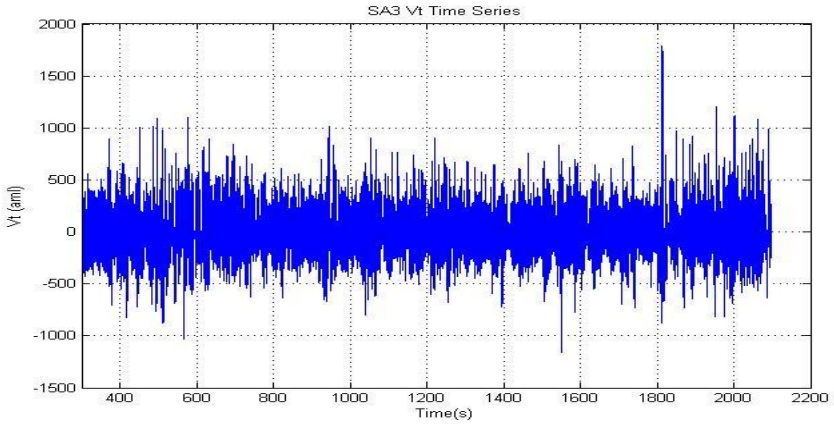

Figure 36: Time series of tidal volume for severe asthma participant 3.

The four figures (Figure 33 to 36) on the previous page (show the time series from two healthy participants and two severe asthma participants. All four participants demonstrated a regular pattern of tidal volume. Within the healthy cohort, seven participants demonstrated this type of regular pattern. Seven participants in the severe asthma cohort also demonstrated this type of regular pattern.

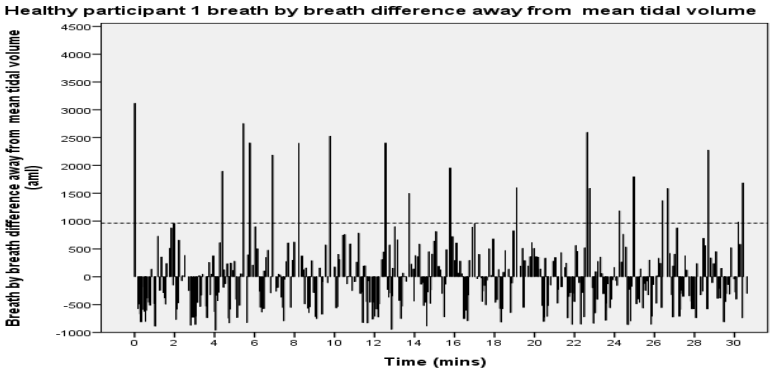

Figure 37: Breath by breath difference away from mean graph for healthy participant 1.

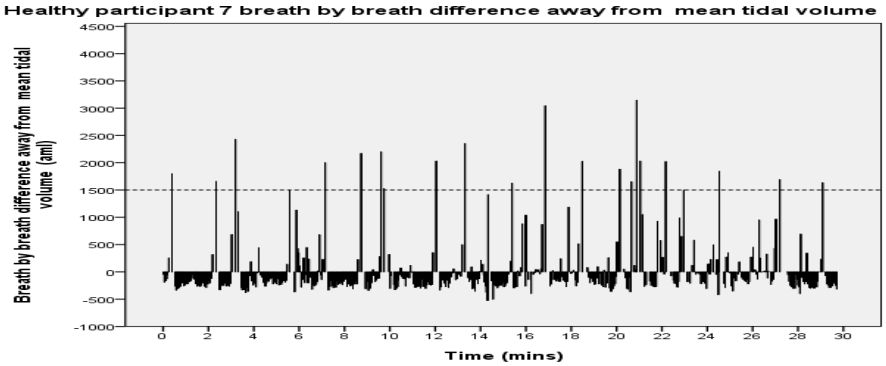

Figure 38: Breath by breath difference away from mean graph for healthy participant 7.

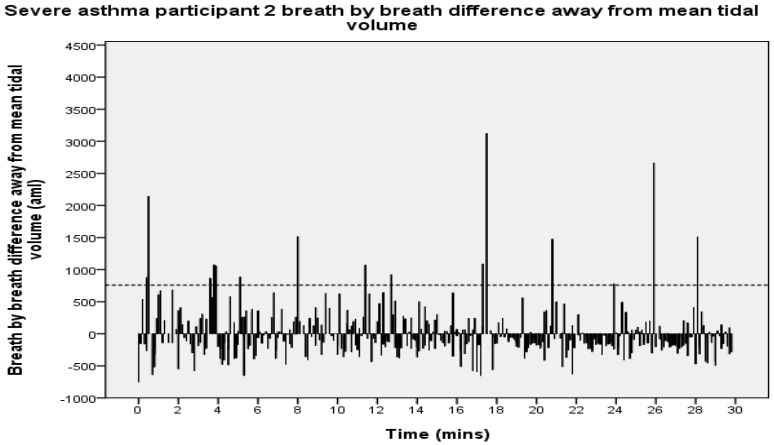

Figure 39: Breath by breath difference away from mean graph for severe asthma participant 2.

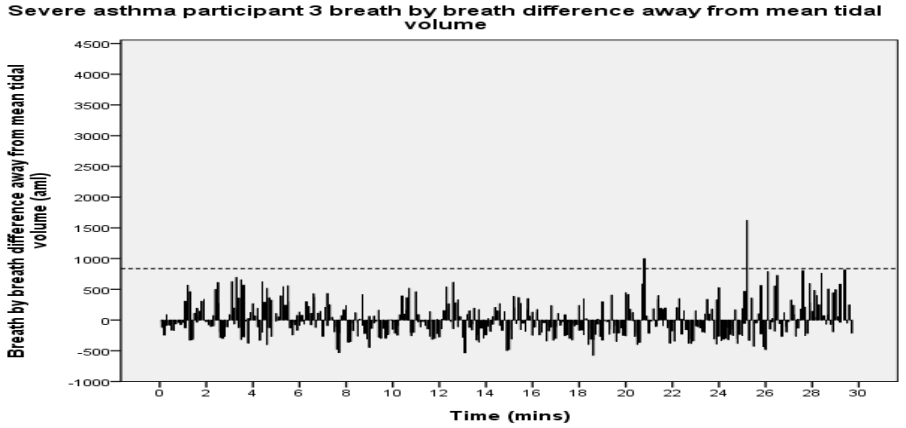

Figure 40: Breath by breath difference away from mean graph for severe asthma participant 3.

The previous four figures (Figures 37 to 40) show the breath by breath difference away from the mean for tidal volume show a regular pattern of distribution of sighs. Breaths of higher than the mean tidal volume and lower than the mean tidal volume are evenly distributed throughout the recording period in healthy participant 1 and severe asthma participants 2 and 3. In healthy participant 7, breaths were predominantly below the mean tidal volume during the first twenty minutes of recording. All four of these participants had different tidal volume variability despite having similar breath by breath patterns of tidal volume and distance away from the mean. Healthy participant 1 had a tidal volume variability of 70% and healthy participant 7 had a tidal volume variability of 90%. Both the severe asthma participants, participants 2 and 3, had lower tidal volume variability, calculated at 53% and 34% respectively.

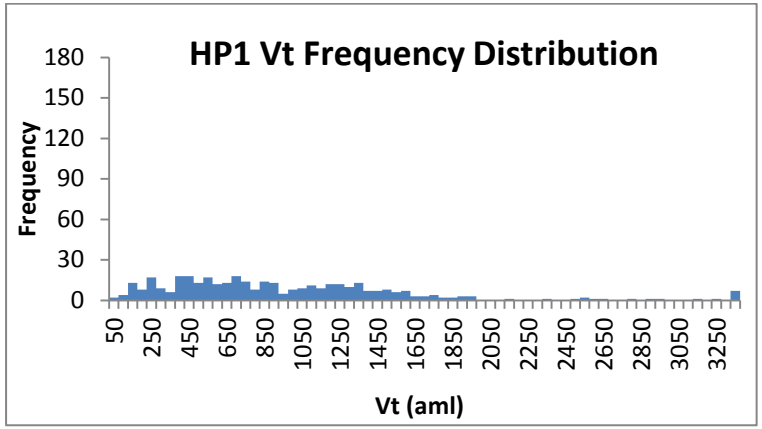

Figure 41: Frequency distribution of tidal volume for healthy participant 1.

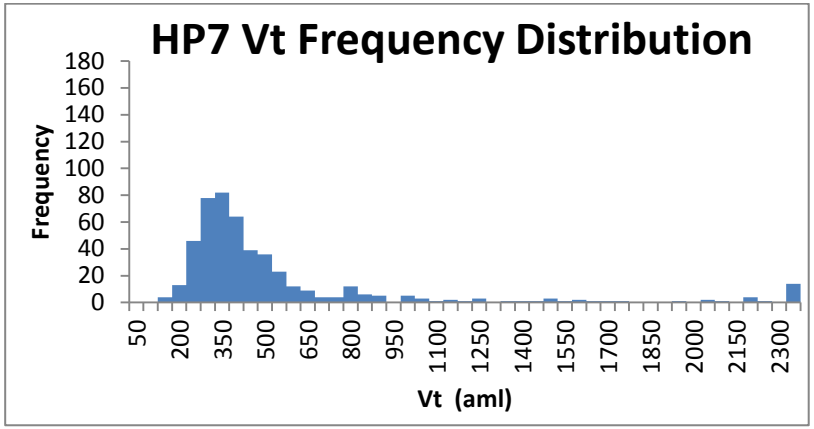

Figure 42 Frequency distribution of tidal volume for healthy participant 7.

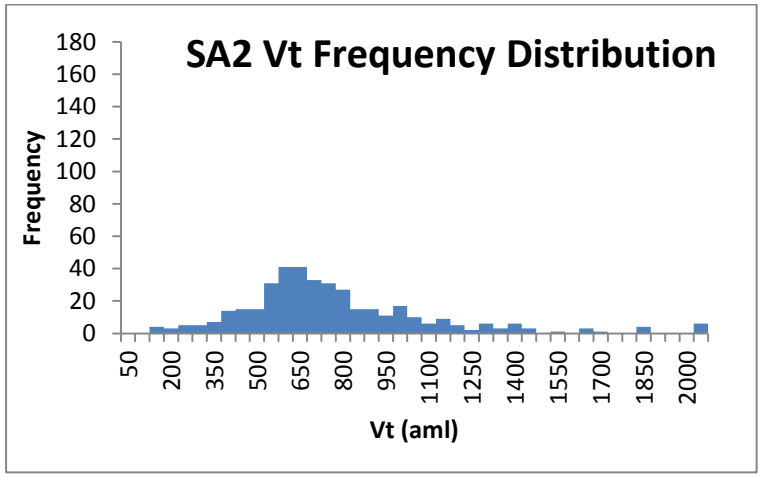

Figure 43: Frequency distribution of tidal volume for severe asthma participant 2.

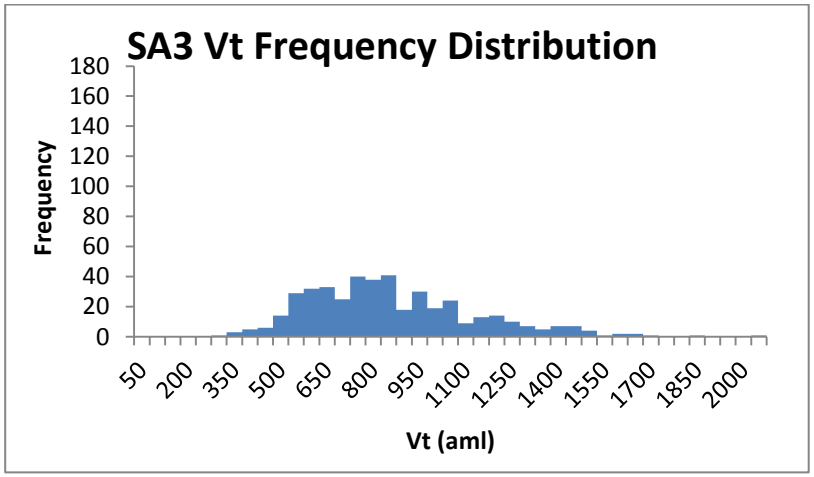

Figure 44: Frequency distribution of tidal volume for severe asthma participant 3.

The frequency distribution graphs for tidal volume (Figures 41 to 44) show that each participant had a differently shaped frequency distribution. Healthy participant 1 and severe asthma participants 2 and 3 all had a flat peak with kurtosis of -0.76, 1.43 and 0.06. Healthy participant 7 had the highest kurtosis of 6, which suggests a sharp peak.

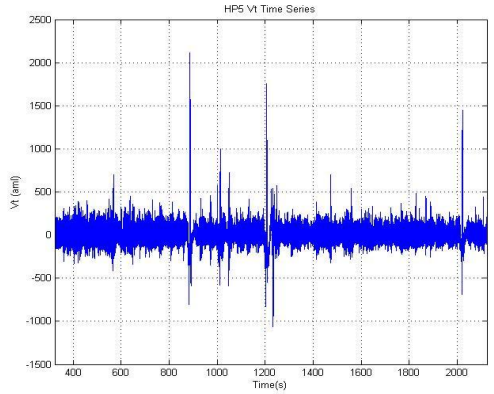

Figure 45: Time series of tidal volume for healthy participant 5.

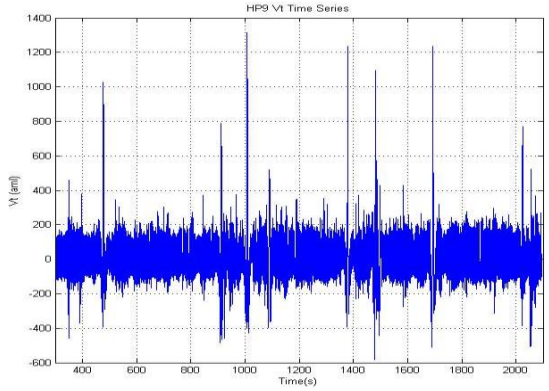

Figure 46: Time series of tidal volume for healthy participant 9.

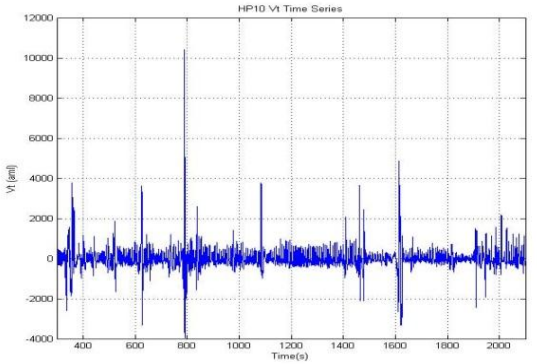

Figure 47: Time series of tidal volume for healthy participant 10.

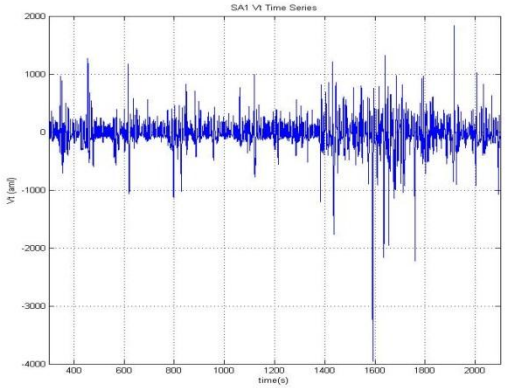

Figure 48: Time series of tidal volume for severe asthma participant 1

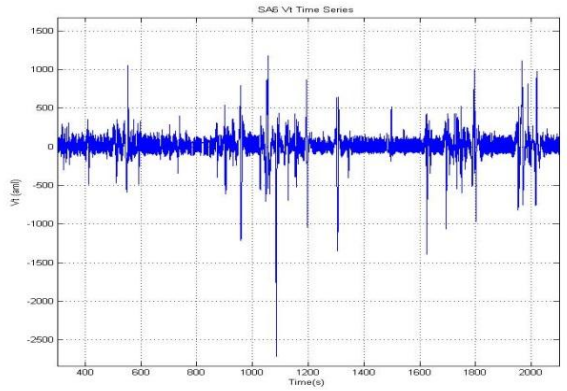

Figure 49: Time series of tidal volume for severe asthma participant 6.

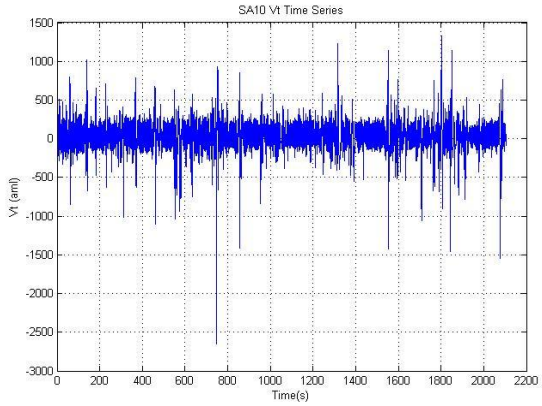

Figure 50: Time series of tidal volume for healthy participant 10.

The graphs above (Figures 45 to 50) showing the time series from the six participants reveal a different pattern from the previous four participants. All six participants (three from the healthy cohort and three from the severe asthma cohort) demonstrate a form of episodic breathing with periods of breathing that contain a large number of high volume breaths, and periods of breathing at near-constant volume.

The next six graphs show the breath by breath difference away from the mean tidal volume (Figures 51 to 56). They demonstrate a form of episodic breathing pattern. In these participants, their breathing pattern contains periods of sighs and breaths that were larger than the mean tidal volume in close succession. These periods are followed by periods of breaths that are predominantly below mean tidal volume. For example, healthy participant 1 had 23% of the total number of their sighs in the first ten minutes of recording, 54% of the total number of sighs recorded between minutes 10 to 16, with the final 23% recorded during the final fourteen minutes. In the final fourteen minutes of recording, the breath sizes were predominantly below the mean tidal volume. In severe asthma participant 1, the first eighteen minutes of the recording contained 32% of the total number of sighs, and breath sizes were predominantly below the mean tidal volume. In the final twelve minutes, 68% of the total number of sighs occurred in close succession between minutes 18 and 30 of the recording, with breath sizes predominantly above the mean tidal volume during this period. A similar trend is also observed in the other healthy participants (9 and 10) and severe asthma participants (6 and 10).

Again, all six selected participants had different tidal volume variability despite having no distinguishable pattern in tidal volume. Healthy participant 5 had a tidal volume variability of 51%, healthy participant 9 had a tidal volume variability of 45%, and the figure was 82% for healthy participant 10. Severe asthma participants 1, 6 and 10 had tidal volume variability of 84%, 89% and 66% respectively.

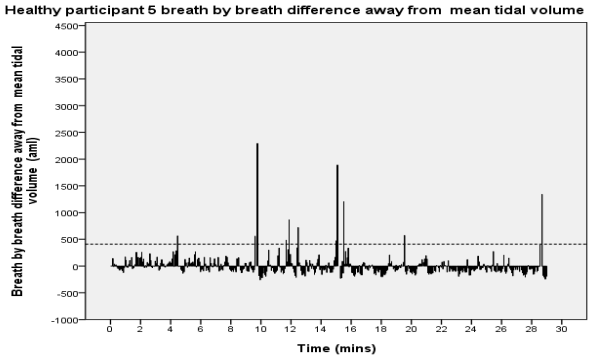

Figure 51: Breath by breath difference away from mean graph for healthy participant 5.

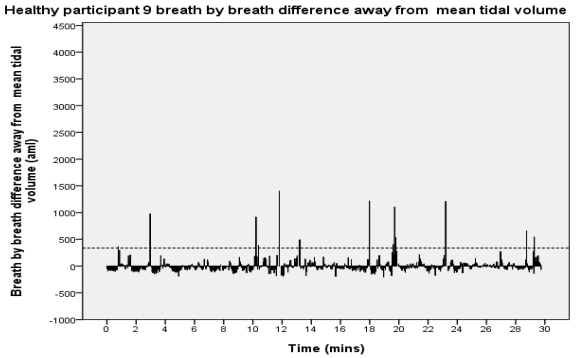

Figure 52: Breath by breath difference away from mean graph for healthy participant 9.

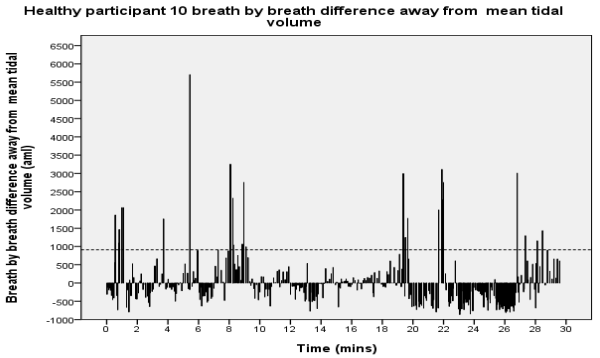

Figure 53: Breath by breath difference away from mean graph for healthy participant 10.

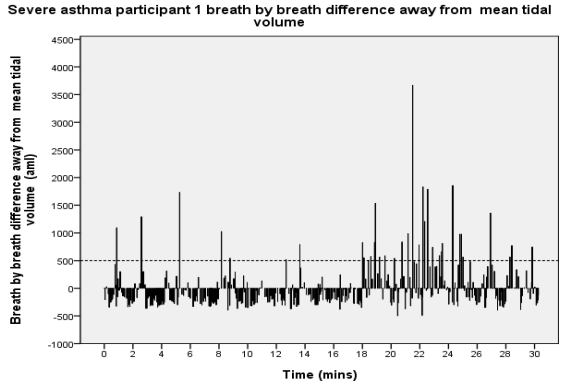

Figure 54: Breath by breath difference away from mean graph for severe asthma participant 1.

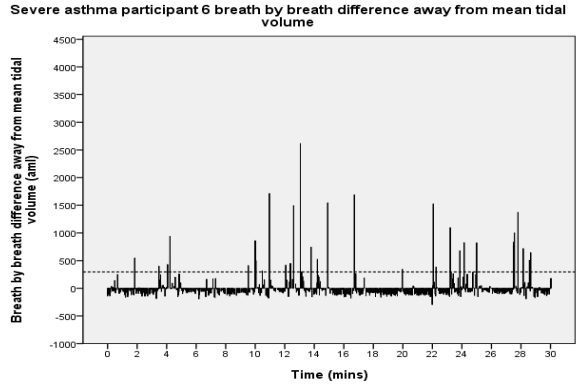

Figure 55: Breath by breath difference away from mean graph for severe asthma participant 6.

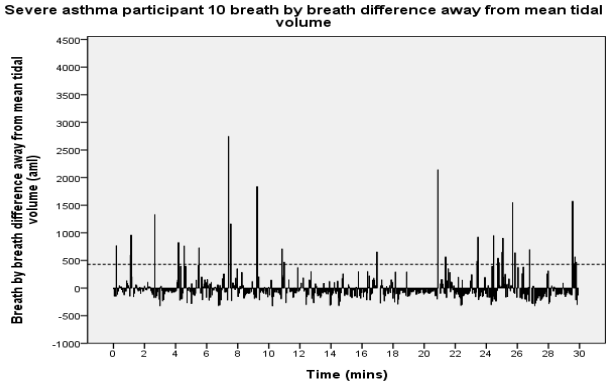

Figure 56: Breath by breath difference away from mean graph for severe asthma participant 10.

The frequency distribution graphs for tidal volume displayed on the next page show that each participant had a differently shaped frequency distribution. Healthy participants 5, 9 and 10 have kurtosis of 1.61, 3.52 and 0.14 respectively. Severe asthma participants 1, 6 and 10 have kurtosis of 8.47, 6.33 and 6.37 respectively. Since healthy participants 5 and 9 also had lower tidal volume variability than severe asthma participants 1 and 6, this might suggest an association between kurtosis and tidal volume variability. However, such an association does not appear to be consistent since severe asthma patient 10 and severe asthma patient 6 had the same kurtosis but there is a 16% difference in tidal volume variability. A further correlation analysis was performed using kurtosis and skewness with tidal volume variability, but no correlation was found between kurtosis and tidal volume variability or between skewness and tidal volume variability.

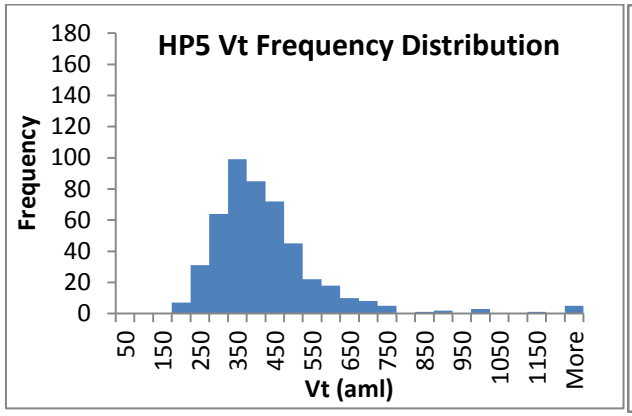

Figure 57: Frequency distribution of tidal volume healthy participant 5.

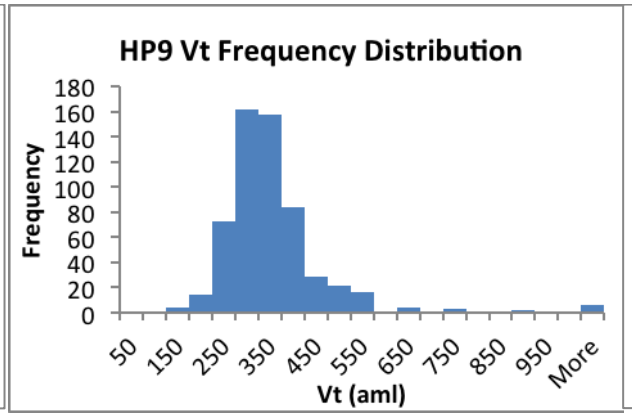

Figure 58: Frequency distribution of tidal volume healthy participant 9.

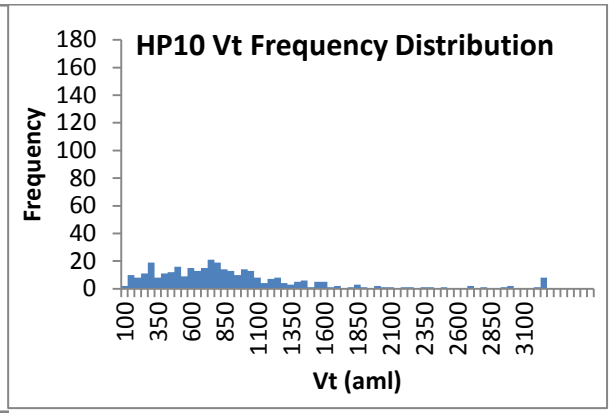

Figure 59: Frequency distribution of tidal volume healthy participant 10.

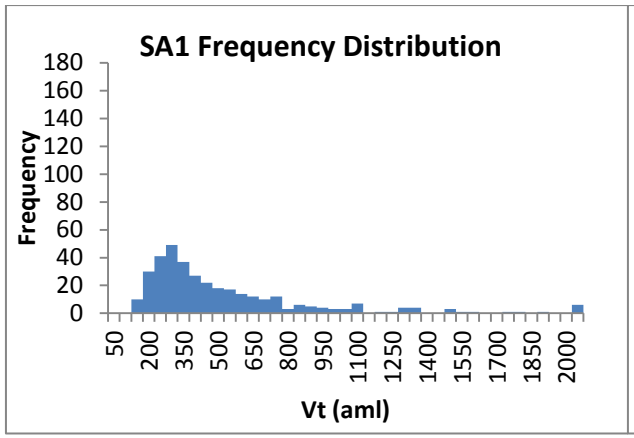

Figure 60: Frequency distribution of tidal volume for severe asthma participant 1.

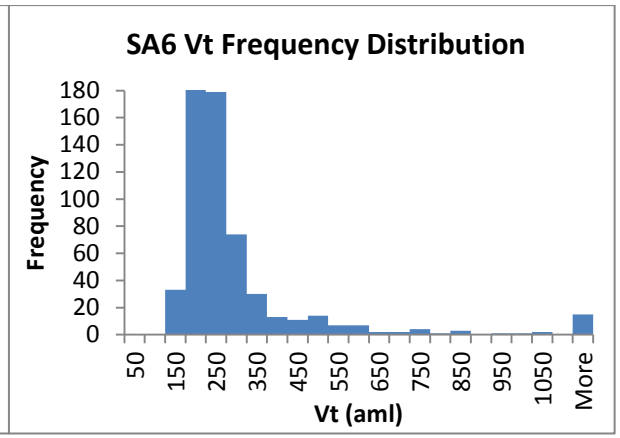

Figure 61: Frequency distribution of tidal volume for severe asthma participant 6.

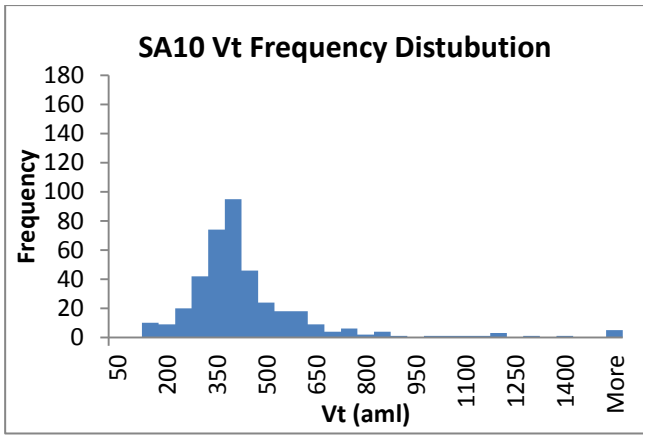

Figure 62: Frequency distribution of tidal volume for severe asthma participant 10.

9.10 Summary

This section looked into the details of the tidal volume pattern of the healthy controls and the severe asthma patients. Various types of pattern were observed, including episodic breathing patterns and different skewness and peaks in the frequency distribution diagram. However, there did not appear to be any identifiable or consistent pattern between individuals or between cohorts.

The following table presents a summary of the findings with respect to the hypotheses described in Section 8.1.

Hypothesis	**Summary of findings**	**Hypothesis Rejected/Supported/Neither?**
H1a: There is a statistically significant between-groups difference in mean relative tidal volume.	The severe asthma cohort recorded a lower mean tidal volume than the healthy cohort. The difference was not statistically significant.	Rejected
H1b: There is a statistically significant between-groups difference in mean expiration time.	The severe asthma cohort recorded a lower mean expiration time than the healthy cohort. The difference was not statistically significant.	Rejected
H1c: There is a statistically significant between-groups difference in mean respiratory rate.	The severe asthma cohort recorded a higher mean respiratory rate than the healthy cohort. The difference was not statistically significant.	Rejected
H1d: There is a statistically significant between-groups difference in mean sigh rate.	The severe asthma cohort recorded the same sigh rate as the healthy cohort. The difference was not statistically significant.	Rejected

H1e: There is a statistically significant between-groups difference in mean end tidal carbon dioxide. level	The severe asthma cohort recorded a lower mean $ETCO_2$ level than the healthy cohort. The difference was not statistically significant.	Rejected
H1f: There is a statistically significant between-groups difference in mean tidal volume variability.	The severe asthma cohort recorded a lower mean tidal volume variability than the healthy cohort. The difference was not statistically significant.	Rejected
H1g: There is a statistically significant between-groups difference in mean expiration time variability.	The severe asthma cohort recorded the same mean expiration time variability as the healthy cohort. There was no statistically significant difference.	Rejected
H1h: There is a statistically significant between-groups difference in mean $ETCO_2$ variability.	The severe asthma cohort recorded a higher $ETCO_2$ level variability than the healthy cohort. The difference was not statistically significant.	Rejected
H1i: There is a statistically significant between-groups difference in mean NQ score.	The severe asthma cohort recorded a higher mean NQ score than the healthy cohort. The difference was statistically significant.	Accepted
H1j: There is a statistically significant between-groups difference in mean HADS scores.	The severe asthma cohort scored the same for mean HADS-A as the healthy cohort. The severe asthma cohort scored lower in mean HADS-D than the	Rejected

	healthy cohort. The differences were not statistically significant.	
H2a: There is a significant correlation between end tidal carbon dioxide level and tidal volume variability.	A stronger negative correlation coefficient was recorded in the healthy cohort than in the severe asthma cohort. The correlation coefficient was not statistically significant.	Rejected
H2b: There is a significant correlation between sigh rate and tidal volume variability.	Sigh rate was significantly correlated with tidal volume variability in the severe asthma cohort and in the healthy cohort.	Accepted
H2c: There is a significant correlation between end tidal carbon dioxide level and sigh rate.	The correlation coefficient was weak in both the severe asthma cohort and the healthy cohort. The correlations were not statistically significant.	Rejected
H2d: There is a significant correlation between end tidal carbon dioxide variability and tidal volume variability.	The severe asthma cohort recorded a weaker negative correlation than the healthy cohort. The correlation was not statistically significant in either cohort.	Rejected
H2e: There is a significant correlation between NQ score and end tidal carbon dioxide level.	The severe asthma cohort and the healthy cohort both recorded a negative correlation between NQ and $ETCO_2$ level. The correlation coefficient was statistically significant in the severe asthma cohort but not in the healthy cohort.	Accepted

H2f: There is a significant correlation between NQ score and sigh rate.	The severe asthma cohort recorded a weak negative correlation and the healthy cohort recorded a weak positive correlation. The correlation coefficients were not statistically significant in either cohort..	Rejected

Table 52: Summary of research findings.

Chapter 10: Discussion

The aim of this study was to obtain new knowledge about the breathing pattern of a group of severe asthma patients. The specific aims of the study were: 1)to explore whether there was a difference in the respiratory parameters of tidal volume, inspiration time, expiration time, respiratory rate, sigh rate, $ETCO_2$ level, tidal volume variability and expiration time variability between a group of healthy people and a group of patients diagnosed with severe asthma; and 2) to explore whether there was a linear association between i) hyperventilation symptoms and resting $ETCO_2$ level; ii) hyperventilation symptoms and sigh rate; iii) $ETCO_2$ level and sigh rate; iv) $ETCO_2$ level and tidal volume variability; v) $ETCO_2$ variability and tidal volume variability; and vi) sigh rate and variability.

Ten healthy participants and ten severe asthma participants completed the study. This small sample size reflects the prevalence of severe asthma (less than 10% of the asthma population), but limits the scope of the thesis in being able to answer the original hypotheses described in Chapter 8.1. This section will discuss the results presented in Chapter 9.

10.1 Breathing patterns parameters

No significant between-cohort differences (healthy versus severe asthma) were found for the mean values of any of the respiratory parameters. This may suggest that there is little difference in terms of these breathing pattern parameters between healthy individuals and severe asthma patients. However, the sample size was small, which may have limited its power to detect such differences if they do exist. The lack of between-group differences is in agreement with existing studies that have investigated breathing patterns in mild to moderate asthma patients during the asymptomatic phase (Tobin *et al*., 1983b; Osborne *et al*., 2000; Delvaux *et al*., 2002). None of these studies showed any statistical differences in tidal volume, expiration time or respiratory rate between healthy volunteers and mild to moderate asthma patients. The results from the current study appear to confirm that there is no distinguishable difference in the breathing pattern parameters of severe asthma patients. All the severe asthma patients except one demonstrated severe airflow obstruction in a lung function test, so there does not appear to be any obvious link between airflow obstruction and breathing pattern parameters.

The lack of difference in respiratory parameters between healthy and severe asthma subjects may be due to the fact that symptoms experienced during the symptomatic phase, such as shortness of breath, wheezing, chest tightness and airflow obstruction, are related to acute bronchoconstriction. Such bronchoconstriction is at least partly reversible with or without intervention (Bousquet *et al*., 2000). Based on the findings of this study and existing evidence on mild to moderate asthma patients, it would appear that the severity of asthma condition does not have an impact on breathing pattern parameters when no active symptoms are present.

This study observed some between-subject differences in the parameters of breathing rate, sigh rate and tidal volume variability. This observation is in agreement with previous studies which reported diversity between-subject in tidal volume, inspiratory and expiration duration and breathing frequency

(Benchetrit, 2000; Bernardi *et al*., 1998; Dejours *et al*., 1961). The results from the current study support the theory that breathing pattern is highly individualised, and therefore it should probably be best considered on an individual basis. Therefore within subject changes are likely to be of more relevant than between group changes if breathing parameters are used as an outcome measure for therapeutic interventions.

10.2 Tidal volume variability

The tidal volume variability observed in the severe asthma cohort was not significantly higher than the variability observed in the healthy cohort. There have been few published studies containing data relating to tidal volume variability in patients with respiratory conditions. One study by Osborne *et al*. (2000) involved twenty-three patients with moderate asthma and seventeen healthy controls, and included some measures of breathing pattern variability. However, this study found no significant differences between the groups. They measured breathing pattern using a heated pneumotachograph over a five-minute recording period, and found that both the asthma group and the healthy group had similar tidal volume variability (19% in the control group and 23% in the mild to moderate asthma sufferers).

Similarly, Kuratomi *et al*. (1985) recorded tidal volume variability in twenty-six healthy individuals and seven asthmatic individuals during an asthma attack and after treatment. The severity of the participants' asthma was not reported in the study. Tidal volume was recorded by impedance pneumography for approximately sixty minutes in a supine position. The results showed that tidal volume variability was significantly higher in asthma individuals during an asthma attack (39%) than for the healthy individuals (26%). However, no difference was observed in tidal volume variability between the healthy and asthmatic individuals post-treatment (22%), when breathing patterns had returned to a stable condition. The results of the study presented in this thesis are in agreement with the findings of these earlier authors, suggesting that there is no difference in the group mean tidal volume variability between healthy and asthma cohorts during the stable period of the condition. However, the variability in tidal volume observed in this study contrasts with standard textbook descriptions of breathing pattern and the common clinical belief that breathing in the healthy population is 'regular' (Rosdahl and Kowalski, 2008, Pollak, 2011, Pryor and Prasad, 2004).

One of the difficulties in interpreting variability in tidal volume is that it is currently not known what level of variability is required for optimal respiratory function. A study by Wysocki *et al.* (2006a) involving fifty-one patients on mechanical ventilators reported a significant difference in tidal volume variability between those who were successful and those who were unsuccessful in weaning off the ventilator. The underlying conditions for requiring mechanical ventilation were not documented. The suggestion was that reduced tidal volume variability was associated with higher respiratory dysfunction. However, it was not reported what type of respiratory dysfunction is associated with altered variability. No difference in tidal volume variability could be detected between the healthy and severe asthma participants involved in this study, which suggests that tidal volume variability may be unrelated to airway obstructive disorder. However, one of the significant differences between the two studies is that Wysocki *et al*.'s patients were sedated and mechanically ventilated, while the participants in this study were awake and breathing spontaneously. Breathing pattern variability in alert participants may be affected by several other factors, such as emotions and mental stress (Wilhelm *et al*., 2001a).

Another study, by Brack *et al*. (2002), investigated tidal volume variability in ten male patients with restrictive lung disease and seven healthy age-matched male participants. Tidal volume was measured by inductive plethysmography over a one hour period. The position and activity during the recording periods were not documented. The results demonstrated that the restrictive lung disease group had significantly lower tidal volume variability than the healthy group. This contradicts the findings of the current study, which were that no difference was found between the healthy and pathology cohorts. The study by Brack *et al*. (2002) included age-matched male participants for comparison, whereas this study was not matched for age or gender. It is not yet known how age and/or gender affect tidal volume variability. Another possibility to explain the difference in results between the Brack *et al*. (2002) study and the present investigation is the difference in pathology. It is possible that restrictive lung disorders have more impact on tidal volume variability than obstructive lung disorders such as asthma.

The difference in choice of activity during the recording period may also contribute to different results between studies. It has been shown that input from the cerebral cortex that allows behavioural and volitional modulation of respiration during wakefulness (Phillipson *et al*., 1978), as well as certain types of mental activity, induce increase in sighs and variability (Vlemincx *et al*., 2009a). The difference between studies may be due to the fact that certain participants found reading a newspaper or a magazine stressful or uninteresting, which might induce more or fewer sighs, which may in turn affect the variability of tidal volume. One of the difficulties of studying breathing pattern is that it can be affected by emotional and/or mental activities. Certain activities, such as watching television, have been reported to induce changes in breathing pattern (Hark *et al*., 2005), and activities that increase mental stress or require attention have been reported to induce sighs (Vlemincx *et al*., 2010). If no activities were given to participants during the recording period, they might be concentrating on their breathing, which may also have an impact on their breathing pattern variability.

10.3 Sigh and variability

10.3.1 Sigh rate between healthy and severe asthma patients

One of the reasons for tidal volume variability is the occurrence of 'sigh' breaths. To date, there does not appear to be a universally accepted definition of a sigh, nor an agreed threshold for normal sigh rate. Several authors have reported various definitions of sighs and different sigh rates in healthy people. Definitions include: twice the mean inspiratory tidal volume (Wuyts *et al*., 2011); 3.5 times the mean expiratory tidal volume (Prys-Picard *et al*., 2006); and twice the mean expiratory tidal volume (Wilhelm *et al*., 2001b). Fredberg (2001) stated that sigh breaths occur naturally at a rate of about ten sighs per hour, and a recent study by Wuyts *et al*., (2011) confirmed this by recording a mean of 1.67 sighs in ten minutes in healthy individuals, which gives an equivalent of approximately ten sighs per hour.

A more recently published study by Velmincx *et al*. (2012) reported that the average number of sighs over a four-minutes recording period was 0.97 in healthy controls. Velmincx *et al*.'s (2012) sigh frequency was measured by the LifeShirt® for thirty-six healthy controls during baseline data collection. Sigh was defined as two times the mean expiration tidal volume in the preceding four minutes. The observed sigh rate in the healthy group in the study reported in this thesis is higher than the sigh rate previously reported for healthy controls. It is likely that the difference between the current study and the study by Velmincx *et al*. (2012) is due to a difference in sigh definition, different lengths of recording period and differences in recording equipment. However, this study has demonstrated no difference in sigh rate between severe asthma patients and healthy individuals, which suggests that on average severe asthma patients do not have more sigh breaths than healthy individuals. This contradicts an existing theory that symptoms of hyperventilation in asthma patients may be associated with frequent or habitual sighing (Gardner and Lewis, 2005). In the study reported in this thesis, participant 1, 6 and 10 demonstrated an episodic breathing pattern where sigh breaths occurred in close successions. All three

participants reported positive NQ scores. This appears to suggest that hyperventilation symptoms may be associated with increased frequency of sporadic sighs, rather than a sustained increase in sigh frequency. It is currently unclear why episodic breathing pattern does not produce symptoms of hyperventilation in healthy participants.

10.3.2 Sigh as a 're-setter' for variability

Sighing is proposed to assist in improving gas exchange and restoring lung volumes (Patroniti *et al*., 2002; Aljadeff *et al*., 1993), to act as a defense mechanism against the collapse of alveoli (Lim *et al*., 2001), and to act as a reset mechanism when variability is abnormally reduced or increased (Baldwin *et al*., 2004; Vlemincx *et al*., 2009a; Vlemincx *et al*., 2009b; Wuyts *et al*., 2011; Vlemincx *et al*., 2012). However, the exact trigger for sighing is currently unknown. Recent studies have proposed a resetting hypothesis which suggests that sigh breaths have a role in reducing respiratory variability (Baldwin *et al*., 2004; Vlemincx *et al*., 2009b; Wuyts *et al*., 2011). The proposed resetting hypothesis states that the human respiratory system, like many other control loop feedback systems, incorporates a 'resetting' mechanism. The role for the resetting mechanism is to reset the system if too much or too little variability exists. However, it is yet to be defined in the literature what constitutes 'too much' or too little' variability The hypothesis that sighs act to reset tidal volume variability, proposed by Baldwin *et al*. (2004), was not investigated in this study. This was due to the large variation both within and between individuals in the number of breaths between sighs. In order to investigate how variability changes before and after a sigh, a window of pre-sigh and post-sigh breaths is needed. As each individual had a different number of breaths between sighs, it was difficult to optimise a suitable window for pre- and post-sigh series; the following schematic gives a visual representation of the problem.

Figure 63: The ideal case scenario representing the ensemble of pre-sigh/post sigh series. *Key: S = sigh breath, NS = non sigh breath.*

Figure 63 is a schematic representing the method used by Baldwin *et al*. (2009b) and Vlemincx *et al*. (2009b), where a fixed number of breaths was available before and after a sigh breath.

In the current study, it was found that it was not possible to implement such a method due to different number of breaths available before and after a sigh breath in different individuals.

Figure 64: Schematic to represent the sigh distribution in the current study.
Key: S = sigh breath, NS = non sigh breath

Figure 64 is a schematic representing the data obtained in the current study. It can be seen that there is no regular number of non-sigh breaths between sighs. Because of this variability, it is not possible to decide when a post-sigh series ends and a pre-sigh series begins

In the studies by Baldwin *et al*. (2009b) and Vlemincx *et al*, (2009b) it was reported respectively that windows of fifteen breaths and ten breaths were likely to contain changes in tidal volume variability between pre- and post-sigh. However, in the study reported in this thesis, it was found that when those windows were applied to the data, they resulted in no sigh series being obtained for certain participants. In the studies by both Baldwin *et al*. (2009b) and Vlemincx *et al*. (2009b), it was unclear how the authors had overcome the problem of sighs that occurred in quick succession with each other. In both studies, it was stated that ‘as many post- and pre-sigh blocks as possible were

constituted in the situation of sighs in close succession'. In Velmincx *et al*. (2009b), twelve participant recorded no sighs and eight participants had such frequent sighs that no 'non-sigh' series was obtained. It is therefore unclear how many complete sigh series were obtained from each participant due to the large between-subject differences in the number of sigh series available from each participant.

Similar results were also reported in a recent study by Wuyts *et al*. (2011), where the same methodology was used to investigate sigh and variability in healthy females with low and negative emotional states. Twenty-one out of the total of forty-eight participants sighed so frequently that no non-sigh series could be created, and a further twenty-seven participants did not sigh during the ten-minute recording period. If not every participant produced a complete data set of sigh and non-sigh series for comparison, then the results should be interpreted cautiously.

Results from these three published studies seem to suggest that in healthy cohorts free from respiratory conditions, there is no regularity of sigh occurrence. Therefore, the occurrence of sighs is probably an individual process depending on the person's respiratory control feedback mechanism. This emphasises the importance of treating breathing as a highly individual process.

10.3.3 Bronchodilatory effect of sigh

One of the theories for why sighs may occur in close succession is that it may be related to the potential bronchodilatory and bronchoprotective effects of deeper breaths (Scichilone *et al*., 2001). It is reported in several studies that a deep breath or a sigh has both bronchodilatory and bronchoprotective effects in healthy individuals, and that such mechanism is diminished or absent in asthma (Kapsali *et al*., 2000). It is not yet known why this difference exists. A study by Scichilone *et al*. (2001) demonstrated that the bronchodilation effect induced by lung inflation can be present in both healthy and asthmatic individuals, but the

bronchoprotective effect is diminished in the asthma population (Scichilone *et al*., 2001). As no differences in sigh frequency were observed between the severe asthma group and the healthy group in this study. It is not possible to comment on the potential for these effects in this sample. However, if this theory also applies to the sample population in the current study, then it might be possible that there is an increased drive to sigh to maximise the bronchodilatory benefit because of its diminished effects.

10.4 End tidal carbon dioxide and tidal volume variability

10.4.1 End tidal carbon dioxide level between healthy and severe asthma groups

The results show that there are no significant between-group differences in mean end tidal carbon dioxide level between the healthy and severe asthma groups. This result is in contrast with the study by Osborne *et al*. (2000), where the population mean $ETCO_2$ level in mild to moderate asthma patients was statistically significantly lower than in the healthy controls (mean difference of 0.39 kPa at $p<0.05$). In the present study the severe asthma cohort also had a lower mean $ETCO_2$ level than the healthy cohort, but the difference was not statistically significant (mean difference of 0.24kPa, $p>0.05$). The recorded mean difference in $ETCO_2$ levels is similar to the difference between mild to moderate asthma patients and a healthy group reported in the study by Delvaux (2002) (mean difference of 0.29 kPa). The small difference reported in Delvaux (2002) had reached statistical significant level (n=80). It is possible that the reason for the lack of a statistically significant difference between the groups in this study was due to the small sample size.

$ETCO_2$ levels are a reflection of arterial carbon dioxide level (Yosefy *et al*., 2004; Russell *et al*., 1990), which is normally controlled within a tight limit via chemoreceptor responses in order to maintain pH balance(West 2005). The difference in $ETCO_2$ was therefore expected to be small because of this tight regulation by chemoreceptors (West, 2005). Therefore it is likely that this study did not have sufficient power to detect the small differences that might exist between healthy and severe asthma groups. Although there was no statistically significant difference between the groups, there were three severe asthma participants who had $ETCO_2$ levels lower than the normal range of 4.66 kPa – 5.99kPa (Murphy *et al*., 2009; Esquinas, 2010). This finding appears to suggest that within the severe asthma cohort, some patients may have abnormally low levels of $ETCO_2$. However, one healthy participant also had an $ETCO_2$ level of

4.66kPa. It is currently unclear how large a difference in $ETCO_2$ level must be in order to be of clinical significance. A limited number of studies have investigated the threshold of changes in $ETOC_2$ that are likely to lead to symptoms of hypocapnia.

10.4.2 Relationship between end tidal carbon dioxide levels and tidal volume variability

The correlation coefficient analysis between $ETCO_2$ and tidal volume variability suggested that there was no significant relationship between ETCO2 and tidal volume variability in this cohort. This contradicted the findings of a previous study by Fiamma *et al*. (2007b) which had shown a significant negative association between $ETCO_2$ and tidal volume variability under experimental conditions. In this study, eight healthy participants (seven men, one woman) were recruited and respiratory parameters were recorded using a heated pneumotachograph during a period of between eight to fourteen minutes while subjects breathed different gas mixtures. The results showed that with a reduction in CO_2 level tidal volume variability was higher than normocapnic, and vice versa with hypercapnic.

One of the key differences between Fiamma *et al*.'s (2007b) study and the work reported in this thesis is that in Fiamma's study participants were asked to breathe a gas mixture to artificially alter $ETCO_2$ level, whereas in this study the $ETCO_2$ level was a result of the person's natural breathing pattern. Participants in the present study were not subject to such a large variation in carbon dioxide as in Fiamma's study. This is because the carbon dioxide content in the gas mixture was more concentrated than the normal atmospheric carbon dioxide level, and therefore it was likely to elicit a physiological response stronger than under normal physiological conditions. A low $ETCO_2$ level in normal breathing conditions is generally caused by hyperventilation, defined as breathing in excess of the body's needs (Gardner & Lewis, 2005).

Variability in this study was defined by the mean and standard deviation of tidal volume, which gives an impression of the overall variability over a period of

time. From the graphs of the breath by breath difference away from the mean tidal volume (Figures 37 to 40, Figures 51 to 56), it can be seen that the variability was related to the frequency of sigh, the size of the sigh, and the mean breath size. Each one of these factors, or a combination of them, could therefore potentially contribute to an increase or decrease of variability in tidal volume. According to respiratory physiology, carbon dioxide level detected by the peripheral chemoreceptor is the primary determining factor of the rate and size of a breath (Nattie, 2006). However, as tidal volume and breathing rate can be changed voluntarily, the arterial carbon dioxide level will therefore be altered as a result of how the person was breathing.

This could explains why participant 3 in the severe asthma group has a different relationship between end tidal carbon dioxide level and tidal volume variability in comparison with the rest of the severe asthma cohort; his breath size was consistently large, giving low end tidal carbon dioxide levels but also low tidal volume variability. In fact, the breathing pattern in participant 3 appears to fit the physiological description of hyperventilation, where an excessive amount of carbon dioxide is being removed (Gardner & Lewis, 2005a). This then results in a low $ETCO_2$ level, which in turn causes hyperventilation symptoms. This participant in the severe asthma cohort has the highest tidal volume (837.22 aml), the lowest $ETCO_2$ level (3.64 kPa) and the highest NQ score (40). The findings of the severe asthma cohort have a stronger negative correlation between $ETCO_2$ levels and tidal volume variability than in the healthy group, suggesting that healthy individuals might be able to maintain their $ETCO_2$ level despite the presence of variable breath sizes

10.4.3 Relationship between end tidal carbon dioxide variability and tidal volume variability.

In this study, a stronger positive correlation coefficient was observed between $ETCO_2$ variability and tidal volume variability in the healthy cohort than in the severe asthma cohort. The positive association agrees with Khoo's theory that fluctuation in arterial carbon dioxide levels stimulates the chemoreflexes to produce ventilatory changes which act to restore carbon dioxide level. Thus a

reduction in arterial carbon dioxide level subsequently results in a decrease in ventilation (Khoo,2000). Khoo (2000) proposed a theory that this type of corrective adjustment does not occur instantaneously. Instead there is a delay in the feedback and control processes, which results in the chemoreflex-mediated response appearing only after much of the original stimulus has occurred. Instead of acting to restore the effect of the original stimulus, the chemoreflex-mediated response could become an over-correction which stimulates the systems in the opposite direction.

Since the respiratory system interacts with other control systems, ventilation variability can also be the result of influences from other organ systems. Variability could be corrected by either chemical or non-chemical drives, and even with same amount of breath to breath variability and the same coefficient of variability, the source of the variability could be quite different. If a system was more responsive to chemical drives, the ventilatory fluctuations would primarily reflect the changes in these chemical drives. If the system was less responsive to chemoreflex feedback, the ventilatory fluctuations would predominantly be the result of the nonchemical components of the system. The observed between-groups difference in correlation between tidal volume variability and end tidal carbon dioxide level variability may suggest that tidal volume variability in the severe asthma population is less chemically driven than in the healthy population, or is more likely to be adjusted by non-chemical components; hence, the weaker association between population mean $ETCO_2$ variability and population mean tidal volume variability.

It is currently unclear whether a system which is more chemically driven is more beneficial than a system which is less so. It is also currently unknown whether this theory plays any pathological role in asthma management, because of insufficient evidence in this area. Due to the small sample size it cannot be certain whether such findings are applicable to the wider severe asthma population, and therefore this theory remains a speculation.

Another possible explanation for the weaker correlation coefficients between $ETCO_2$ variability and tidal volume variability and between $ETCO_2$ and tidal volume variability in the severe asthma cohort than in the healthy cohort might be that severe asthma patients have lower peripheral chemoreceptor gains than the healthy volunteers. Central chemoreceptors provide most of the steady-state tonic response, whereas peripheral chemoreceptors provide a rapid response to changes in arterial carbon dioxide (Nattie, 2006). It is therefore possible that if the peripheral chemoreceptor is less sensitive to changes in carbon dioxide level, there will be a weaker correlation between $ETCO_2$ level variability and tidal volume variability.

While the concept of reduced peripheral gain was not tested specifically in this thesis, the concept has been demonstrated in patients diagnosed with panic disorder. Wilhelm (2001b) conducted a test on sixteen patients diagnosed with panic disorder and fifteen diagnosed with general anxiety disorder. Tidal volume was monitored by an RIP system over a thirty-minute rest period. $ETCO_2$ level was continuously monitored by a capnograph. The relationship between sigh and $ETCO_2$ was assessed by creating sigh series that consisted of three breaths preceding a sigh and three breaths after the sigh. $ETCO_2$ levels for the corresponding breaths were compared against changes in tidal volume. The panic disorder group demonstrated a slower recovery in $ETCO_2$ level and tidal volume after a sigh compared with healthy controls. On average, the panic disorder group had not returned to pre-sigh levels of either $ETCO_2$ or tidal volume by the end of third breath, whereas the healthy controls had. This slow recovery process may indicate a lower gain for peripheral chemoreceptors, since a higher gain from the peripheral chemoreceptor would have been indicated by a rapid rebound in $ETCO_2$ level and tidal volume. If severe asthma patients have a slow response from peripheral chemoreceptors they would take longer to response to changes in carbon dioxide level, and the ventilator response may then over-correct so that the response itself becomes a new stimulus. This may result in more fluctuation and higher variability in carbon dioxide level, and less association between carbon dioxide level variability and tidal volume variability.

It is currently unclear if a potentially lower peripheral chemoreceptor gain has any role to play in the pathology of asthma. However, this hypothesis that some severe asthma patients are less sensitive to changes in carbon dioxide level contradicts an existing hypothesis, which suggests that some asthma patients are more sensitive to changes in carbon dioxide (Buteyko, 1990). The theory proposed by Buteyko was that asthma patients were more sensitive to changes of carbon dioxide level and that small changes in carbon dioxide level could induce hyperventilation. There is some evidence that demonstrates an increase in sensitivity to carbon dioxide in the airway smooth muscles of asthma patients (van den Elshout *et al*., 1991), but no published study has been identified which demonstrates altered sensitivity to carbon dioxide in the control of breathing with asthma.

10.5 Individuality in tidal volume variability

In this small study, it has been demonstrated that although there were no overall differences between groups in tidal volume variability, there appear to be individualised patterns in the tidal volume time series and frequency distribution histograms, despite similar tidal volume variability between individuals. From the time series each severe asthma participant has their own pattern of breathing, but there is no specific asthma pattern in terms of tidal volume, frequency of sighs or distributions of sighs. The numbers of sighs per recording, or the sigh rate, are frequently documented in the literature but the distribution of sighs is rarely documented. These time series and the breath by breath difference away from the mean graphs clearly demonstrate that there was some difference in the pattern of distribution of sighs between individuals, despite little difference in the overall number of sighs between groups or between individuals.

For example, severe asthma participants 5 and 6 both had the same sigh rate of 1.07. However, their tidal volume variability varied considerably, with a mean difference of 20%. The distribution of sighs also varied considerably between the two individuals, with severe asthma participant 6 demonstrating an episodic tidal volume pattern while severe asthma participant 5 showed a relatively steady distribution of sighs. Episodic breathing refers to periods of breaths containing a large number of either sigh breaths and/or breaths which are predominantly above mean tidal volume. This is preceded/succeeded by a period of breaths that contain comparatively fewer sighs and/or breaths which are predominantly below mean tidal volume. The occurrence of such episodes, however, does not appear to be regular or predictable. For example, it could happen at the beginning and/or the end of the recording period, or in the middle part of the recording. An example of this was seen in severe asthma participant 1, where 68% of the total number of sighs occurred in close succession between minutes fifteen and twenty-five of the recording, whereas severe asthma participant 10 had 34% of the total number of sighs occurring in the first ten minutes and 55% of the total number of sighs recorded in the final ten

minutes. Only 3% of the total number of sighs was recorded in the middle ten minutes.

A third example is severe asthma participant 8, in which sigh breaths occur regularly, approximately one sigh every four to six minutes. However, severe asthma participant 1 and severe asthma participant 8 both had a similar level of tidal volume variability (84% and 79% respectively) and similar sigh rates (1.13/min, 1.07/min respectively) when summary calculations were performed, despite the observed underlying episodic difference.

Based on this observation, it is speculated that the distribution of sighs may be a more important factor to consider than the simple number of sighs per recording period. Sigh breaths in the literature are often reported as averages over the recording period and expressed as either the number of sighs per minute (Wuyts *et al*., 2011) or the total number of sighs recorded during the recording period (Prys-Picard *et al*., 2006). No published studies have been identified that report the distribution of sigh breaths. Distribution of sigh breaths is an area where it is difficult to quantify due to the difficulty of selecting a representative period. As seen in this study, when a specific period was considered and averaged out, the average number of sighs was often similar across participants. It is therefore proposed that visual analysis of the distribution of time series and the breath by breath difference away from the mean may be a useful alternative way to analyse sigh distribution and breathing pattern in future.

The frequency distribution histograms of tidal volume (Figures 41 to 44, Figures 57 to 62) suggest that different individuals have different frequency distribution patterns of tidal volume. Some participants had frequency distributions that were more positively skewed, whereas some participants had a distribution that was close to a normally distributed pattern. Some participants had a higher frequency curve with a sharp peak (high kurtosis), while some participants had a frequency distribution graph with a flat peak. This suggest that some participants had a higher proportion of breaths at low tidal volume with

occasional breaths at high tidal volume, while other participants had breaths with more equal proportions of low and high tidal volume. These differences are obscured when averaged data are used.

These patterns in frequency distribution do not appear to be related to respiratory parameters, nor do they show consistency with all respiratory parameters among the sample population. The frequency distribution graphs show that there might be an association between ‘normality’ and tidal volume variability. For example, severe asthma participants 1, 6, 7 and 10 and healthy participant 7 had tidal volume variability over 80 % and skewness of over 2.5. However, this pattern was not consistent within the cohort, because severe asthma participant 7 and healthy participant 10 both had tidal volume variability over 80% but skewness of 1.13 and 1.30 respectively.

The observation of the differences in skewness, kurtosis and tidal volume variability would suggest that a similar level of tidal volume variability could arise when the individual: 1) takes a high proportion of breaths at low volume and a smaller proportion of breaths at high volume (high skewness, high kurtosis); 2) takes equal proportions of breaths at high and low volume with occasional occurrences of breaths that are of even higher volume (high skewness, low kurtosis; 3) takes equal proportions of breaths at both high and low volume without the occurrence of breaths of even higher volume (low skewness, low kurtosis). It is currently unclear whether the observed pattern in tidal volume distribution and tidal volume variability has any physiological meaning, or simply represents a shape for the patterns of breathing for the individuals concerned, given the lack of correspondence with other recorded respiratory parameters between and within groups and individuals. In the existing literature surrounding asthma, breathing pattern has rarely been considered on an individual basis (Tobin *et al*., 1983b; Kuratomi *et al*., 1985; Veiga *et al*., 2010). However, given the complexity of asthma, which is likely to be a collection of different phenotypes rather than a single, specific disease with specific mechanism (BTS, 2009), it is perhaps logical to consider asthma breathing pattern on an individual basis rather than as a pathological group.

The results of the research in this thesis have clearly demonstrated the individuality of breathing pattern variability, and shown that it does not follow a consistent pattern between groups or between individuals. Individuality in breathing pattern was documented in early literature, but at that point it was associated with a high consistency of breathing pattern within single subjects. Proctor & Hardy (1949) recorded the patterns of air flow by pneumotachograph. Sixty one normal individuals age from new born baby to 60 years old were included in the study. The airflow patterns from the pneumotachogram recorded were superimposed within subject and between subjects. The comparisons of airflow patterns of consecutive cycles and cycles taken on different days from a single subject demonstrated high consistency. The comparison of pneumotachogram between different subjects however, differed widely. These results suggest the individuality in airflow patterns. However, interpretation of the result from the study is limited as the exact recording protocol was not reported. It was also unclear how many sets of pneumotachogram were superimposed and checked for consistency.
Benchetrit (2000) reported an observation based on unpublished data that either regular or irregular breathing patterns were reproducible for the same subjects under the same conditions. As this observation was based on unpublished data, such findings need confirmation. However, if this observation is accepted as being applicable to the wider population, it is not surprising that no specific pattern was found in this study in either the healthy group or the severe asthma group.

Given the lack of observed pattern in tidal volume variability within the healthy group or the severe asthma group, it is speculated that breathing pattern may be regarded as an individual ‘trait’ that is unique to the individual.

10.5.1 Implication of individuality of breathing pattern

In existing published studies, the breathing patterns of asthma patients were often compared with a group of healthy controls matched according to age, gender and weight. The individuality in breathing pattern found in this thesis and in earlier literature casts some doubt on whether the matching criteria would yield adequate control samples for the comparison of breathing components. This is because there does not appear to be a 'normal pattern' that exists in people without any pathology. It is not yet fully understood what factors are associated with the components of breathing pattern. As Dejours *et al*. (1961) pointed out 50 years ago, there is an infinite number of combinations of breathing pattern components which can achieve adequate ventilation, and therefore individual results might be more meaningful than group results.

The findings about the individuality of breathing pattern also cast some doubt on the adequacy of existing studies that investigated or described breathing patterns in terms of the averages of timing and volume components. More information may be derived from examining the within-subject variability of these parameters.

10.6 Questionnaire scores

10.6.1 Nijmegen Questionnaire

The results have shown that this small severe asthma group had a statistically significantly higher mean Nijmegen score than the healthy group. If these severe asthma patients are representative of the wider population, this suggests that the severe asthma population does experience hyperventilation symptoms more frequently than the healthy population. Within the severe asthma cohort, five participants (50%) had a positive Nijmegen score. This finding is in line with a study by Thomas *et al*. (2005b), which reported that 30% of the mild to moderate asthma population had a Nijmegen score of over twenty-three. No previous figures have been published for the prevalence of hyperventilation symptoms in a severe asthma population. Although a higher percentage was observed in this study, the sample size is too small to know this is representative of the severe asthma population.

10.6.2 Relationship between Nijmegen score and $ETCO_2$ level

The Nijmegen questionnaire was originally developed to detect hyperventilation syndrome based on presenting symptoms (van Dixhoorn & Duivenvoorden, 1985). To date, there is limited published data to demonstrate that there is a significant relationship between NQ score and $ETCO_2$ level. $ETCO_2$ is a physiological indicator of hyperventilation; therefore, a lower $ETCO_2$ level should theoretically be associated with symptoms and hence a higher NQ score would be expected. Therefore a negative correlation between NQ score and $ETCO_2$ would be expected.

The results from this sample population demonstrated that $ETCO_2$ level is significantly negatively correlated with NQ score in the severe asthma population. However, this may not always be the case, because the NQ asks the patient to score according to the frequency of the symptoms experienced within the last seven days, while $ETCO_2$ level was measured during the data collection period. This might be the reason why some participants had similar

levels of $ETCO_2$, but did not report experiencing symptoms of hyperventilation – e.g. severe asthma participant 4 [who did not have hyperventilation symptoms (NQ = 18)] had an $ETCO_2$ level of 4.96 kPa, while severe asthma participant 6 had a similar $ETCO_2$ level of 4.97kPa but did have hyperventilation symptom (NQ=32).

10.6.3 Relationship between NQ score and sigh

Increased sigh rate is believed to be one of the factors that contributes to hyperventilation syndrome (Gardner & Lewis, 2005b). An increased sigh rate has been reported in a case study of difficult-to-treat asthma (Prys-Picard *et al*., 2006). The results of this study demonstrated a weak positive relationship between sigh rate and NQ score. If sigh rate is believed to contribute to hyperventilation syndrome, then it is necessary to establish normal sigh frequency. Based on the observations in this study, it is speculated that sigh rate is unlikely to be a contributing factor to NQ score.

10.6.4 Hospital and Anxiety Depression Scale

The results for the HADS scores demonstrated no between-group difference in anxiety and depression level for either asthma or healthy groups. The separate mean group scores for both anxiety and depression were within the 'normal' range (Zigmond & Snaith, 1983). However, despite the normal mean HADS and NQ scores, the Spearman's rank correlation coefficient demonstrated a strong association between the NQ score and anxiety score ($r = 0.75$) and depression score ($r = 0.84$). On inspection of the individual HADS and NQ scores, out of the five participants with severe asthma who had a positive NQ score of 23 or over, three participants had an 'abnormal' level of anxiety (an anxiety score 8 or above) while one participant had 'abnormal' level of depression (a depression score above 8). These results suggest that for these individuals their hyperventilation symptoms may be associated with anxiety or depression. This finding reflects reports from other studies that have also demonstrated a relationship between hyperventilation and anxiety and depression levels (Lum,

1981). This association is not consistent, however, because even in this small severe asthma sample there was one individual who had an NQ score of 23 (i.e. a positive score), but who was negative for anxiety and depression. Within the healthy group, there was no significant relationship between HADS scores and NQ scores. A few participants were positive for anxiety, but none of these also had a positive NQ score.

10.6.5 Asthma Control Questionnaire

As described in Section 8.1, the Asthma Control Questionnaire was designed to measure both the adequacy of asthma control, and changes in asthma control which occur either spontaneously or as a result of treatment. Asthma control is defined as the minimisation of: symptoms, activity limitation, bronchoconstriction, and rescue β_2-agonist use. In these severe asthma participants the control of symptoms had a significant positive association with reported hyperventilation symptoms as measured by the NQ score. The ACQ score ranges from 0 to 6, with 0 being totally controlled and 6 being severely uncontrolled. A score of less than 0.75 has been defined as ‘well-controlled’, and a score of greater than 1.5 has been defined as ‘poorly controlled’ (Juniper *et al*., 1999). As would be expected in a severe asthma population, no participants reported total control and nine out of the ten participants had a score over 1.5, indicating poor control.

10.7 Limitations

This study has several limitations that limited the scope of the interpretation of the results.

10.7.1 Sample size

One of the limitations of this study was the sample size. One of the reasons for the small sample size is that the severe asthma population itself is a small population. It has been estimated that approximately 5% to 10% of the total asthma population belongs to the severe asthma patient group (Busse *et al*., 2000). The number of suitable patients identified during the recruitment process (n=22) represented 8% of the total number of asthma volunteers on the database. Ten patients did not accept the invitation to take part because of the long travelling distance, and a further two patients had a possible serious pathology that required further investigation. It is acknowledged that a larger sample size would enhance the generalisabilty of the results and the validity of the statistical analysis. However, this was not feasible within the timeframe of the PhD. As this study aimed to investigate breathing pattern in patients affected by asthma in isolation, the study criteria excluded patients with any other medical conditions that required medical interventions. This further limited the number of eligible patients.

However, although this was a small sample size by usual standards, it is not uncommon for published studies of breathing patterns to have a similar sample size. For example, Fiamma (2007a) recruited a sample size of eight participants when investigating tidal volume variability and complexity, while Veiga *et al*. (2010) recruited five participants with mild and severe airflow restrictions and six participants with a moderate airflow restriction.

The small sample population increases the chance of type II error, where the null hypothesis is falsely accepted (Rubin, 2009). The results from this study are likely to contain type II errors since the between-group mean difference is very

small in all recorded respiratory parameters. One way of minimising type II error is to perform a power calculation for the primary outcome measures, in order to determine an adequate sample size to give a sufficient power to detect effects of a given size. A power sample calculation was not carried out in this study because insufficient data regarding breathing pattern in the severe asthma patient group was available to determine an adequate sample size. The data obtained in this study may be used as a reference for power calculation in future studies.

10.7.2 Capnograph

The sampling frequency of the capnograph, which operated on a four-second epoch, and the lack of ability to export raw signals both limited the interpretation of $ETCO_2$ level and analysis of the interaction between $ETCO_2$ level and tidal volume. It is acknowledged that it is important to understand how the equipment calculates the parameters it produces. Several attempts were made to contact the representative of the manufacturer to address the issue of signal exporting and to understand the algorithm used by the capnograph software to calculate the $ETCO_2$ level. However, the manufacturer was not forthcoming with the information, and at the time of writing there has yet to be a satisfactory reply from the manufacturer.

The four-seconds sampling epoch limited the interpretation of $ETCO_2$ variability because the sampling of four seconds does not necessary reflect breath-by-breath variability, as was recorded for tidal volume. Therefore the association between $ETCO_2$ variability and tidal volume variability might not represent a direct comparison.

10.7.3 $ETCO_2$ levels in severe asthma patients

It is not known what the possible relation between $ETCO_2$ levels and arterial blood gases might be for severe asthma patients. Therefore this study took $ETCO_2$ levels as a reflection of arterial carbon dioxide, based on the recommendation by Osborne *et al.* (2000) that $ETCO_2$ is a measurement of

arterial blood gases in mild to moderate asthma because they have little ventilation-perfusion (V/Q) mismatch. However, it has been reported that some patients with chronic stable severe asthma, either symptomatic or asymptomatic, may have ventilation/perfusion (V/Q) mismatch (Wagner *et al*., 1987; Wagner *et al*., 1978). The presence of V/Q mismatch alters the correlation between arterial carbon dioxide levels and $ETCO_2$ levels. The reading from capnography will often underestimate arterial carbon dioxide levels as the non-perfused alveoli carbon dioxide concentration is zero and the perfused alveoli carbon dioxide concentration is normal (Liu *et al*., 1992). The $ETCO_2$ reading recorded from the severe asthma group in this study therefore may not necessary reflect the arterial carbon dioxide levels. A capnogram would be a useful tool to validate the $ETCO_2$ values if V/Q mismatch is suspected. In this study, a capnogram was not obtained as the capnograph device did not support the export of the capnograpm. Several attempts were made to contact the manufacturer but no suitable response was received from the company.

Arterial blood gases remain the gold standard to measure carbon dioxide levels. However, this method is deemed to be invasive and may alter the breathing patterns of individuals.

10.7.4 Visual analysis of episodic breathing pattern

In the discussion section, it was proposed that visual analysis of the sigh breath distribution in a time series and the breath by breath difference away from the mean graph may be a useful alternative way to analyse sigh distributions and individual differences. However, visual analysis is a subjective method and therefore lacks objectivity. This lack of objectivity may lead to different interpretations of breathing patterns when interpreted by different raters, leading to weaknesses in the interpretation of the results. The observed episodic breathing pattern previously discussed was the author's interpretation, and therefore may be interpreted differently by another rater. Hence no firm conclusion could be drawn from this analysis at this stage. Currently, there is no clear guideline or criteria for the interpretation of the time series or breath by

breath difference away from the mean graph. Further research is required to develop robust criteria to define an ‘episodic’ breathing pattern to ensure the reliability of the analysis.

10.7.5 Time varying nature of breathing pattern

Another limitation of interpreting patterns in time series is that breathing is time varying in nature. A breathing pattern recorded at one time and the breathing pattern recorded at another time might be quite different for a given individual. For example, if the breathing pattern was recorded at one time and again fifteen minutes later, and different pattern was observed, then it would need to be decided whether such a difference was a natural variation, or whether it was reflecting longer term or significant changes in breathing. Also, when breathing is being recorded, one can never be sure when the ‘start’ of any observable pattern is. If the starting point for each individual is different, then one cannot be sure if the different patterns observed between individuals are genuinely different, or are simply due to a difference in the starting time which cause the pattern to appear to be different when in fact it is the same, if the recording period is sufficiently long enough to allow the repeat of the pattern to be recorded.

In this study the repeatability of breathing pattern was not measured due to time constraints, and therefore no conclusion could be drawn regarding whether the observed pattern is repeatable in individuals. However, according to Proctor and Hardy (1949), the pneumotachogram of consecutive cycles and cycles taken on different days were highly consistent within subjects. The comparison of pneumotachograms between different subjects, however, differed widely. Unpublished results reported in Benchetrit (2000) also suggested that either regular or irregular breathing patterns were reproducible for the same subjects under the same conditions. Currently there is very limited evidence which demonstrates the repeatability of breathing patterns, and further research is essential to establish if the observed pattern is repeatable to determine whether observed differences are genuine between-subject differences.

10.7.6 Calibration method for severe asthma patients

The calibration method used in this study had not been validated for the severe asthma patients. As discussed in the previous chapter (Section 4.7.1.2), the calibration technique is based on the Konno and Mead model (1967) where changes of cross-sectional area of the chest wall are measured. It was discussed in the literature review (Section 2.3.2.1) that asthma patients often have persistent use of accessory muscles to generate further lung expansion to overcome increased airway resistance. The contractions of accessory muscles create chest wall movement in the cranial direction (vertical) as well as the dorsal (lateral) direction. Such a pattern of movement may violate the assumption of the Konno and Mead model (1967) that the chest wall has two degrees of freedom, since it only considers cross-sectional area. Muscle activations were not recorded in this study and therefore could not confirm if accessory muscles participated during quiet breathing in the severe asthma group. Therefore it is not known if there is any violation of the Konno and Mead (1967) model assumption. The potential impact of the tidal volume estimation was also unknown since no literature was found during the literature search which reported this issue.

10.8 Further research

10.8.1 Variability

The study reported in this thesis demonstrated a wide range of variability in respiratory parameters between individuals. The results obtained from the study reported in the thesis could be used to provide data for power calculations for a larger study. This would ensure that any future study would have sufficient power to detect significant differences between healthy and severe asthma patients, if any such differences exist.

We currently do not know what constitutes a normal or optimal level of within-subject variability in respiratory parameters. To establish this, it will be necessary to recruit a large, representative sample of people and record their breathing patterns over a period of thirty minutes. A better understanding of the variability in respiratory parameters would help to establish whether variability could be used as either a selection criterion to target interventions, or as an outcome measure for evaluating therapeutic interventions.

The researcher proposes that breathing pattern should be considered on an individual basis due to the lack of any consistent pattern between individuals. It is currently not known if all elements of breathing pattern is stable over time – i.e. if patterns are consistently reproducible from one day to another in patients with different pathologies. It is therefore recommended that the reproducibility of breathing patterns within individuals should be studied by monitoring breathing patterns on separate occasions, but under the same conditions. If the breathing pattern is found to be reproducible, this has the potential to be used as an individualised outcome measure.

10.8.2 Carbon dioxide levels

The work in this thesis found no evidence that fluctuations in carbon dioxide level were associated with fluctuations in any specific elements of breathing

pattern in the asthma group. This contradicts existing unproven claims that some asthma patients are hypersensitive to changes in carbon dioxide level, so that a small change in carbon dioxide level could elicit a ventilatory response (Buteyko, 1990). Currently there is no evidence that asthma patients respond differently to changes in carbon dioxide levels. It would therefore be useful to investigate the ventilatory response to changes in carbon dioxide levels in severe asthma patients.

10.8.3 Sigh breaths

Another area that could be investigated in further detail is the distribution of sigh breaths. Both healthy individuals and severe asthma patients in this research demonstrated an episodic breathing pattern where a large number of sighs occurred in close succession. This type of pattern may contribute to hyperventilation symptoms in some severe asthma patients. Further study is essential to investigate whether such a pattern is physiologically relevant, and why it is associated with hyperventilation symptoms in asthma patients but not healthy subjects. This may throw some light onto why lower end tidal carbon dioxide levels without signs of hyperventilation have been repeatedly reported in some asthma patients.

10.8.4 Breathing pattern interventions

Published trials of breathing pattern modification in patients diagnosed with asthma have consistently reported positive results. The reported benefits include improved symptom control, improved quality of life and the reduction of medication used. The research in this thesis found no difference in respiratory parameters between severe asthma patients and healthy individuals. Therefore it is currently still unclear which patients are likely to obtain the greatest benefit from this type of intervention, or if such breathing interventions have any impact on respiratory parameters. Further research into the mechanics of breathing pattern is required to understand the mechanisms behind breathing pattern interventions. A better understanding would enable a more targeted approach to identifying suitable patients for this type of intervention.

10.8.5 Summary

Further research with regard to the variability in respiratory parameters, chest wall motion and response to changes in carbon dioxide level is required to further our understanding of the pattern of breathing in severe asthma patients. By gaining a better understanding of these aspects within and between individuals, it may enable us to improve patient care by targeting specific deficits in breathing parameters and relate specific changes in breathing pattern to improved asthma symptoms.

10.8 Conclusion

Although between group mean differences did not reach statistically significant levels, some differences in breathing pattern parameters have been observed between individuals among the healthy population and severe asthma patients. Within each group the inter-individual differences were greater than the differences between groups. Given the wide range of breathing patterns observed, it is proposed that breathing pattern should be considered on an individual basis. The term ‘breathing pattern’ may itself be misleading, because each person has their own individual idiosyncrasies related to breathing. Therefore it is probably not appropriate to compare specific groups of people with pathologies and expect to find any characteristic ‘pattern’.

The work in this thesis cast some doubt over the adequacy of describing breathing pattern purely by referring to averages of timing and volume components. More information may be derived from examining the within-subject variability of these parameters. Studies conducted by clinicians who have investigated timing and volume components did not report any differences in breathing pattern, whereas studies conducted by engineers who have looked for recognisable patterns have reported differences in air flow and volume patterns. It is recommended that further research on breathing patterns should explore beyond the averaging of volume and timing components, and perhaps investigate the possibilities of developing tidal volume or tidal flow profiles for individuals with and without respiratory conditions.

The high variability of tidal volume observed in this study contrasts with published studies that have reported lower variability. It is likely that this difference is due to the length of recording period. Shorter periods of recording are unlikely to reveal the total extent of within-subject variability. It has been demonstrated that longer recording times reveal a form of episodic breathing pattern characterised by the sporadic occurrence of sigh breaths in quick succession; therefore, it is proposed that recording time should not be less than thirty minutes if the variability of tidal volume is to be analysed.

The amount of variability observed in this study also contrasts with standard textbook descriptions and the common clinical belief that breathing in the healthy population is ‘regular’ (Rosdahl & Kowalski, 2008; Pollak, 2011; Pryor & Prasad, 2004). It is currently unclear how or if the observed variability relates to physiology or whether it is more related to other factors (e. g. emotional state).

The lack of significant association between tidal volume variability, hyperventilation symptoms and $ETCO_2$ levels suggests that it is unlikely that a ‘variable breathing pattern’ is a key contributor to symptoms of hyperventilation or any observed reduction in $ETCO_2$ level. Therefore, overall tidal volume variability might not be suitable as a selection criterion or an outcome measure for interventions such as breathing retraining, which aims to ‘regularise’ breathing pattern. However, further work is needed to establish whether specific elements of breathing, such as response to sigh breaths, are likely to be stable over time and/or respond to therapeutic interventions.

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Appendix I - Reliability and Validity of the LifeShirt – Risk Assessment form

UNIVERSITY OF Southampton
School of Health Professions and Rehabilitation Sciences

RISK ASSESSMENT FORM

To be completed in accordance with the attached guidelines

Principal Investigator: Wai Lo
Title of Project: Reliability and Validity of the LifeShirt
Ethics No: 08/-028 ver 2

Activity:

This project aims to evaluate the quality of signals collected by the LifeShirt garment when compared with the laboratory standard of respiratory monitoring system (pneumotachograph) at rest and during exercise. In the quiet breathing session, participants will be seated in a comfortable chair, wearing both LifeShirt garment and pneumotachograph. Respiratory parameters will be simultaneously monitored by the two equipment. A video will be played during recording period for entertainment. After 35 minutes of recording a 5 minutes break will be given. On returning to the laboratory, participants will wear the monitoring systems and will be asked to perform light exercise, assessed by the Borg scale of perceived exertion, on an exercise bike. Exercise session will last for 25 minutes and data will be simultaneously recorded by the two sets of equipment.

Location(s):

Gait Laboratory, Southampton General Hospital

Significant Hazards:

The potential hazards are this study will be the potential cross contamination of the facemask and the tubing circuit by individuals.
The researcher will be working alone during the data collection procedure
Participants who are unaccustomed to exercise might experience muscle soreness after exercising and up to 48 hours.

Who might be exposed/affected:
Participants

Existing control measures:

Infection control policy
Hand hygiene policy
Lone working policy
School Health and Safety Policy,
Single use disposable filter
A warm down period of 2-3 minutes is added in the protocol.
Exercise induced muscle soreness are self-limiting.

Risk evaluation: Low / Medium / High

Can the risk be further reduced: Yes / No

Further controls required:

No further controls are required.

Date by which further controls will be implemented:

Are the controls satisfactory: Yes / No

Date for reassessment: 25/07/2008

Completed by: …… …… ……
name signature date

Supervisor/manager: …… …… ……
If applicable name signature date

Risk Assessment Reviewed by Ethics Committee

Appendix II - Reliability and Validity of the LifeShirt® Participant Information Sheet

Project Title:	Reliability and Validity of the LifeShirt®
Ethics Submission No:	SHPRS-Ethics 08-028

You are invited to take part in a post-graduate student research project which looks at the accuracy of a new garment that monitors your breathing. Garments like these are being developed to help patients avoid having to come into hospital so often for tests.
Before you decide if you want to participate, it is important for you to understand why the research is being done and what it will involve for you, so please read this leaflet carefully. Talk about it with your family or friends if you want to.
Thank you for reading this.

Background

If scientists or doctors want to know more about your breathing pattern, at the moment they have to bring you into a laboratory and ask you to breathe in and out through a mask or mouthpiece. We know that some people find this a cause for anxiety, which makes them breathe differently to normal. We are currently testing a new piece of equipment called a LifeShirt, which is supposed to be able to measure your breathing without any facemask or mouthpiece, and may be able to be used at home in the future. If it is accurate and reliable it could be used to monitor people at home, or to test the effects of new therapies. In order to establish if it is accurate and reliable, we need to compare the new garment with the information we get from using a facemask. This needs to be done firstly while you are relaxing in a chair and then while you are exercising on a static bicycle

Why have I been chosen?

You have been approached because you are attending the University of Southampton, aged between18-35, currently a non-smoker and consider yourself to be healthy. We need approximately 50 people like you to take part in the study.

Do I have to take part?

No. Participation in the research is entirely voluntary. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without

giving a reason. A decision to withdraw at any time, or a decision not to take part, will not bear any consequences.

What will happen to me if I take part?

If you decide to participate, you should contact the researcher (Wai Lo, also known as Ambrose) who will answer any questions you may have (please see the end of this form for contact details). If you express an interest in taking part in this study, a researcher will ask you some questions designed to determine whether it is appropriate for you to take part or not. If you are suitable then you will be invited to attend the Gait Laboratory at Southampton General Hospital for one session which will last for approximately one and a half hours. During your visit you will be given the information sheet to read again and have an opportunity to ask questions. If you agree to take part then you will be asked to sign the consent form and complete a screening questionnaire to ensure you meet the criteria.

Your weight and height will be measured, and some measures of your chest taken to make sure we select the correct LifeShirt to fit you properly. The researcher (or a female assistant, if preferred) will then help you to put on the appropriate sized garment. It is a sleeveless vest that zips up the front. Ideally you should wear it next to your skin, but women can wear their underclothing. After the satisfactory fit of the LifeShirt you will then be asked to put on a facemask. You will be then be asked to sit down and try to relax in a comfortable chair with back rest and arms where recording of your breathing pattern will begin. During the recording, a video clip will be played for your entertainment. After 35 minutes of video watching, recording will cease and you may remove the facemask. A five minutes break will be given before the exercise session begins.

After the break the researcher will ask you to perform mild/moderate exercise on an exercise bicycle. Again the researcher will help you to put on the facemask when you are seated comfortably on the exercise bicycle. You will be asked to exercise lightly so that there is a slight increase in your heart rate. The exercise period will be for 25 minutes. After the 25 minutes recording will cease, the LifeShirt will be removed and you will be free to go.

What do I have to do?

You are not expected to do anything different from your normal routine. However, we would like you not to perform physical exercises and not to have alcohol or non-prescription drugs on the day you are due to attend. As you will be performing exercise we would also ask you to attend the session in comfortable clothing. The LifeShirt should be worn next to the skin. We should like you to be able to remove your outer clothing for your upper body, so please wear appropriate underclothing.

What are the side effects of any treatment received when taking part?

No treatment will be applied during the session. There is no known harm from using the recording equipment and therefore this study presents minimum foreseeable risk. Some people find wearing the facemask a little uncomfortable, if you find you are one of these people and the discomfort becomes intolerable, you will be advised to withdraw from the study. If you are unaccustomed to exercise, you may experience muscle soreness up to 48 hours after exercise. This is normal body response and should not be problematic.

What are the possible benefits of taking part?

There is no direct benefit for you from taking part in this study. However, the information we get might help improve the management of people with breathing conditions such as asthma, hyperventilation syndrome, COPD and other breathing disorders.

What if there is a problem?

If you have any concerns about any aspect of this study, you should ask to speak with the researcher or supervisors who will do their best to answer your questions.
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. You can contact the university using the contact number below. Alternatively you can contact the research supervisor of this project or the researcher using the contact details at the end of the form.

Will my taking part in the study be kept confidential?

Yes. All the information which is collected about you during the course of the study will be kept confidential.

You will be assigned a code number in order to give you anonymity. Your contact details and any information collected for the study will only be looked at by authorised persons from the representatives of regulatory authorities, and by authorised people from the University of Southampton to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site. Data Protection Act 1998 will strictly be followed at all times.

Who has reviewed the study?

This study was reviewed and given a favorable ethical opinion for conduct in the University of Southampton by the internal Ethics Committee of the School of Health Sciences.

If you are willing to participate in the study please complete the consent form. You may keep this information sheet for future reference.

Contact Detail

Researcher: Wai Lo BSc MCSP
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Thank you for considering taking part in this study, and taking the time to read this information sheet.

Appendix III – Screening questionnaire

Project Title:	Reliability and Validity of the LifeShirt ® during Quiet breathing and Exercise
Ethics Submission No:	SHPRS-ETHICS 08-28

UNIVERSITY OF Southampton
School of Health Sciences

Please answer the following questions:

Are you aged between 18-35? Y / N

Are you a non-smoker? Y / N

Are you able to ride an exercise bicycle? Y / N

Do you recently suffer from respiratory tract infection (Within 4 weeks) Y / N

Are you current receiving treatment from a health practitioner. Y / N

Do you have history of serious cardiovascular, respiratory or neurological impairment? Y / N

Participant No:

Height:

Weight;

Chest: **Abdomen:**

LifeShirt size

Appendix IV – Participant consent form

UNIVERSITY OF Southampton
School of Health Sciences

Project Title:	Reliability and Validity of the LifeShirt® During Quiet breathing and Exercise
Ethics Submission No:	SHPRS-Ethics-08-028 ver 2

Name of Researcher: Wai Lo
Research Supervisors: Dr. Anne Bruton
Dr. Anna Barney

Please initial to confirm

I confirm that I have read and understand the information sheet dated 14/10/2008 for the above study.	
I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
I understand that all data collected during the study may be looked at by responsible individuals from the University of Southampton or from regulatory authorities. I give permission for these individuals to have access to my data.	
I agree to take part in the above research study.	

_____ _____ _____

Signature of Participant Date Name of Participant

_____ _____ _____

Signature of Researcher Date Name of Researcher

When complete, 1 copy for participant: 1 copy for researcher site file

Appendix V – Risk Assessment form for healthy participant

UNIVERSITY OF Southampton
School of Health Sciences

Research and Enterprise Services Office
Form RA1/November 2008/V1.0

Risk Assessment Form

IMPORTANT
Please make sure that you use the most up to date version of this information by checking with the Research and Enterprise Services Office (RESO) before you start. If you have any queries please contact the Head of Research & Enterprise Services (Susan Rogers, ssr@soton.ac.uk or 023 8059 7942).

Please read the following before completing this form

Where are you sending your application for ethics approval?

If you are applying via the School of Health Sciences Ethics Committee your risk assessment will be considered as part of your application.

If you are applying via the Integrated Research Approval System (IRAS) then please make sure you obtain the appropriate signatures on this Risk Assessment document as described below
If this is a student project this risk assessment needs to be completed by the student (applicant) and supervisor (reviewer).
If this is a staff project this risk assessment needs to be completed by the Principal Investigator (applicant) and reviewed by the head of the actual research programme/area/unit relevant to the proposal.
If this is a staff project and the risk assessment is completed by a Research Assistant/Fellow, then it needs to be checked by the Principal Investigator and reviewed by the head of the actual research programme/area/unit relevant to

the proposal. If the Principal Investigator is head of the actual research programme/area/unit relevant to the proposal, then the Director of Research needs to be the reviewer

If the Principal Investigator completes the risk assessment and the applicant is the head of the actual research programme/area/unit relevant to the proposal then it needs to be reviewed by the Director of Research (reviewer).

A copy of the approved risk assessment form is then sent to the School Health and Safety Officer by the RESO Office

If you are an international student undertaking your research fieldwork entirely within your own country this risk assessment needs to be completed by you (applicant) and supervisor (reviewer)

Applicant Name:	Wai Leung Ambrose Lo
Project Title:	Reliability and validity of the LifeShirt
Type of project: (Please tick)	Staff Student
Supervisor's Name: *(if relevant – see point i above)*	Dr Anne Bruton, Dr Anna Barney
Principal Investigator's Name: *(if relevant- see point iii above)*	Wai Leung Ambrose Lo

Part 1: To be completed by the Applicant in accordance with the guidelines attached below (pages 4 & 5)
1a. Who will this research involve? *(please provide a brief description of your proposed sample and/or research site)* This research will involve healthy non-smokers.
1b. Where will this research take place?

(please provide a brief description of the research environment e.g. NHS trust premises) This study will take place in either a laboratory at the University of Southampton or the Gait Laboratory at the Southampton General Hospital. Both premises are University of Southampton premises.
1c. Will any of this research involve you working alone? *(for example working outside of office hours in your office or a lab-based environment or conducting interviews with patient in their own homes)* <u>Yes</u> *If none of the planned research involves you working alone please skip question 1d and go straight to 1e* *If any of your work will involve working alone please complete question 1d before moving on to the next section*
1d. You have indicated that some or all of this research will involve you working alone, what will this involve? This will involve the researcher working alone in the laboratory with the participants. However, the premises are supported by relevant staff. *Please also read the lone interviewing guidance (RA2), discuss and complete the lone interviewing checklist (RA3) with your supervisor/team/line manager/head of research group and submit this with the contact procedure form RA4 to the Research and Enterprise Services Office for sign off prior to completing your first interview / data collection session..*
1e. What are the health & safety issues/risks arising from the proposed research? The researcher will be working alone during the data collection procedure. Participants who are unaccustomed to exercise might experience muscle soreness after exercising and up to 48 hours.
1f. Who might be affected?

The research and the participants might be affected.		
1g What existing precautionary measures are in place? Lone working policy A warm down period of 2-3 minutes is added in the protocol. Exercise induced muscle soreness are self-limiting.		
Signatures:		
Applicant name	Applicant signature	Date
Wai Lo		24/03/2010

Please now submit this completed form with your study proposal/protocol to the appropriate person for review (please see note 1 on page 1)

Appendix VI – Ethics amendment for the healthy group

25 March 2010

Dear Maggie,

I am writing to request an amendment of the existing study 'Reliability and validity of the LifeShirt' (Ethics submission No: SHPRS-ETHICS 08-028). Having analysed the original data, I have found that I need to collect further data for a larger sample as recruitment was slow (n=11 out of 50 needed) and that some additional measures would also be useful in describing and characterising the sample population. It was also felt that removing the use of the facemask might facilitate recruitment. A summary of the amendments is provided in the table below.

Original study	Amendment
1. Sample group age 18-35	No upper age limit i.e. anyone 18 or above
2. Recruitment – poster, internet, word of mouth	Same - plus email to students with permission from Heads of Schools. Poster slightly reworded (attached)
3. Measurement a) Height, weight, BMI, chest and abdomen measurement b) Breathing pattern with both LifeShirt and pneumotachograph during quiet breathing and exercise	a) Same b) Same - without the pneumotachograph PLUS c) Lung function test d) End-tidal carbon dioxide level e) Questionnaires
4. Procedure Consent Measurement 3a (above) Measurement 3b: Participant watching video during the quiet	Same consent form Same procedure for measurement 3a (above) PLUS End-tidal carbon dioxide level will be taken after measurement 3a has been taken Measurement 3b: Participant will be filling in two questionnaires during the quiet breathing

breathing	Lung function test will be carried out after the recording of quiet breathing End-tidal carbon dioxide level again will be taken after the moderate exercise.
5. Location Gait Laboratory at the Southampton General Hospital	Participants will be offered a choice of Gait Laboratory at the Southampton General Hospital or a laboratory at building 45, University of Southampton
6. Participant information sheet	See highlighted section

Details and justification of the amendments:

1. The upper age limit of the sample population has been removed to increase the generalisability of the findings and to facilitate recruitment.

2. To encourage recruitment, permission is sought to email the student population in various Schools, with permission from their Heads of Schools.

3. The same measurements will be taken with the addition of two brief questionnaires (attached with this application), the Hospital and Anxiety Depression questionnaire and the Nijmegen hyperventilation questionnaire. Each scale will take only a few minutes to complete and additional reading material (e.g. newspaper, magazines) will be provided during the quiet breathing. This is to distract participants from thinking about their breathing. Questionnaires are being used to find out anxiety level and presence of hyperventilation symptoms as they are likely to have some influence on breathing pattern.

4. Additional data of lung function and end-tidal carbon dioxide level will be collected by laboratory standard equipment of spirometer and capnograph. The additional data will aid the characterisation of the sample population.

5. The procedure of the data collection will be slightly different to enable the collection of the additional information. The consenting procedure will be the same. The duration of the recording period will also remain the same but with the addition of end-tidal carbon dioxide being monitored noninvasively at the beginning of the quiet breathing period and at the end of the exercise period. Lung function tests will be performed at the end of the quiet breathing period to avoid effects on end-tidal carbon dioxide level.

6. To make it convenient for participants to attend the session, a choice of location (either Gait Laboratory at Southampton General

Hospital or a laboratory at the University of Southampton) will be offered to participants.

7. The participant information sheet had been amended to reflect the minor changes in the data collection procedure (section 'Background' and 'What will happen to me if I take part'). Change has been made to the section 'What if there is a problem' to keep up to date with the current university complaints procedure. Please see the attached document in which changes are highlighted.

8. A new risk assessment has also been completed and is enclosed within the application.

In summary, I requested the specific amendments as follow:

- Removal of upper age limits
- Study invitation to university students via email, with the permission of Heads of Schools
- Additional measurements of end-tidal carbon dioxide, lung function
- Additional use of two questionnaires
- Changes to procedure: record end-tidal carbon dioxide before the recording of breathing pattern, fill in questionnaire and read during the quiet breathing period; lung function data to be taken after quiet breathing period; record end-tidal carbon dioxide post-exercise.

Wai (Ambrose) Lo

PhD Student

School of Health Sciences

University of Southampton

Appendix VII – Participant information sheet

UNIVERSITY OF Southampton
School of Health Sciences

Project Title:	Reliability and Validity of the LifeShirt®
Ethics Submission No:	SHPRS-Ethics 08-028

You are invited to take part in a post-graduate student research project which looks at the accuracy of a new garment that monitors your breathing. Garments like these are being developed to help patients avoid having to come into hospital so often for tests.
Before you decide if you want to participate, it is important for you to understand why the research is being done and what it will involve for you, so please read this leaflet carefully. Talk about it with your family or friends if you want to.
Thank you for reading this.

Background
If scientists or doctors want to know more about your breathing pattern, at the moment they have to bring you into a laboratory and ask you to breathe in and out through a mask or mouthpiece. We know that some people find this a cause for anxiety, which makes them breathe differently to normal. We are currently testing a new piece of equipment called a LifeShirt, which is supposed to be able to measure your breathing without any facemask or mouthpiece, and may be able to be used at home in the future. If it is accurate and reliable it could be used to monitor people at home, or to test the effects of new therapies. Before it can be used in patients with respiratory disorders it is important for us to collect some breathing pattern information from healthy people. This needs to be done firstly while you are relaxing in a chair and then while you are exercising on a static bicycle

Why have I been chosen?

You have been approached because you are attending the University of Southampton, aged between 18 and above, currently a non-smoker and consider yourself to be healthy. We need approximately 50 people like you to take part in the study.

Do I have to take part?

No. Participation in the research is entirely voluntary. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not bear any consequences.

What will happen to me if I take part?

If you decide to participate, you should contact the researcher (Wai Lo, also known as Ambrose) who will answer any questions you may have (please see the end of this form for contact details). If you express an interest in taking part in this study, a researcher will ask you some questions designed to determine whether it is appropriate for you to take part or not. If you are suitable then you will be invited to attend either a laboratory at Building 45, University of Southampton or the Gait Laboratory at Southampton General Hospital for one session which will last for approximately one and a half hours. During your visit you will be given the information sheet to read again and have an opportunity to ask questions. If you agree to take part then you will be asked to sign the consent form and complete a screening questionnaire to ensure you meet the criteria.

Your weight and height will be measured, and some measures of your chest taken to make sure we select the correct LifeShirt to fit you properly. The researcher (or a female assistant, if preferred) will then help you to put on the appropriate sized garment. It is a sleeveless vest that zips up the front. Ideally you should wear it next to your skin, but women can wear their underclothing. After the satisfactory fit of the LifeShirt you will then be asked to wear a piece of

tubing which goes around the back on the head and rests just under your nose. You will be then be asked to sit down and try to relax in a comfortable chair with back rest and arms where recording of your breathing pattern will begin. During the recording, there will be two short questionnaires which you will be asked to fill in about how you are feeling on the day. Additional reading material (e.g. newspaper, magazines) will be provided for you to read for the remainder of the recording period. After 35 minutes, recording will cease. The tube under your nose will be removed. You will then be asked to blow into a machine to measure your lung capacity. This involves taking deep breaths and blowing as hard as you can into the machine. You will repeat this three times and the highest reading will be recorded. You will have a five minute break after the lung function test.

After the break the researcher will ask you to perform moderate exercise on an exercise bicycle, while still wearing the garment. You will be asked to exercise so that there is an increase in your heart rate. The exercise period will be for 20 minutes. After 20 minutes recording will cease and you will be encouraged to 'warm-down' i.e. to continue cycling gently for a period of 2-3 minutes. You will then wear the plastic tube under your nose again for several minutes. After this the session is ended and all recording equipment will be removed and you will be free to go.

What do I have to do?

You are not expected to do anything different from your normal routine. However, we would like you not to perform physical exercises and not to have alcohol or non-prescription drugs on the day you are due to attend. As you will be performing exercise we would also ask you to attend the session in comfortable clothing. The LifeShirt should ideally be worn next to the skin. We should like you to be able to remove your outer clothing for your upper body, so women should wear appropriate underclothing.

What are the side effects of any treatment received when taking part?

No treatment will be applied during the session. There is no known harm from using the recording equipment and therefore this study presents minimum foreseeable risk. If you are unaccustomed to exercise, you may experience muscle soreness that can start up to 48 hours after exercise and may persist for a few days. This is a normal body response and should not be problematic. If you are concerned, or the discomfort persists for more than 5 days, please seek guidance from your Primary Care physician and let the researcher know.

What are the possible benefits of taking part?

There is no direct benefit for you from taking part in this study. However, the information we get might help improve the management of people with breathing conditions such as asthma, hyperventilation syndrome, COPD and other breathing disorders.

What if there is a problem?

If you have a concern or a complaint about this study you should contact Susan Rogers, Head of Research & Enterprise Services, at the School of Health Sciences (Address: University of Southampton, Building 67, Highfield, Southampton, SO17 1BJ ; Tel: +44 (0)23 8059 7942; Email: S.J.S.Rogers@soton.ac.uk). If you remain unhappy and wish to complain formally Susan Rogers can provide you with details of the University of Southampton Complaints Procedure."

Will my taking part in the study be kept confidential?

Yes. All information about you that is collected in this study will be kept strictly confidential. You will be assigned a code number in order to give you anonymity. Your contact details and any information collected for the study will only be looked at by authorised persons from the representatives of regulatory authorities, and by authorised people from the University of Southampton to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal

your identity will be disclosed outside the research site. Data Protection Act 1998 will strictly be followed at all times.

Who has reviewed the study?

This study was reviewed and given a favourable ethical opinion for conduct in the University of Southampton by the internal Ethics Committee of the School of Health Sciences.

If you are willing to participate in the study please complete the consent form.

You may keep this information sheet for future reference.

Contact Details

Researcher: Wai Lo BSc MCSP
MPhil/PhD student
School of Health Sciences
University of Southampton
+44 238059 4727
wlal106@soton.ac.uk

Supervisor: Dr Anne Bruton PhD
Reader in Respiratory Rehabilitation
School of Health Sciences
University of Southampton
+44 238059 5283
ab7@soton.ac.uk

Dr Anna Barney PhD
Senior Lecturer
Institute of Sound and Vibration Research
University of Southampton
+44 238059 3734
ab3@soton.ac.uk

Thank you for considering taking part in this study, and taking the time to read this information sheet.

Appendix VIII – Participant invitation letter

Southampton NHS
University Hospitals NHS Trust

UNIVERSITY OF Southampton
School of Medicine

Allergy Asthma and Inflammation Research Group
IIR Research Division
Mail Point 810
Level F, South Block
Southampton General Hospital
Tremona Road
Southampton, SO16 6YD
phh1@southampton.ac.uk

Peter Howarth
BSc (Hons), DM, FRCP

Date

Dear

Ethics Committee ref: 09/H0502/37

Introduction to the Wessex severe asthma cohort

I am writing to invite you to take part in a PhD research study which aims to enhance our understanding of the causes and investigate possible new treatments for people with severe asthma.
We hope to enroll 30 patients with severe asthma as participants in this study from Southampton or Wessex. Your name has been selected as you have

taken part in a previous study ‘Introduction to the Wessex severe asthma cohort’. The attached patient information sheet will give you more information about this part of the study and what will be expected of you.

This is an extension of the study ‘Introduction to the Wessex severe asthma cohort’ and will form part of a postgraduate research programme. It has been reviewed by the University of Southampton and has been approved by the NHS Research Ethics Committee. If you do not wish to participate in this study then please disregard this letter and attached patient information sheet. Please be assured that if you do not wish to participate in this study it will not influence any health care you receive currently or in the future.

Please read carefully the attached **Patient Information Sheet**.

If you would like more information please ring Wai Lo who is coordinating this study,
on (023) 8079 8427.

We appreciate your help in this important research.

Yours sincerely,

Dr Peter Howarth
Consultant Physician
Head of Clinical Allergy Services
Southampton University Hospitals Trust

Appendix IX – Wessex Asthma Cohort Participant Information Sheet

University of Southampton
School of Health Sciences

Project Title: Wessex Severe Asthma Cohort

R&D office number: RHM MED0834

You are invited to take part in a research project which looks at breathing pattern in patients with asthma.
Before you decide if you want to participate, it is important for you to understand why the research is being done and what it will involve for you, so please read this leaflet carefully. Talk about it with your family or friends if you want to. Thank you for reading this.

Background

We have reasons to believe that people with asthma may have a different breathing pattern in comparison with people without asthma. It is possible that a difference in breathing pattern may contribute to some of the symptoms that people with asthma may experience. Until recently, testing this theory meant measuring breathing with equipment attached by a mask or mouthpiece. We now have a way to measure breathing pattern without either of these, which involves just wearing a sleeveless garment. In order to establish if there is any difference in breathing pattern between asthma patients and healthy individuals, we would like to record the breathing pattern of people with asthma.

Why have I been chosen?

You have been approached because you are aged 18 or above, you have received a diagnosis of asthma from your doctor, and your asthma symptoms are difficult to control. We would like to recruit 20 people like you to take part in the study.

Do I have to take part?

No. Participation in the research is entirely voluntary. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your treatment now, or in the future.

What will happen to me if I take part?

If you decide to participate, please contact the researcher (Wai Lo, known as 'Ambrose') who will answer any questions you may have (please see the end of this form for contact details). If you still want to participate in the project, you will be invited to attend the Gait Laboratory at Southampton General Hospital for one session which will last for approximately one hour and fifteen minutes. During your visit you will be given the information sheet to read again and have an opportunity to ask questions. If you agree to take part then you will be asked to sign a consent form and complete a screening questionnaire to ensure you meet the study criteria.

Your weight, height, chest and abdomen measurement will be measured to make sure the breathing pattern garment fits you properly. The researcher will then show you how to put on the garment and you can then get changed in private. After the satisfactory fit of the garment you will be asked to wear a plastic tube with tips that rest under your nose. This device is to measure the amount of carbon dioxide in the air that you are breathing out.

You will be then be asked to sit and relax in a comfortable chair with back and arm rests where recording will begin. During the recording, there will be three short questionnaires (Hospital Anxiety and Depression Scale, Asthma Control questionnaire and Nijmegen questionnaire) which you will be asked to fill in. Additional reading material (e.g. newspaper, magazine) will be provided for you to read for the remainder of the recording period. After 30 minutes, recording will cease and you will be given a five minutes break before the exercise session begins.

After the break the researcher will ask you to perform moderate exercise on an exercise bicycle. You will be wearing the breathing pattern garment during the exercise period. You will be asked to exercise at a moderate level for 20 minutes. After the 20 minutes recording, you will be asked to wear the nasal tube again to measure the carbon dioxide in the air you are breathing out. After this, the session is ended and you will be free to leave the laboratory.

What do I have to do?

You are not expected to do anything different from your normal routine. However, we would like you not to perform physical exercises and not to have alcohol or drugs on the day you are due to attend. As you will be performing exercise we would kindly ask you to attend the session in comfortable clothing. The breathing pattern garment should be worn next to the skin. We should like you to be able to remove your outer clothing from your upper body, so please wear appropriate underclothing.

What are the side effects of any treatment received when taking part?

No treatment will be applied during the session. There is no known harmful effect from using the recording equipment which presents minimal foreseeable risk. Muscle soreness is a potential side effect from unaccustomed exercise and may occur 24-48 hours after exercise. This is a normal body response and it should not be problematic. There is also a potential risk of an exercise induced asthma attack. However, as you would be exercising at only moderate intensity, it is unlikely that this would happen. The laboratory is a controlled environment within the hospital, and you would have immediate medical access if required. If you know you have a tendency to exercise induced asthma, we ask you to let us know before taking part.

What are the possible benefits of taking part?

There is no direct benefit from you taking part in this study. However, the information we get might help improve the management of people with asthma in future. You will also receive a token sum of £20 towards your time and travel costs.

What if there is a problem?

If you have a concern or a complaint about this study you should contact Susan Rogers, Head of Research & Enterprise Services, at the School of Health Sciences (Address: University of Southampton, Building 67, Highfield, Southampton, SO17 1BJ ; Tel: +44 (0)23 8059 7942; Email: S.J.S.Rogers@soton.ac.uk). If you remain unhappy and wish to complain formally Susan Rogers can provide you with details of the University of Southampton Complaints Procedure.

Will my taking part in the study be kept confidential?

You will be assigned a number in order to give you anonymity. Your contact details and any information collected for the study will only be looked at by authorised persons from the representatives of regulatory authorities, and by authorised people from the University of Southampton to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site. Data Protection Act 1998 will strictly be followed at all times.

Who has reviewed the study?

This study was reviewed and given a favourable ethical opinion for conduct in the Southampton General by the Local NHS Ethics Committee. Ethics number:

If you are willing to participate in the study please fill in the reply slip, indicating your interest in taking part in the study. Alternatively you can also express your interest using the contact details below. If you respond, the researcher will contact you to arrange a suitable date for attending. You may keep this information sheet for future reference.

Thank you for reading this information sheet.

Contact Detail

Researcher: Wai Leung Ambrose Lo BSc MSC MCSP
PhD student
School of Health Sciences
University of Southampton
+44 238059 4727
wlal106@soton.ac.uk

Supervisor: Dr Anne Bruton PhD
Reader in Respiratory Rehabilitation
School of Health Sciences
University of Southampton
+44 238059 5283
ab7@soton.ac.uk

Dr Anna Barney PhD
Senior Lecturer
Institute of Sound and Vibration Research
University of Southampton
+44 238059 3734
ab3@soton.ac.uk

Appendix X - Reply Form

UNIVERSITY OF Southampton
School of Health Sciences

Introduction to the Wessex severe asthma cohort
(Breathing pattern recording)
Ethics Committee ref: 09/H0502/37

I, (Please insert your full name)……. am interested in taking part in the breathing pattern recording phase of the study 'Introduction to the Wessex severe asthma cohort'

I am happy to be contacted by the researcher conducting this part of the study i.e. Mr. Wai Lo (known as Ambrose)
.

My contact details are:
Postal
address:……
……
……
Email:
……
Telephone:…….

…….. ……..
Signature Date

Please return this form to:
Mr. Wai Lo
PhD student
School of Health Sciences, Bg 45
University of Southampton
SO17 1BJ

Appendix XI -Abstract for European Respiratory Society Congress 2010

Lo, W. Bruton, A. Barney, A. (2010) 'Does garment-embedded respiratory inductive plethysmography show equivalent changes in respiratory parameters to a pneumotachograph?'. European Respiratory Journal At European Respiratory Society Congress, Barcelona, Spain 18-22 Sept 2010

Title: Does garment-embedded respiratory inductive plethysmography (RIP) show equivalent changes in respiratory parameters to a pneumotachograph (P_{NT})?

Aim: P_{NT} is the gold standard for measuring respiratory parameters. RIP is a validated technology, recently embedded into garments. This study investigates the validity of this new approach.

Method: Data were simultaneously recorded by the garment-embedded RIP and P_{NT} in 11 healthy volunteers. Tidal volume (V_t) and expiration time (T_e) were recorded per breath during 30 minute periods of rest and 20 minute periods of moderate exercise on a cycle ergometer. Matched cycles were those recorded by both devices, unmatched cycles by one device only.

Results: *Matched cycles.* Mean V_t between devices was strongly correlated for rest and exercise (r=0.8). No statistical difference in V_t variability was observed between devices during either period. ICC for T_e was 0.8 during rest and exercise. Significant differences in T_e between devices were observed in all participants at rest (μ=0.5sec) but not during exercise (μ=0.05sec).

Unmatched and matched cycles. Mean V_t between devices was strongly correlated for rest and exercise (r=0.8). V_t variability was higher than for matched cycles alone during both periods but no statistically significant differences between devices were observed. ICCs for T_e were 0.9 at rest and 0.8 during exercise. Significant differences in T_e between devices were observed in all participants at rest (μ=0.6sec) but not during exercise (μ=0.05sec).

Conclusion: RIP records proportionate changes of V_t relative to P_{NT} and results for matched cycles only were not different to those for matched and unmatched cycles together. Breath by breath analysis using RIP without P_{NT} is therefore feasible which may be clinically advantageous.

Appendix XII – Abstract: Association Respiratory Technology and Physiology Conference, 2010

Lo, W. Bruton, A. Barney, A. (2010) 'The validity of an instrumented garment to measure relative changes in respiratory parameters during quiet breathing and exercise. At Association of Respiratory Technology and Physiology, Heathrow, London 28 -30th Jan 2010

Title: The validity of an instrumented garment to measure relative changes in respiratory parameters during quiet breathing and exercise.

Breathing pattern monitoring outside a clinical setting is desirable in patients with respiratory disorder, as it would give a more accurate picture of patients' breathing pattern during real life activities rather than just a 'snap shot' during a routine clinical visit. This could lead to earlier diagnosis of developing complications and result in appropriate treatment being given more swiftly. In the past, the primary limitation to recording accurate breathing pattern non-invasively outside the laboratory has been reported to be technical difficulties with recording equipment. In recent years, various instrumented garments have been developed which claim to allow accurate breathing monitoring outside the clinical setting.

Aims: To explore the validity of a respiratory inductive plethysmography (RIP) based garment in measuring relative changes of respiratory parameters, during quiet breathing and exercise.

Methods: This study used a concurrent design where respiratory parameters were simultaneously measured by RIP and pneumotachograph (P_{NT}). The RIP garment was calibrated with a variation of the Qualitative Diagnostic Calibration[1]. Eleven healthy volunteers (age between 18-35) were recruited in the study. Tidal volume (V_t) and expiration time (T_e) were simultaneously recorded on a breath by breath basis during a session of 35 minutes of quiet breathing and 25 minutes of mild exercise. Signals were analysed in MatLab.

Results: The correlation of coefficient of mean V_t between RIP and P_{NT} was significantly correlated ($p<0.001$) at 0.8 during quiet breathing and 0.8 exercise. Mean coefficient of variation of V_t between devices was significantly correlated ($p<0.001$) at 0.8 during quiet breathing and 0.8 during exercise. The mean difference in T_e between RIP and P_{NT} was 0.5 sec during quiet breathing and 0.1 sec during exercise. Significant differences in T_e between devices were observed in all participants during quiet breathing and in seven participants during exercise. Bland & Altman analysis showed that RIP had overestimated T_e than P_{NT}. 95% limits of agreement of T_e was -1.0 to 1.9 during quiet breathing and -0.69 to 0.74 exercises. ICC of T_e between RIP and P_{NT} for quiet breathing was 0.8 and 0.9 for exercise. Standard error of measurement between devices was 0.5 sec during quiet breathing and 0.3 sec during exercise.

Conclusions: The results of this study demonstrated that the RIP garment can accurately measure relative changes in tidal volume during both quiet breathing and exercise. Results also supported that the garment is as good as the P_{NT} in measuring variability of tidal volume V_t in breathing. Although a significant difference in T_e was observed, the differences between devices were relatively small and are unlikely to be of clinically significant.

Appendix XIII -Abstract for European Respiratory Society Congress 2012

Lo, W. Bruton, A. Barney, A. (2012) 'Can breathing pattern parameters be differentiated between healthy and severe asthma patients?' At European Respiratory Society Congress, Vienna, Austria

Title: Can breathing pattern parameters be differentiated between healthy and severe asthma patients?

Background: Abnormal breathing patterns during acute episodes of asthma are common. However, currently little is known about breathing pattern parameters (BPP) in severe asthma (SA) patients during the asymptomatic phase, or how they relate to those in the healthy population.

Aim: To determine which BPP differentiate SA patients from healthy volunteers.

Method: Ten SA patients and 10 healthy volunteers were recruited. BPP were monitored over a 30 minute period by respiratory inductive plethysmography garment. Recorded respiratory parameters included: 1. Tidal volume (Vt); 2. Variability in tidal volume (VVt); 3. Expiration time (Te); 4. Symptoms of hyperventilation (SH); 5. End-tidal carbon dioxide ($ETCO_2$). [VVt was assessed by coefficient of variation (CV)]. Time series of breath by breath Vt were inspected for abnormal pattern. SH were assessed by Nijmegen questionnaire (NQ). [$ETCO_2$ was monitored by capnography (sampled at 4 seconds epoch)]. Differences between healthy volunteers and SA patients were explored using one-way ANOVA.

Results: Mean NQ score was higher in SA patients than in healthy volunteers ($p=0.00$). $ETCO_2$ levels were significantly correlated with NQ score ($r =-0.8$, $p <0.01$) in the SA patients but not in healthy volunteers ($r= -0.6$, $p>0.01$). Time series analysis revealed sporadic episodes of frequent sighs in both groups. No significant differences between groups for any BPP were identified.

Conclusion

The recorded BPP did not differentiate between the SA patients and healthy volunteers in our small study. The higher SH found in the SA group do not appear to be associated with differences in BPP. This study raised doubt that there is a 'pattern' that is common within the SA population and therefore BPP must be considered on an individual basis.

Appendix XIV – Individual breathing pattern graphs.

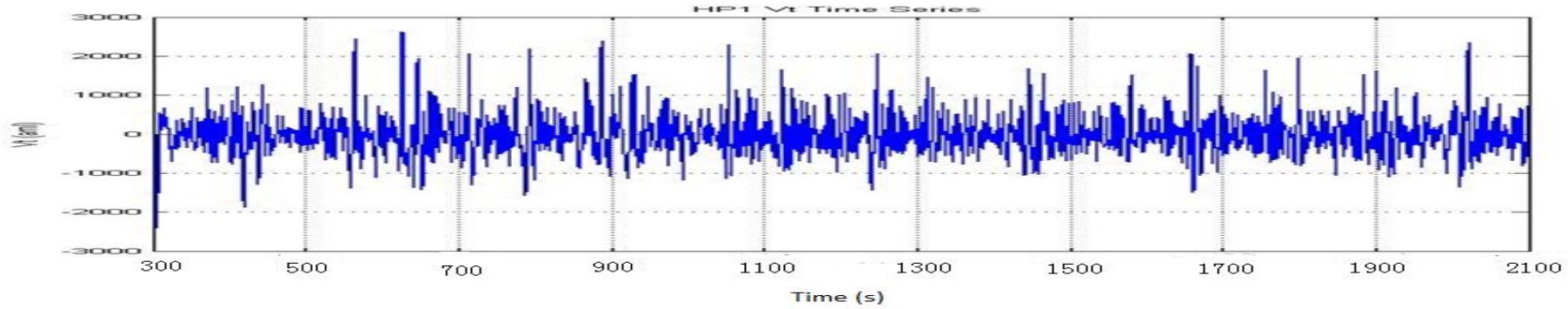

Figure 65: Time series for tidal volume for healthy participant 1

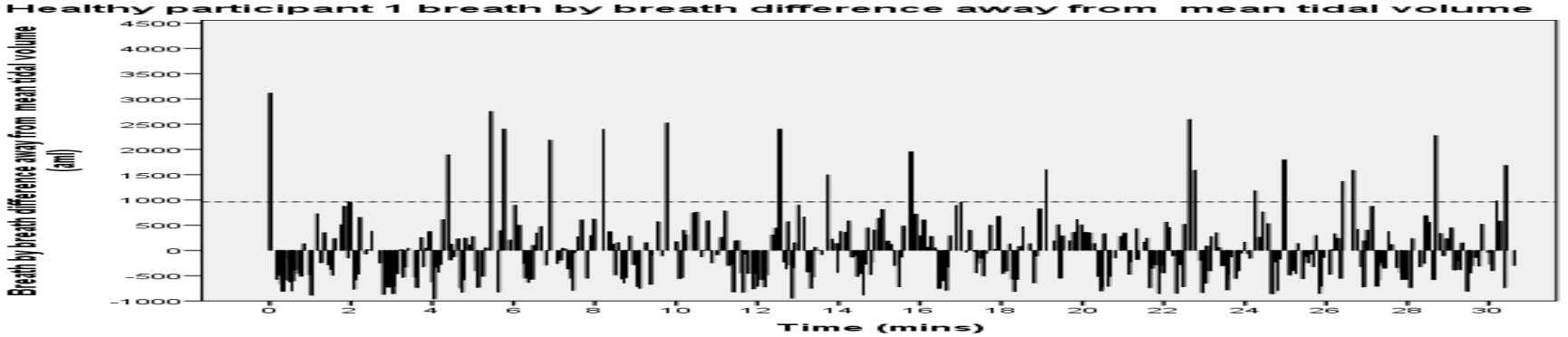

Figure 66: Breath by breath difference away from mean graph for healthy participant 1

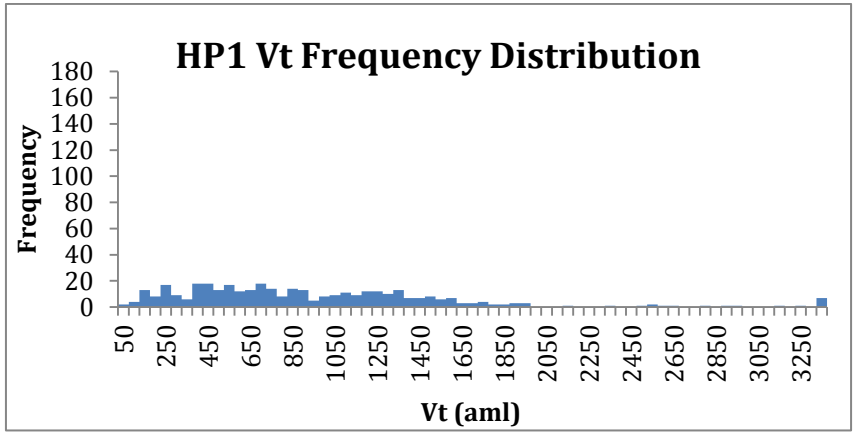

Figure 67: Frequency distribution of tidal volume for healthy participan1

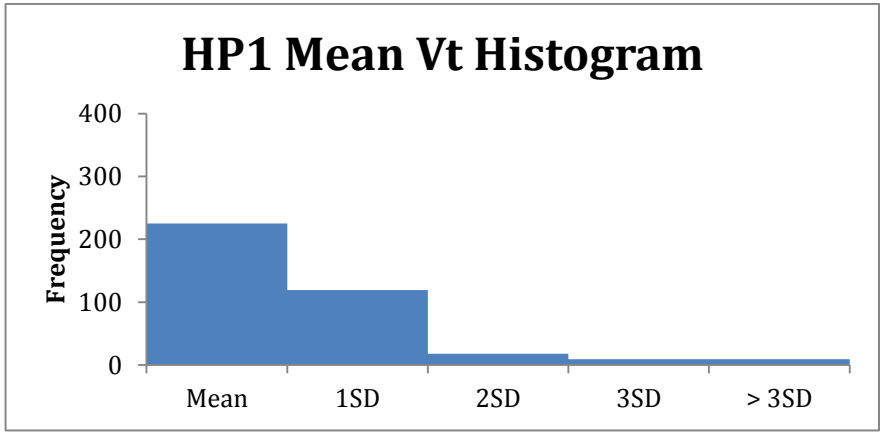

Figure 68: Histogram of tidal volume for healthy participant 1.

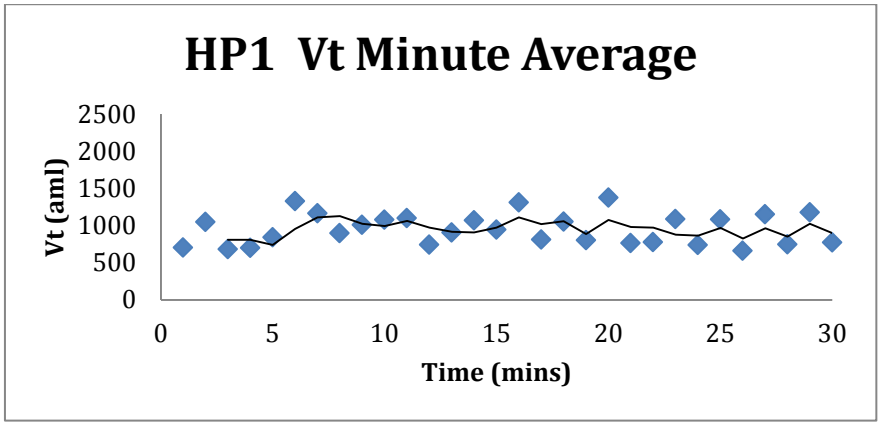

Figure 69: Tidal volume minute average for healthy participant 1.

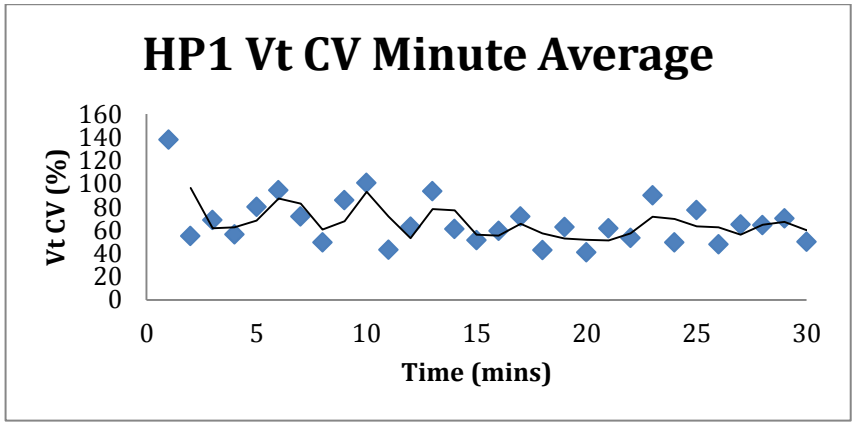

Figure 70: Tidal volume variability minute average for healthy participant 1.

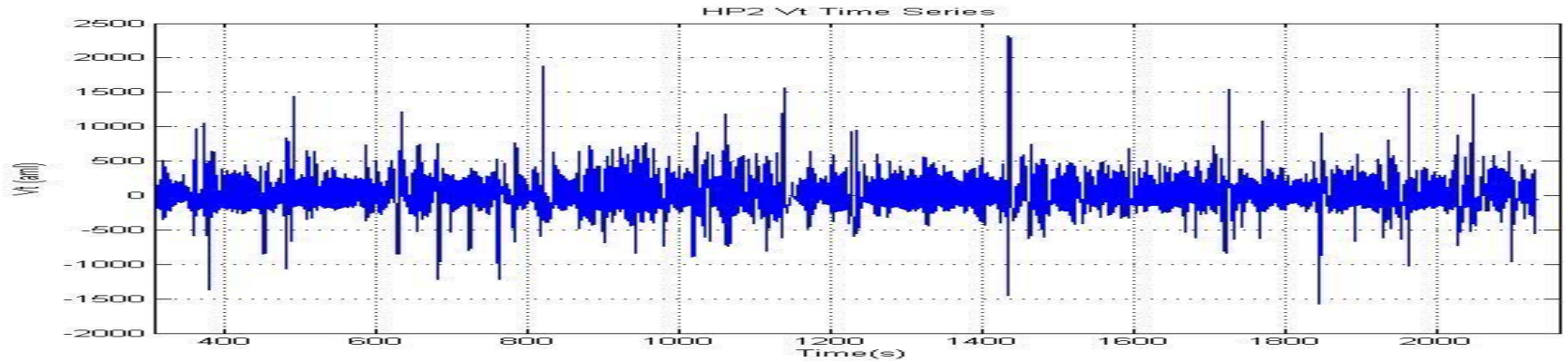

Figure 71: Time series for tidal volume for healthy participant 2

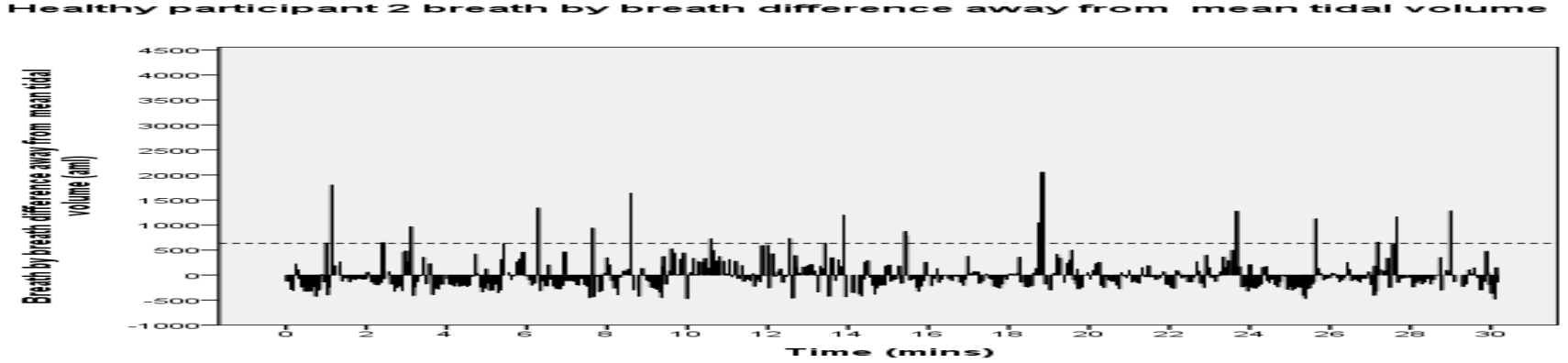

Figure 72: Breath by breath difference away from mean graph for healthy participant 2

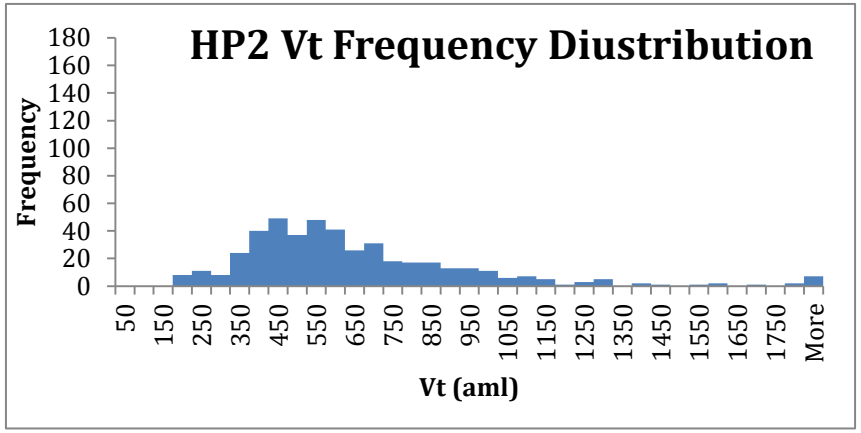

Figure 73: Frequency distribution of tidal volume for healthy participant 2.

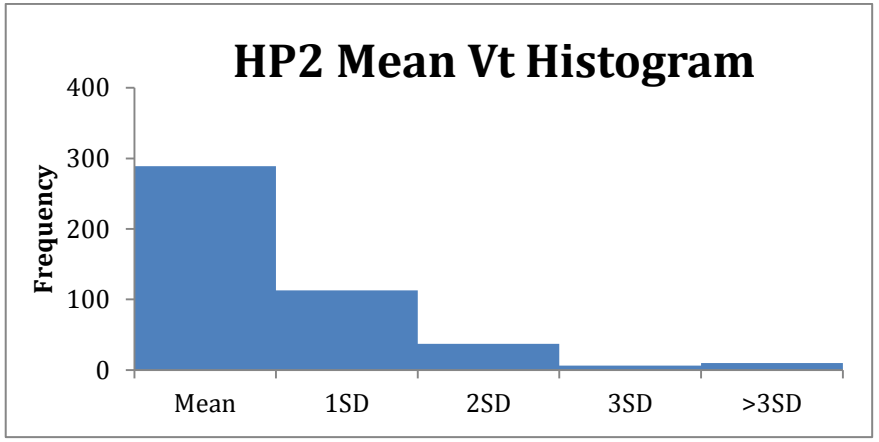

Figure 74: Histogram of tidal volume for healthy participant 2.

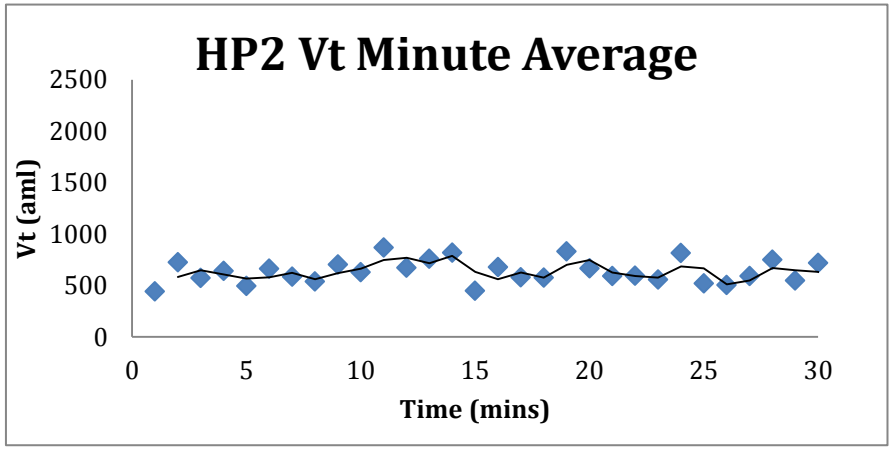

Figure 75: Tidal volume minute average for healthy participant 2.

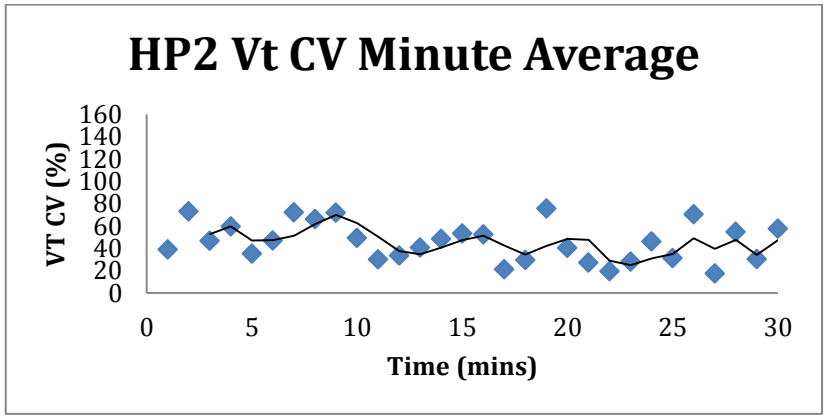

Figure 76: Tidal volume variability minute average for healthy participant 2

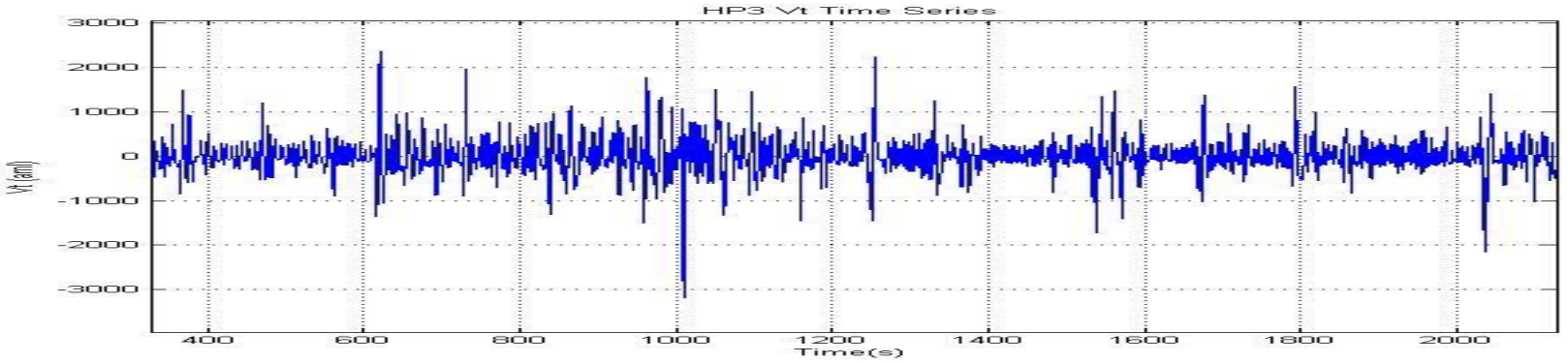

Figure 77: Time series for tidal volume for healthy participant 1

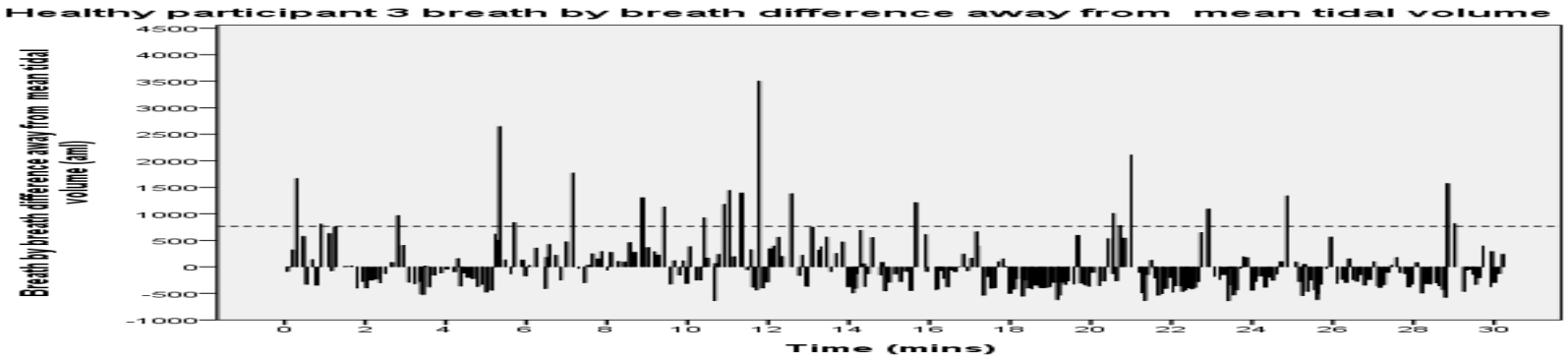

Figure 78: Breath by breath difference away from mean graph for healthy participant 3.

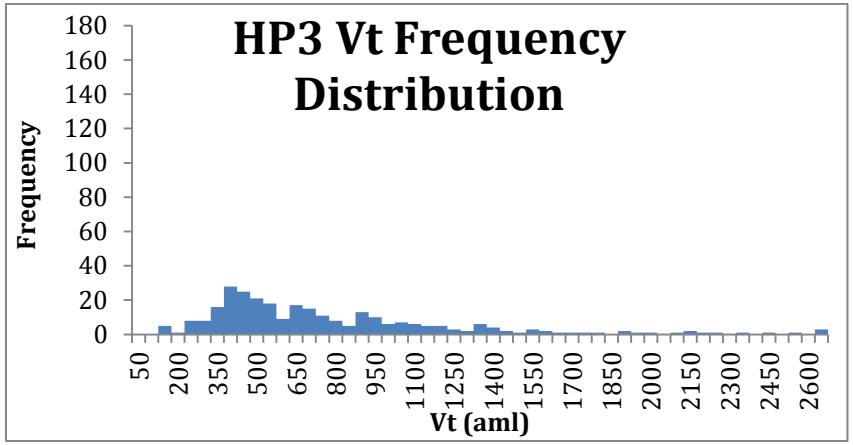

Figure 79: Frequency distribution of tidal volume for healthy participant 3.

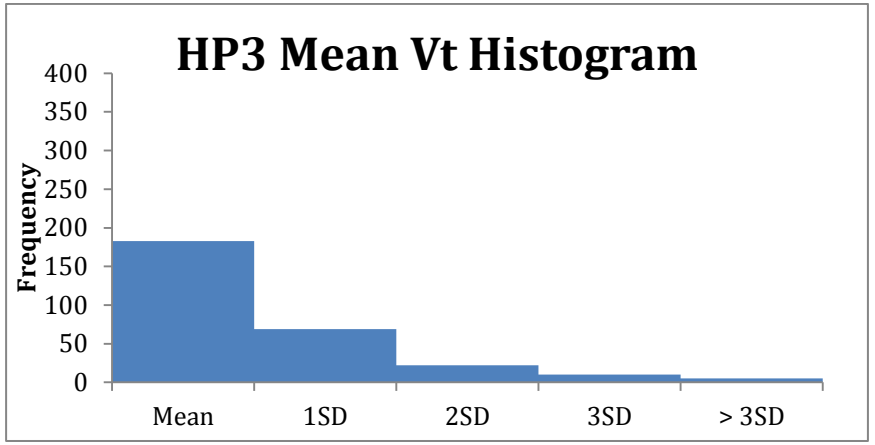

Figure 80: Histogram of tidal volume for healthy participant 3.

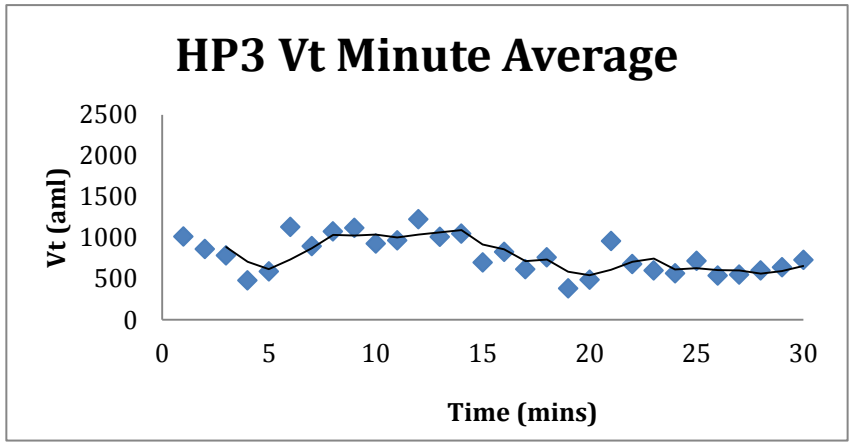

Figure 81: Tidal volume minute average for healthy participant 3.

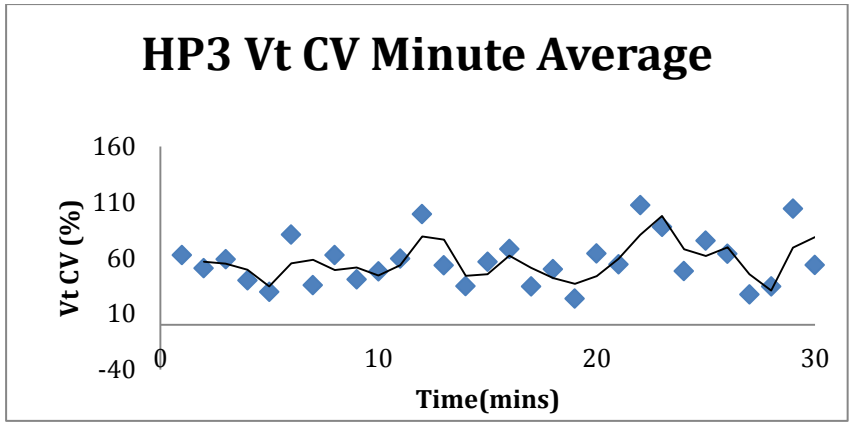

Figure 82: Tidal volume variability minute average for healthy participant 3.

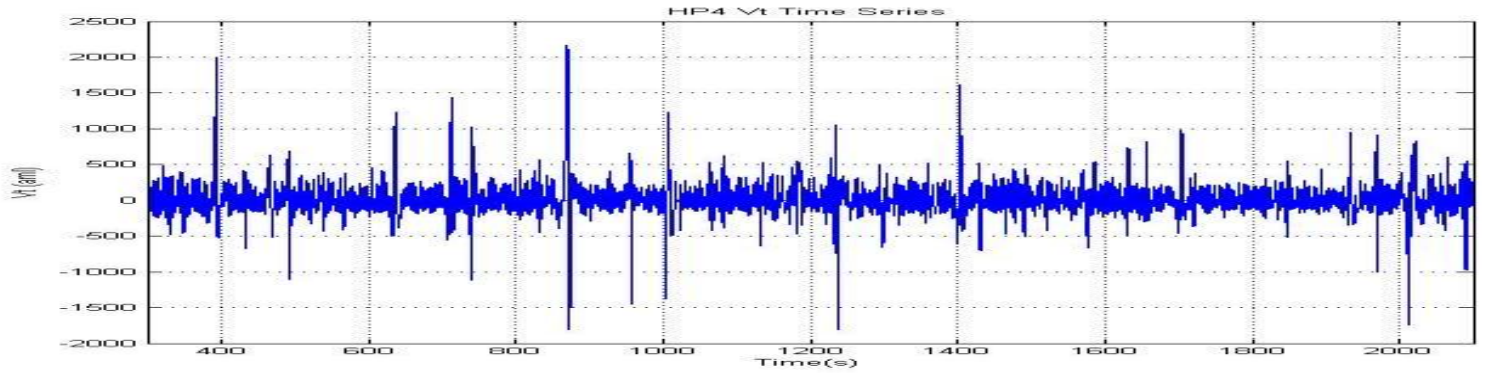

Figure 83: Time series for tidal volume for healthy participant 4.

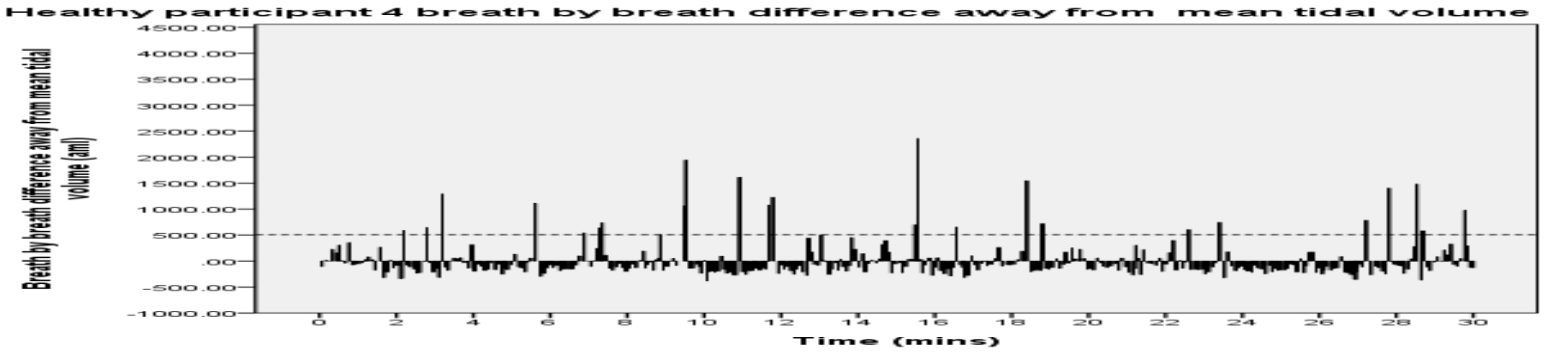

Figure 84: Breath by breath difference away from mean graph for healthy participant 4.

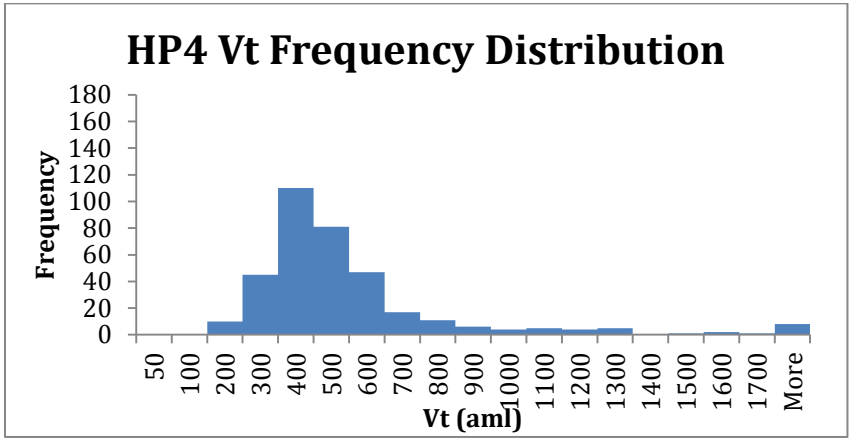

Figure 85: Frequency distribution of tidal volume for healthy participant 4.

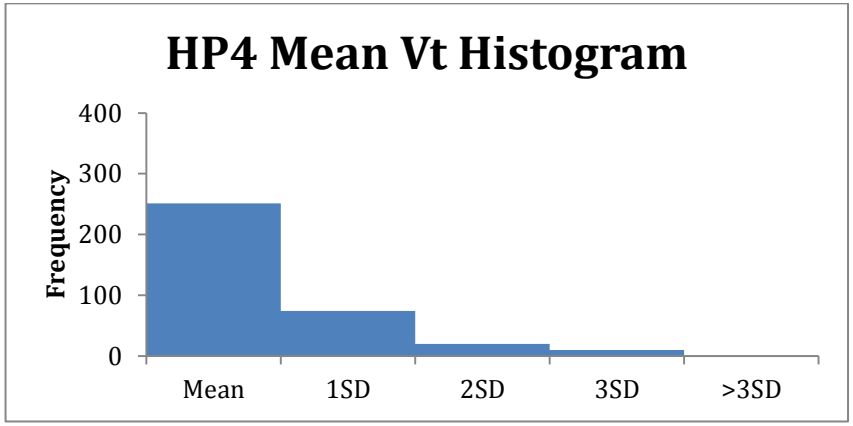

Figure 86: Histogram of tidal volume for healthy participant 4

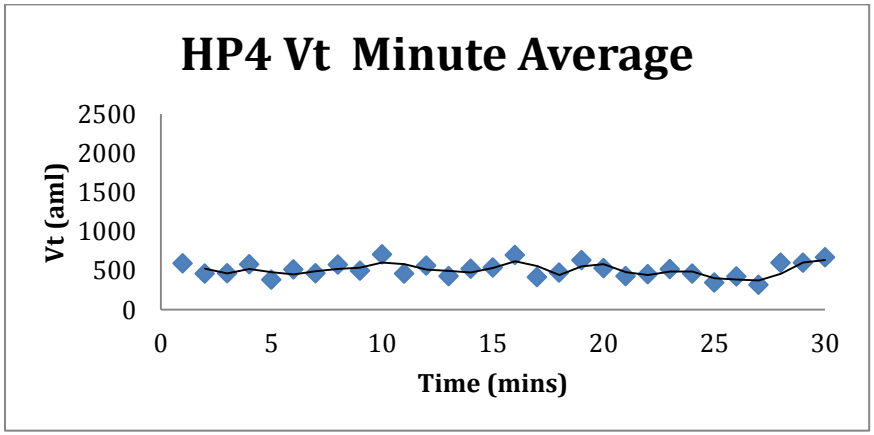

Figure 87: Tidal volume minute average for healthy participant 4

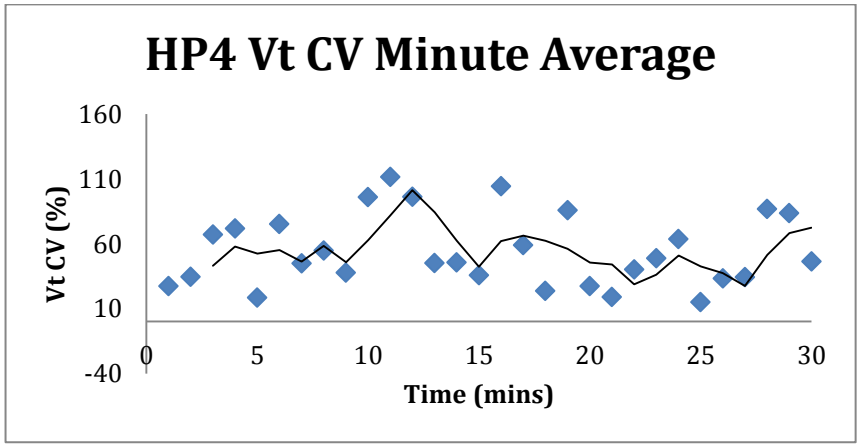

Figure 88: Tidal volume variability minute average for healthy participant 4.

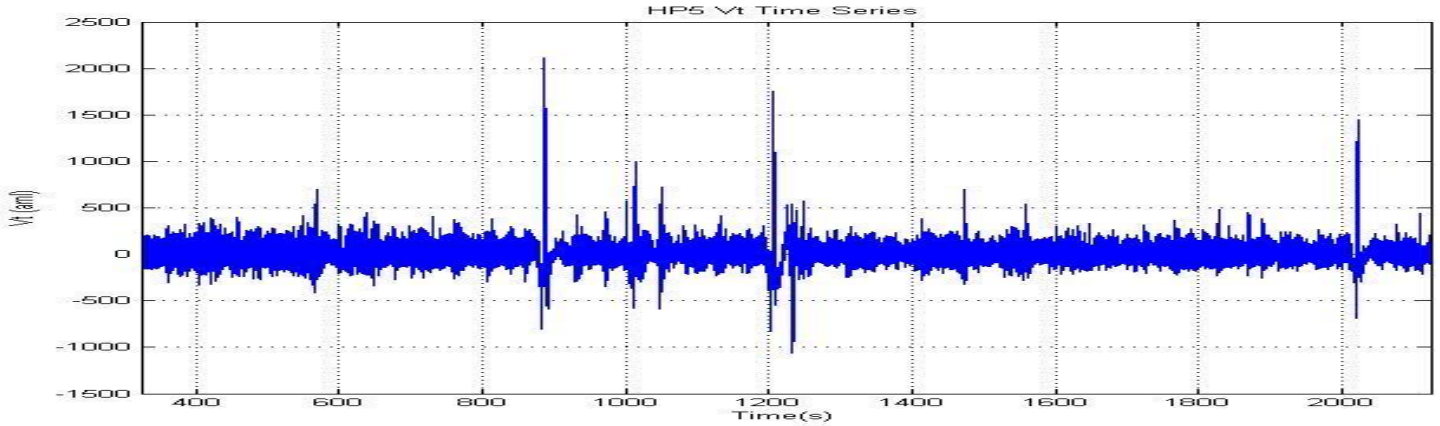

Figure 89: Time series for tidal volume for healthy participant 5.

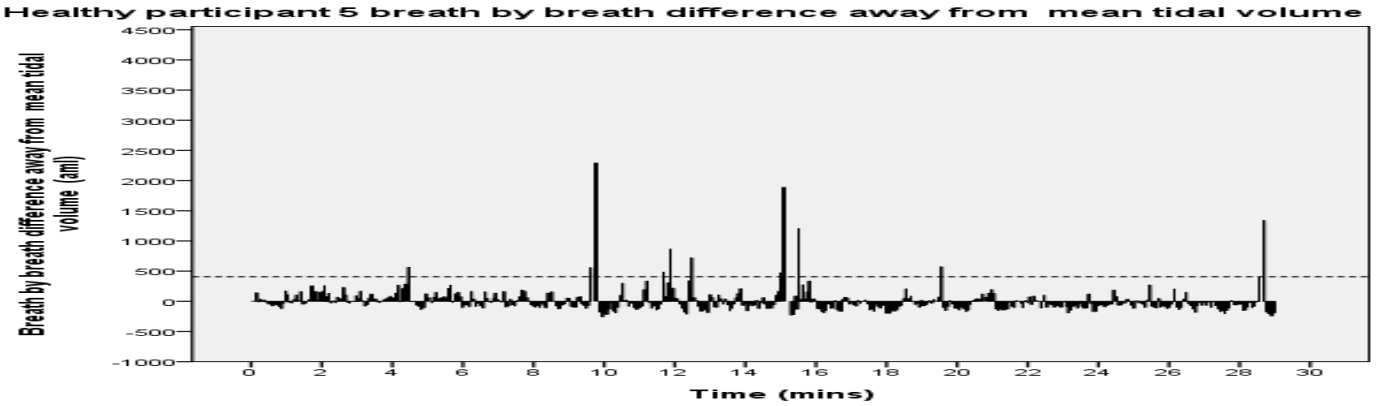

Figure 90: Breath by breath difference away from mean graph for healthy participant 5.

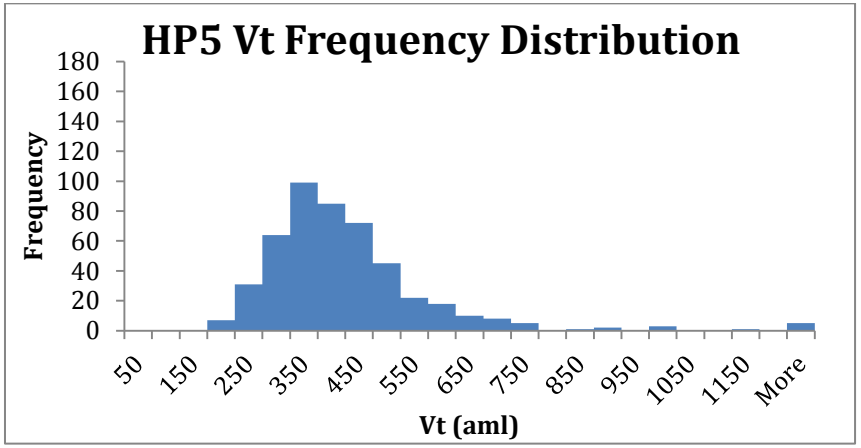

Figure 91: Frequency distribution of tidal volume for healthy participant 5

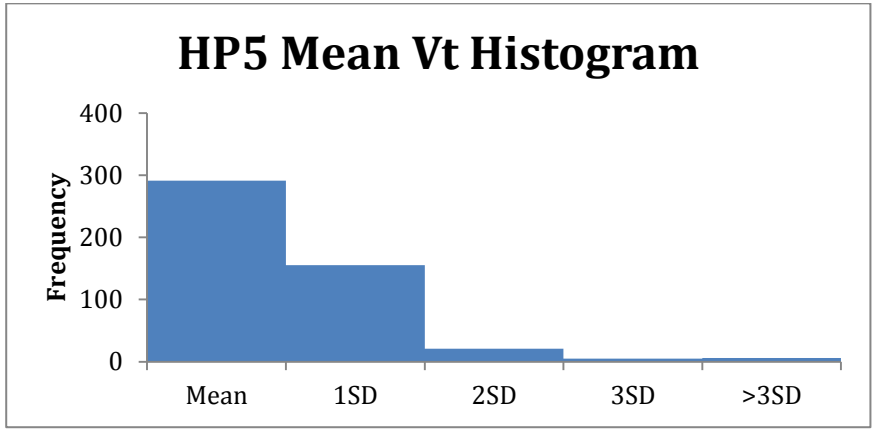

Figure 92: Histogram of tidal volume for healthy participant 5.

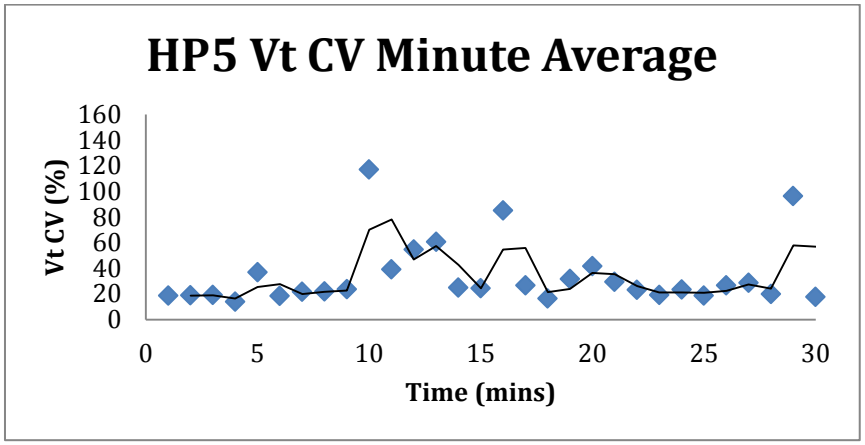

Figure 93: Tidal volume minute average for healthy participant 5.

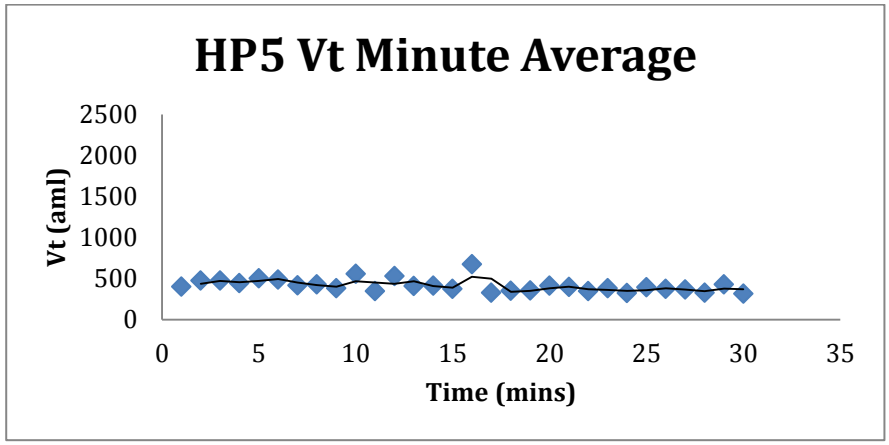

Figure 94: Tidal volume variability minute average for healthy participant 5.

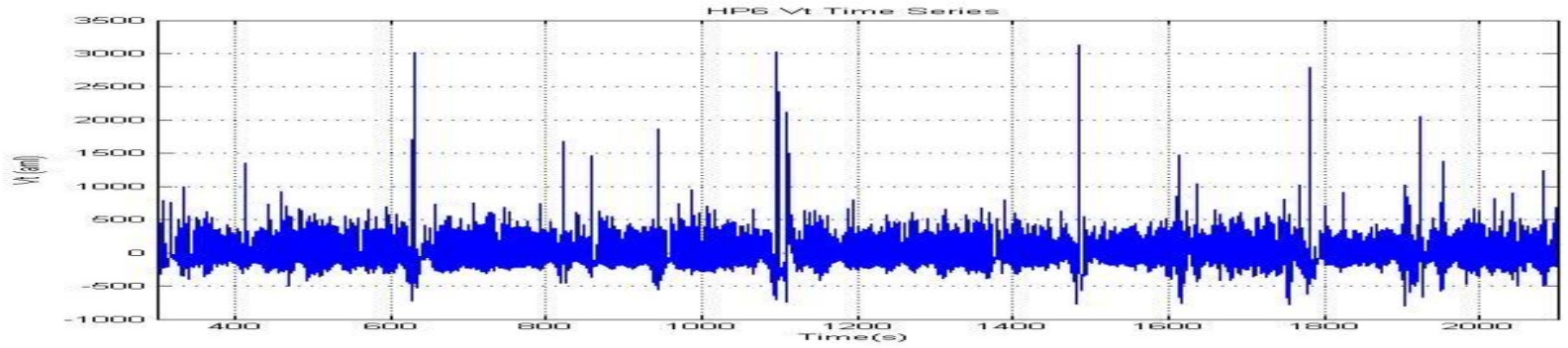

Figure 95: Time series for tidal volume for healthy participant 6.

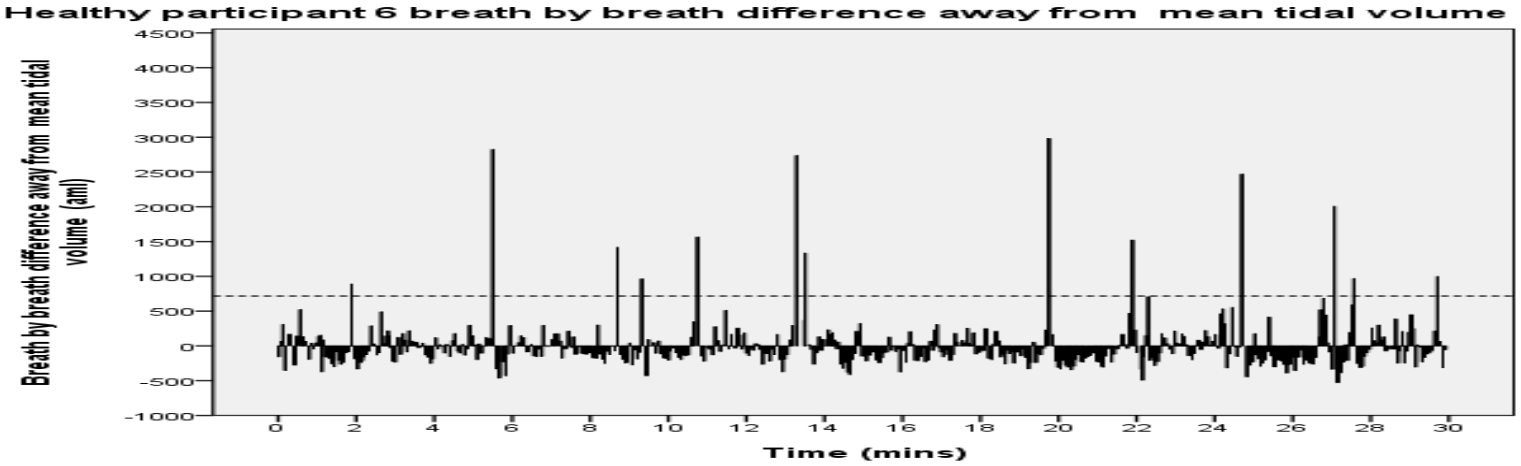

Figure 96: Breath by breath difference away from mean graph for healthy participant 6.

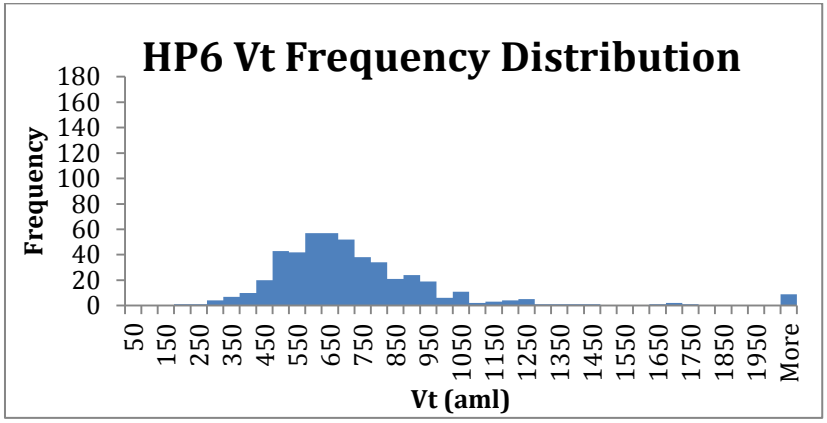

Figure 97: Frequency distribution of tidal volume for healthy participant 6.

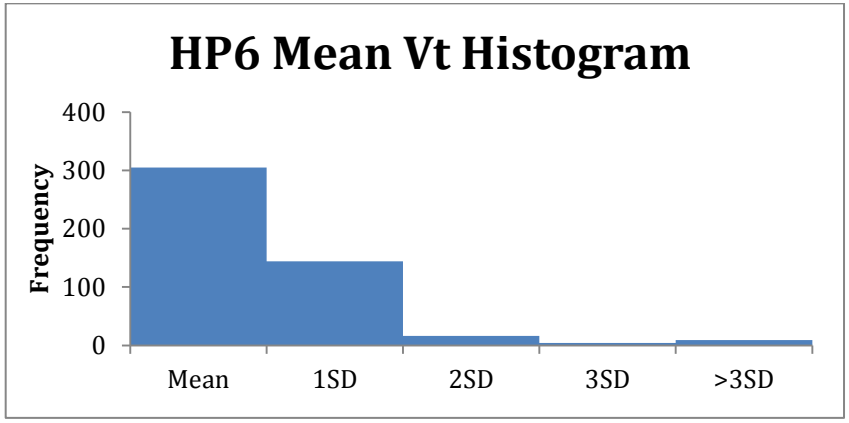

Figure 98: Histogram of tidal volume for healthy participant 6.

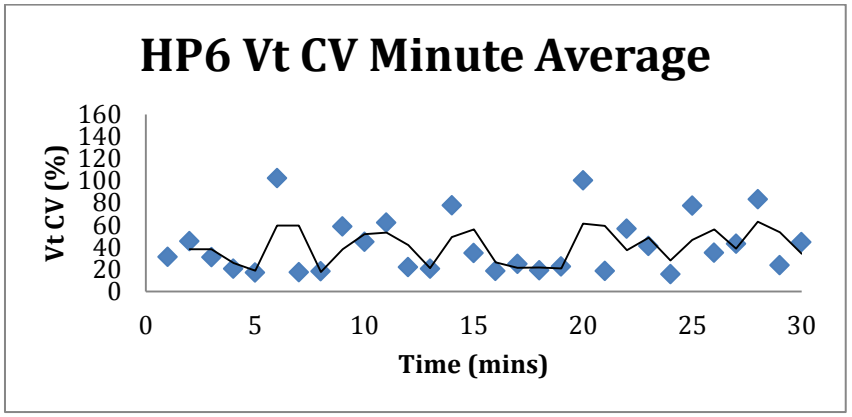

Figure 99: Tidal volume minute average for healthy participant 6.

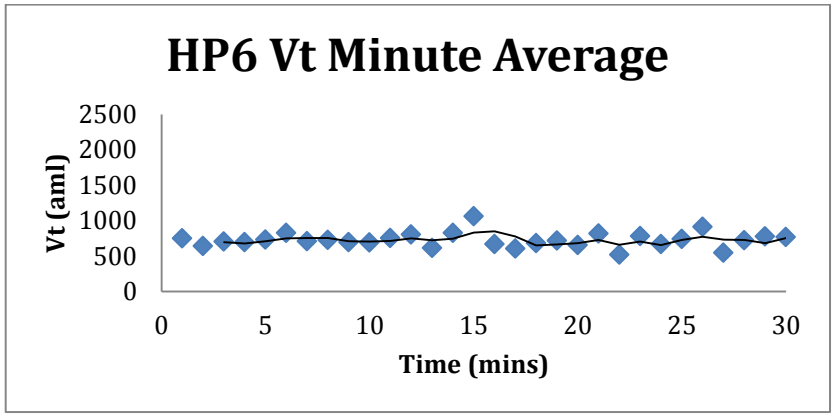

Figure 100: Tidal volume variability minute average for healthy participant 6.

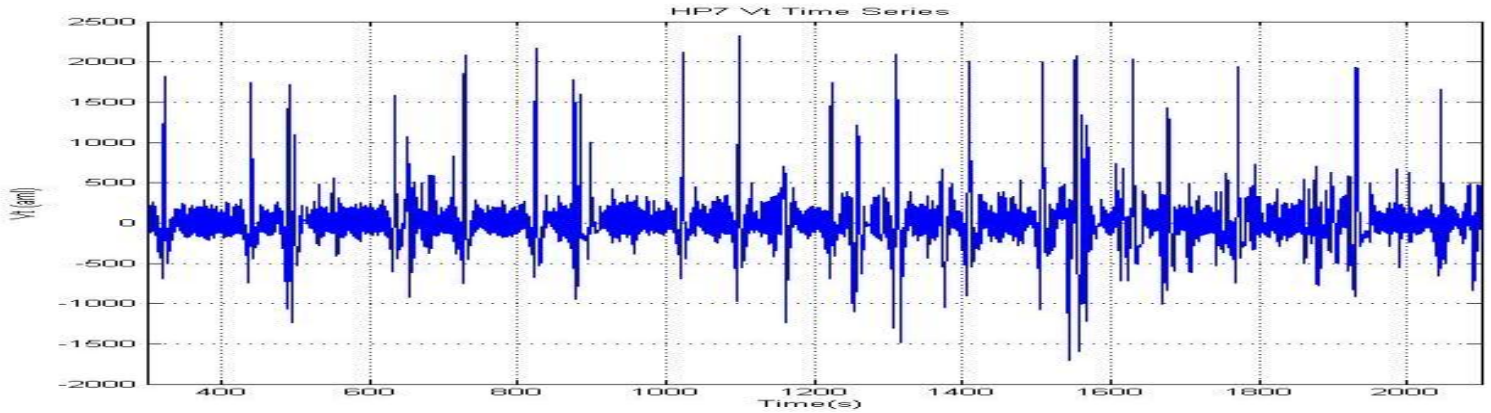

Figure 101: Time series for tidal volume for healthy participant 7.

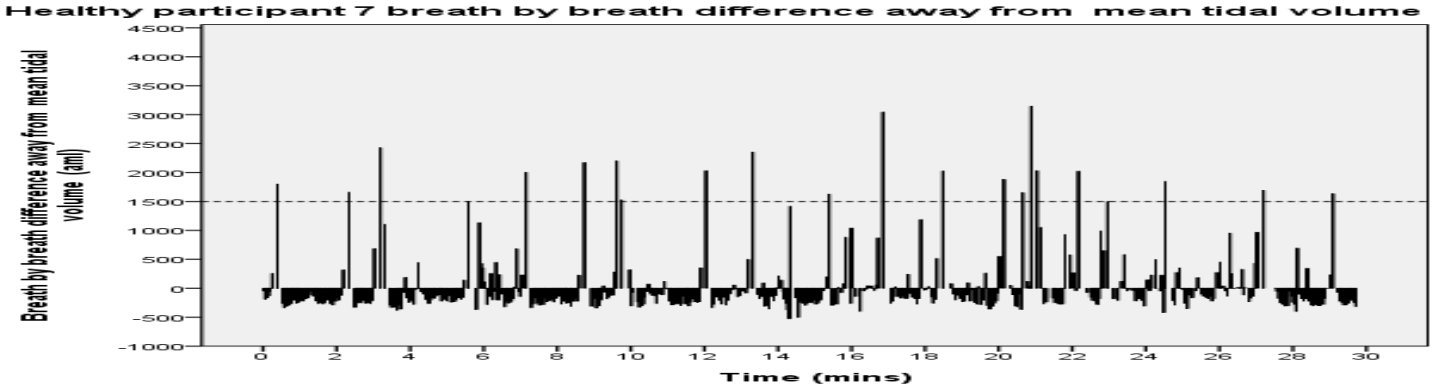

Figure 102: Breath by breath difference away from mean graph for healthy participant 7.

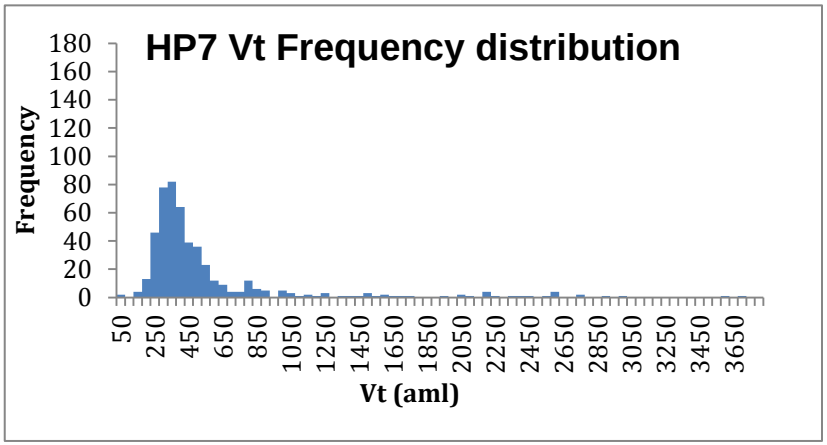

Figure 103: Frequency distribution of tidal volume for healthy participant 7.

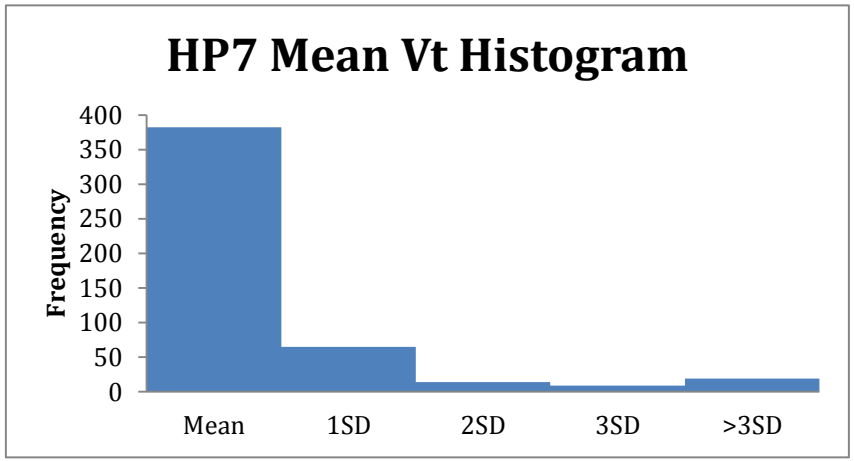

Figure 104: Histogram of tidal volume for healthy participant 7.

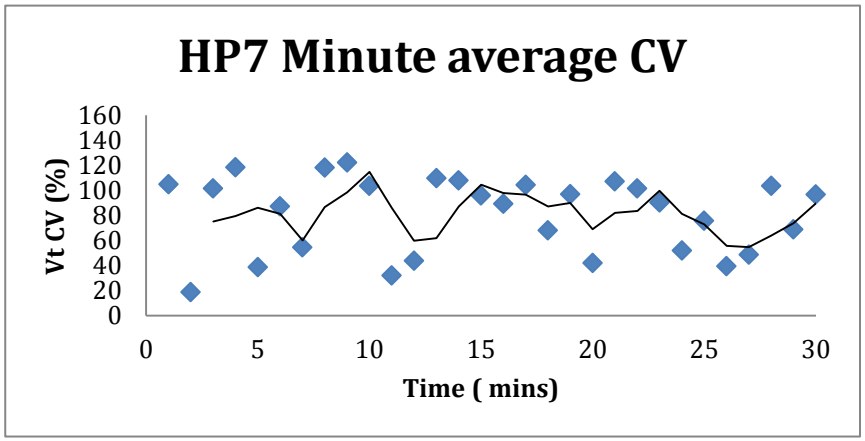

Figure 105: Tidal volume minute average for healthy participant 7.

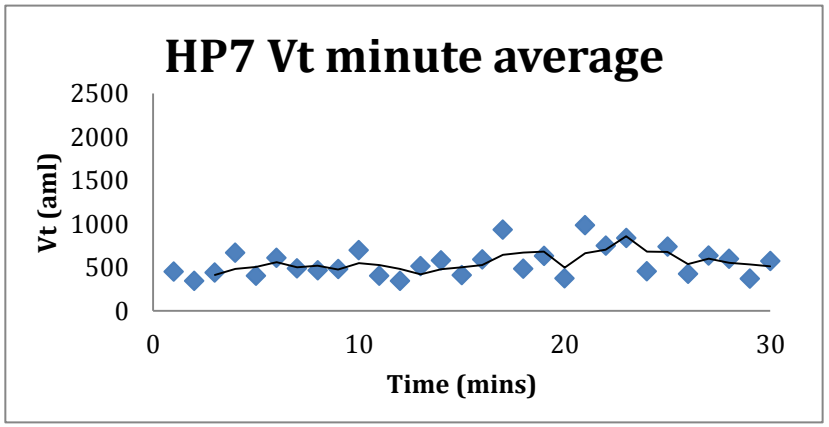

Figure 106: Tidal volume variability minute average for healthy

participant 7.

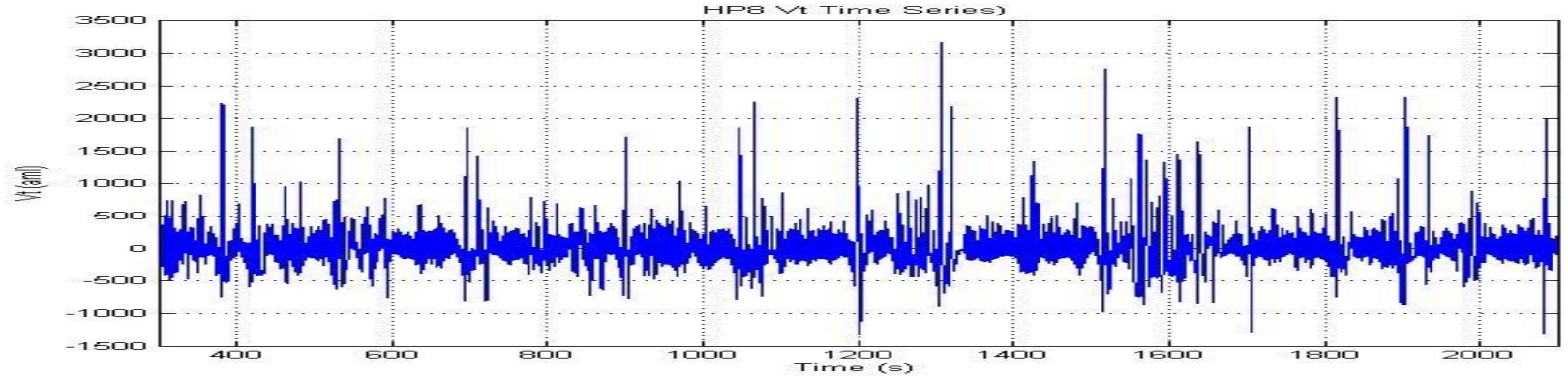

Figure 107: Time series for tidal volume for healthy participant 8.

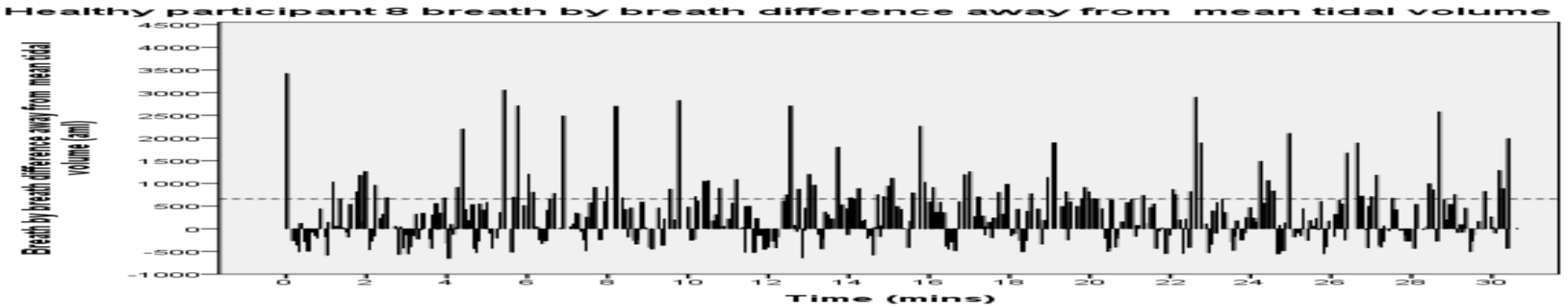

Figure 108: Breath by breath difference away from mean graph for healthy participant 8.

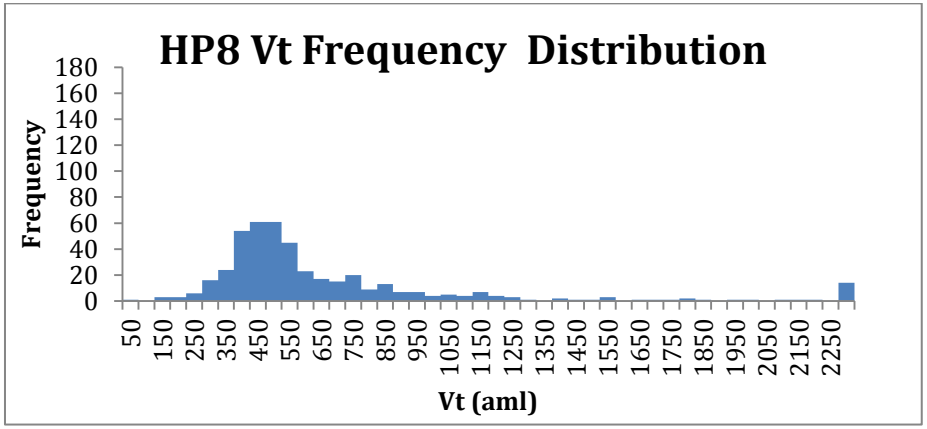

Figure 109: Frequency distribution of tidal volume for healthy participant 8.

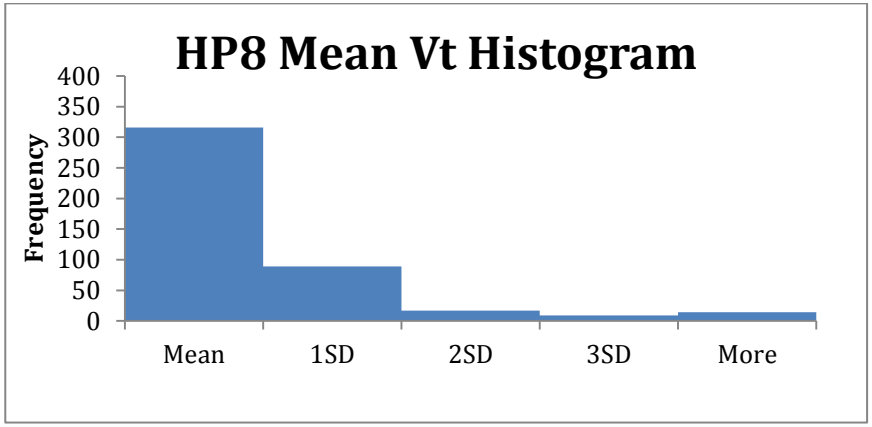

Figure 110: Histogram of tidal volume for healthy participant 8.

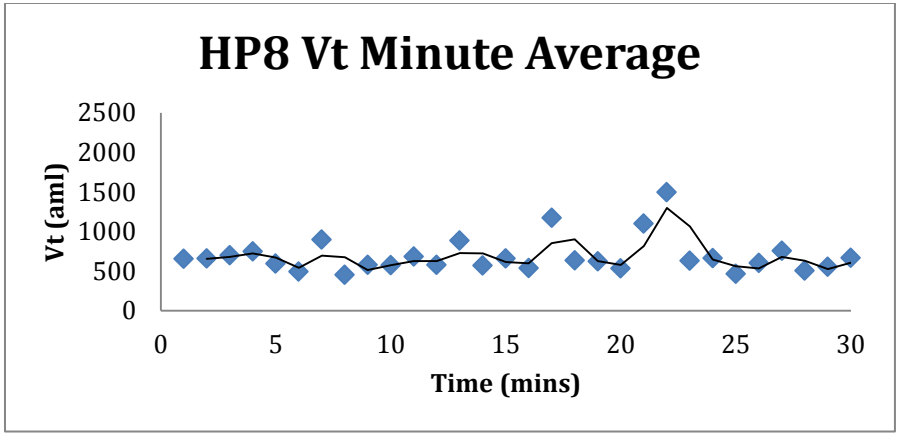

Figure 111: Tidal volume minute average for healthy participant 8.

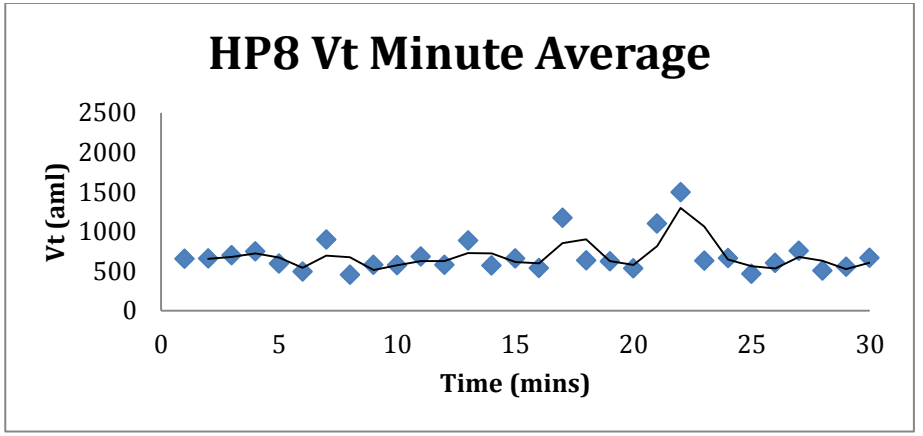

Figure 112: Tidal volume variability minute average for healthy participant 8.

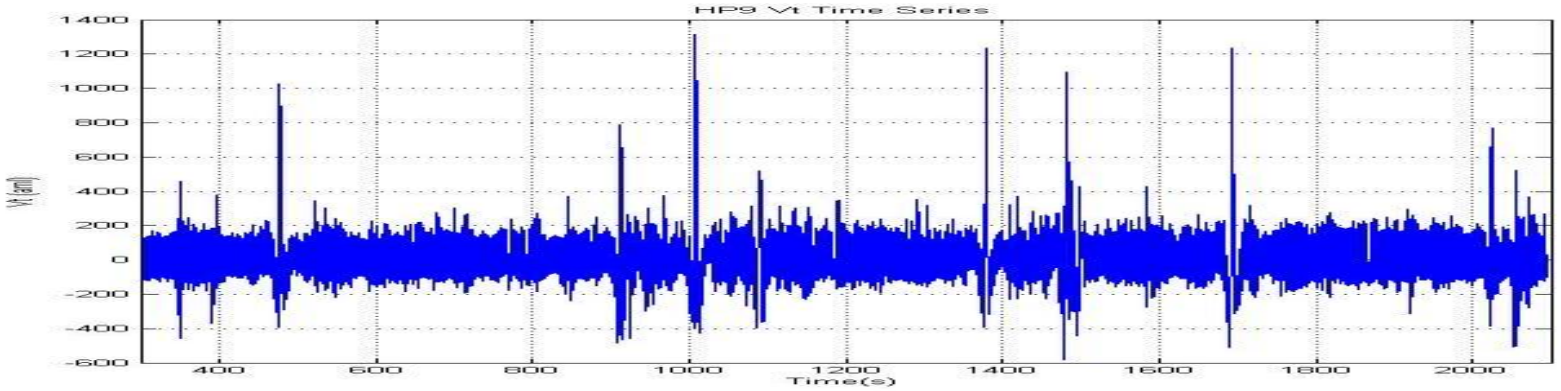

Figure 113: Time series for tidal volume for healthy participant 9.

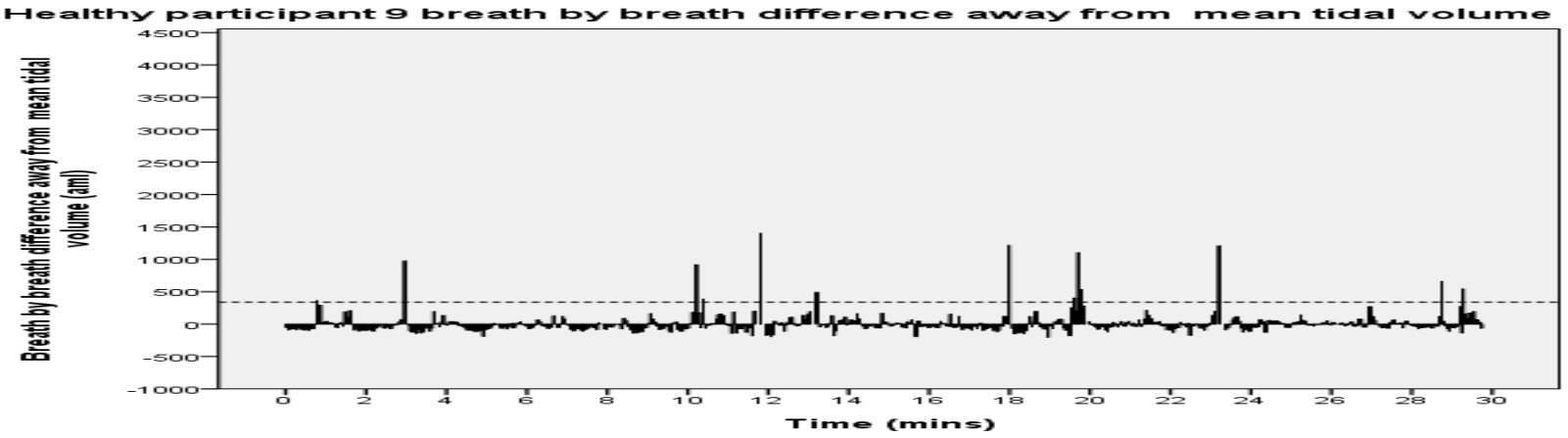

Figure 114: Breath by breath difference away from mean graph for healthy participant 9.

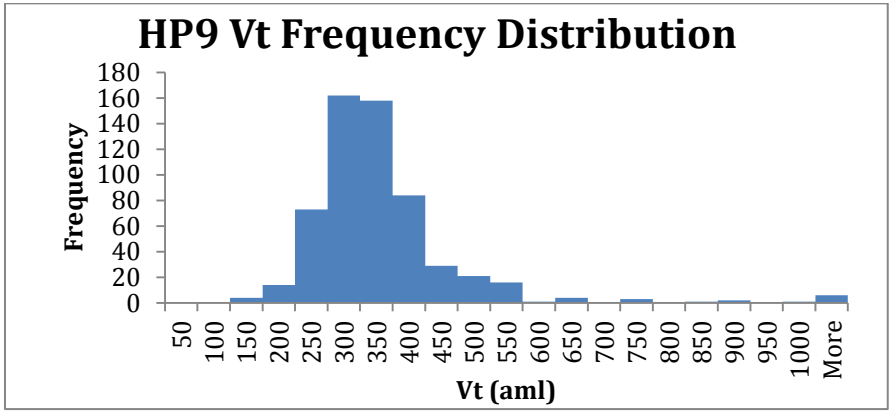

Figure 115: Frequency distribution of tidal volume for healthy participant 9.

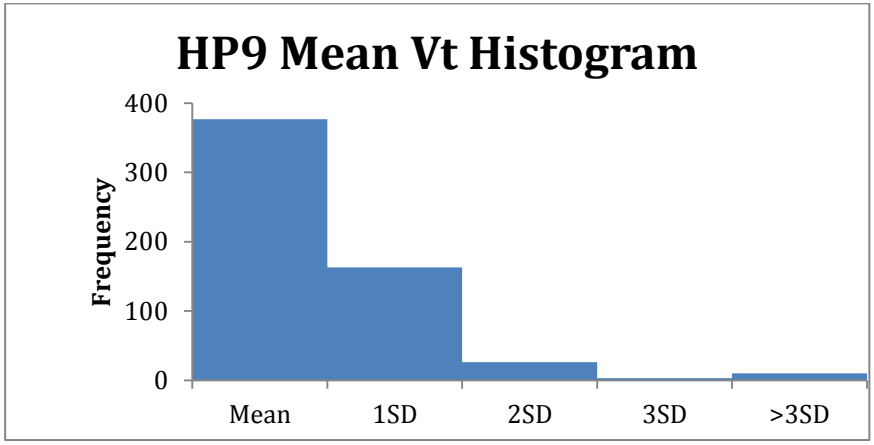

Figure 116: Histogram of tidal volume for healthy participant 9.

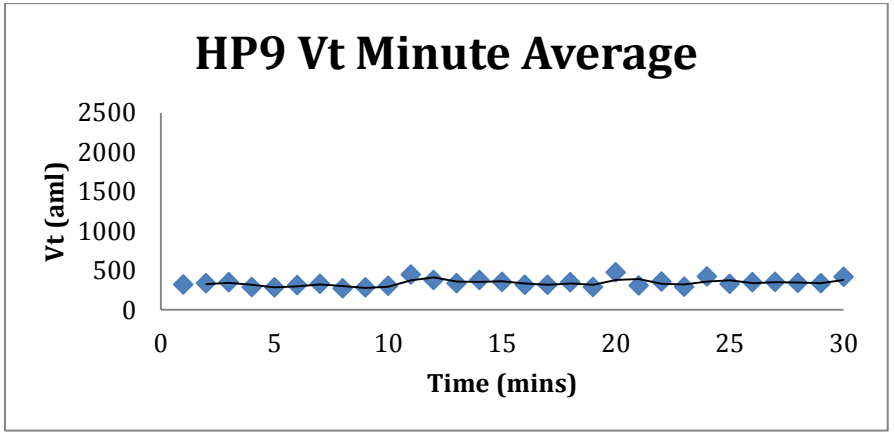

Figure 117: Tidal volume minute average for healthy participant 9.

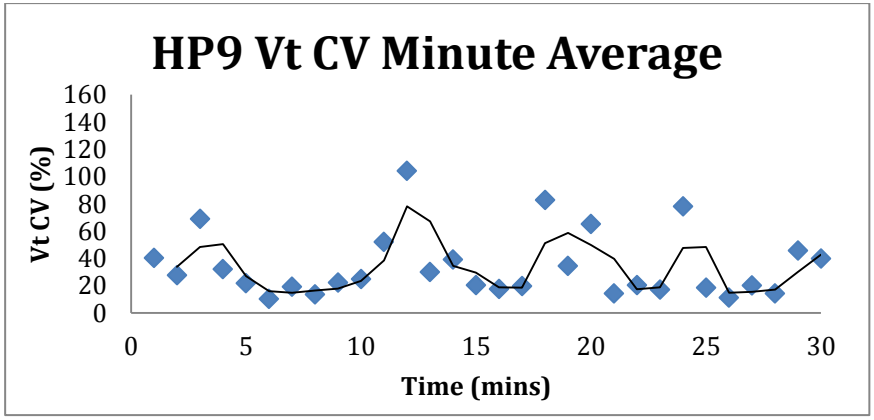

Figure 118: Tidal volume variability minute average for healthy participant 9.

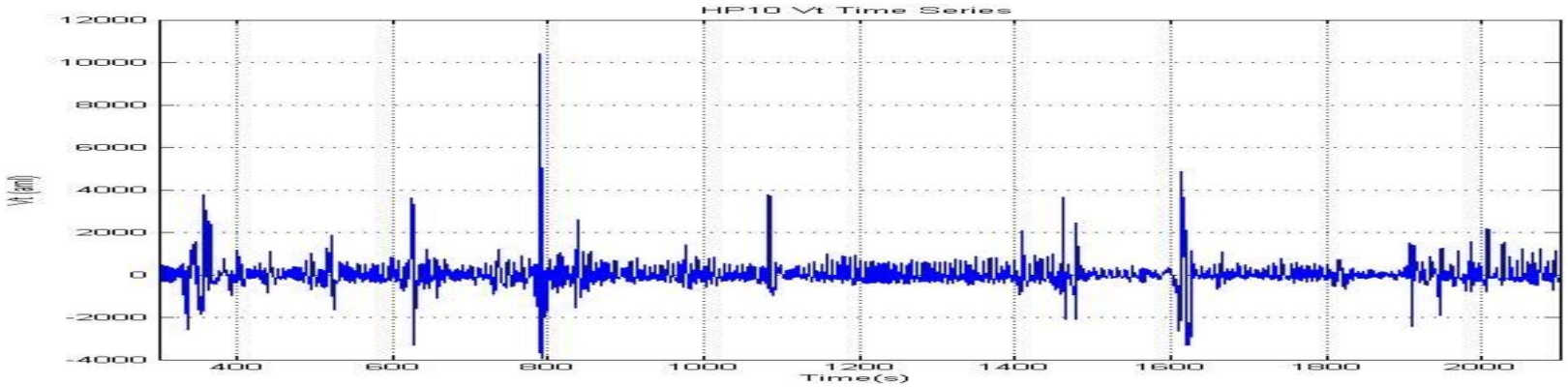

Figure 119: Time series for tidal volume for healthy participant 10.

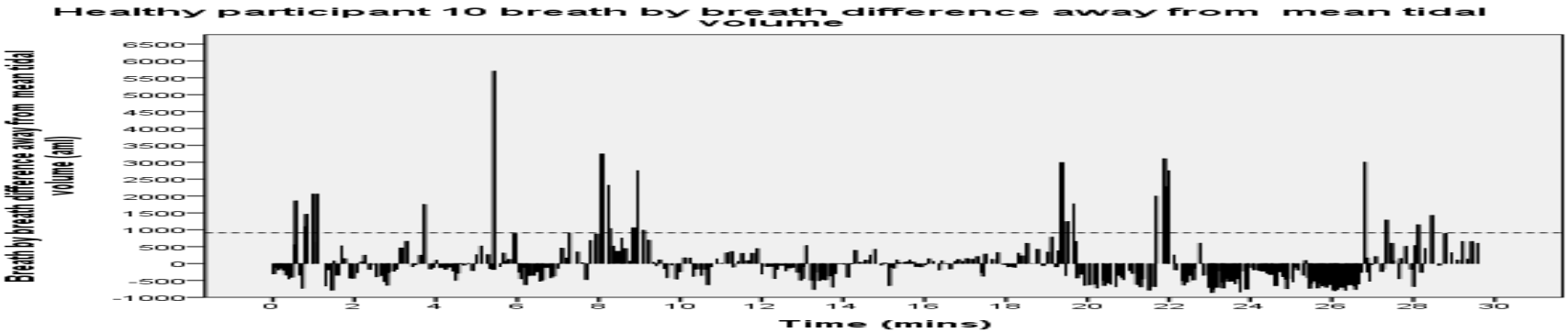

Figure 120: Breath by breath difference away from mean graph for healthy participant 10.

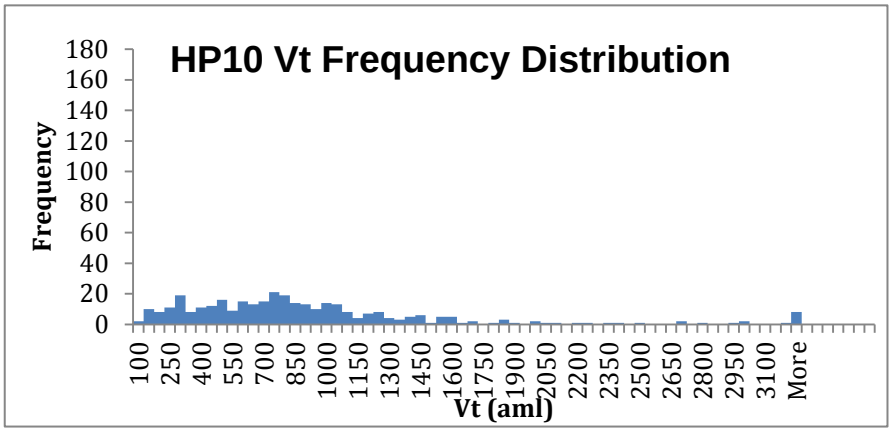

Figure 121: Frequency distribution of tidal volume for healthy participant 10

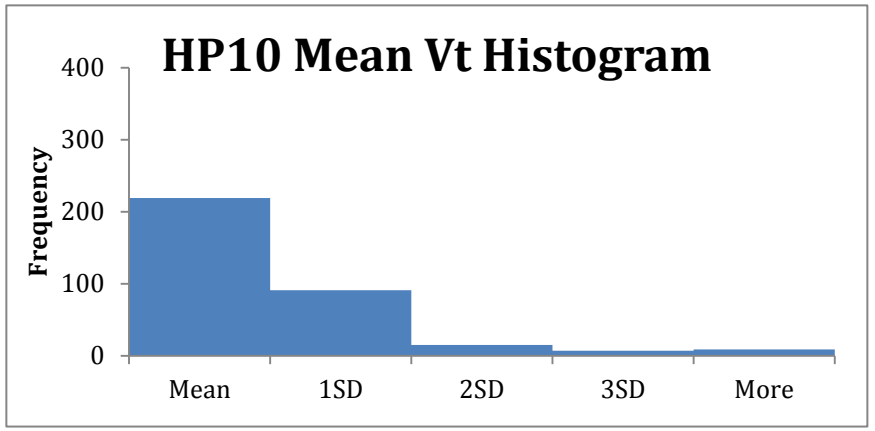

Figure 122: Histogram of tidal volume for healthy participant 10.

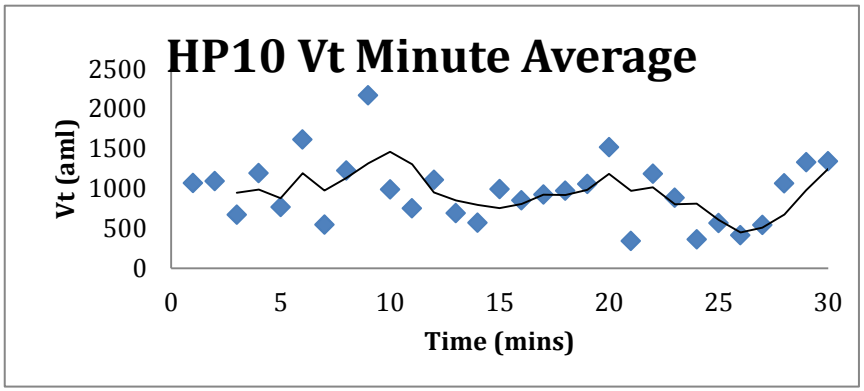

Figure 123: Tidal volume minute average for healthy participant 10.

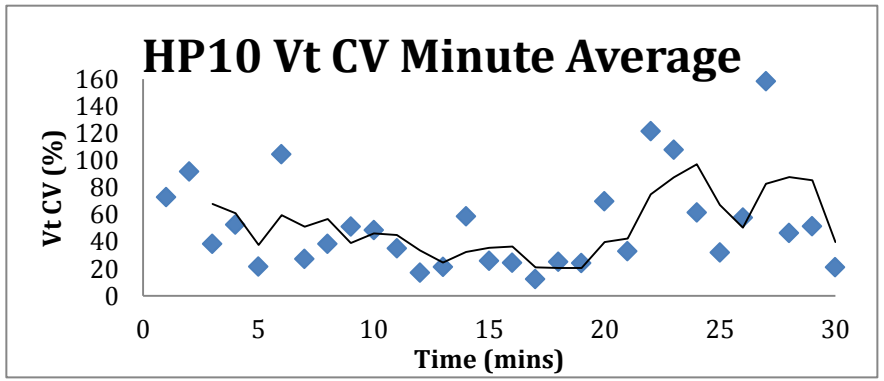

Figure 124: Tidal volume variability minute average for healthy participant 10.

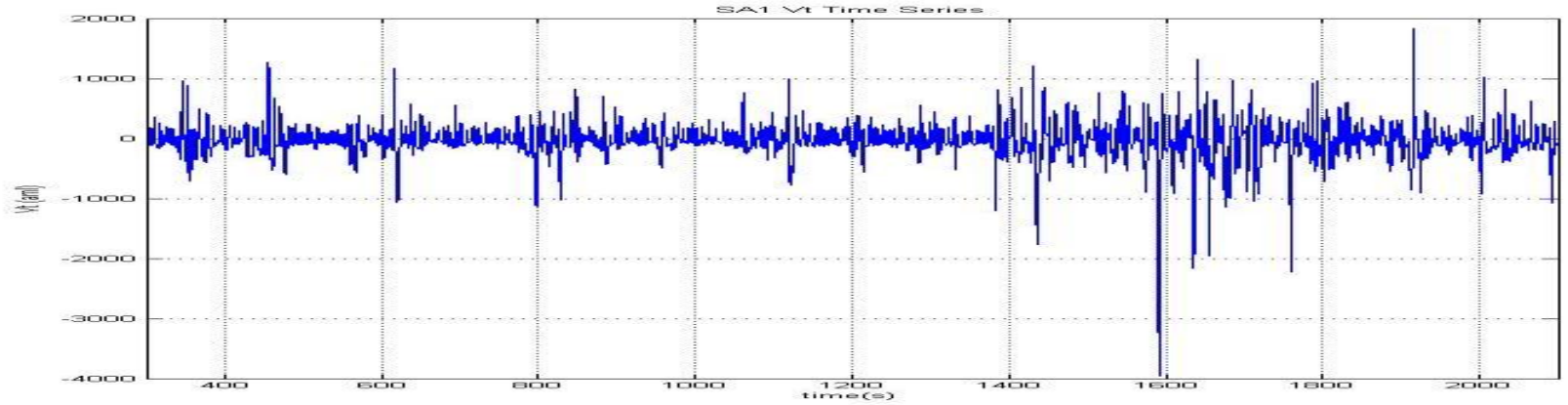

Figure 125: Time series for tidal volume for severe asthma participant 1.

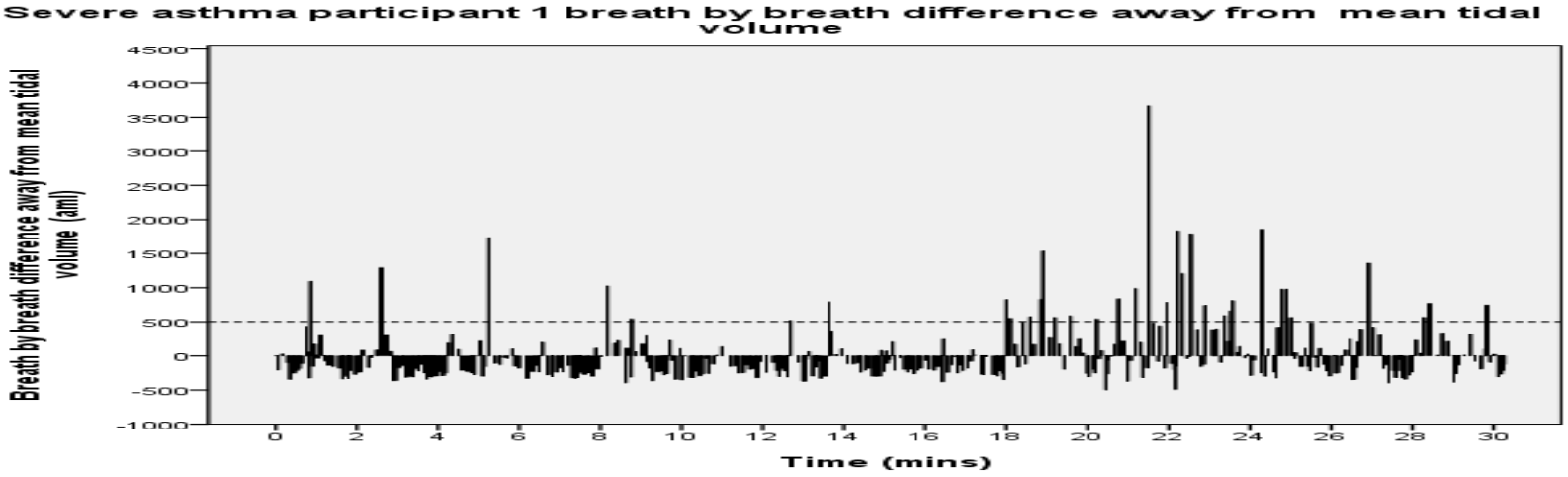

Figure 126: Breath by breath difference away from mean graph for severe asthma participant 1.

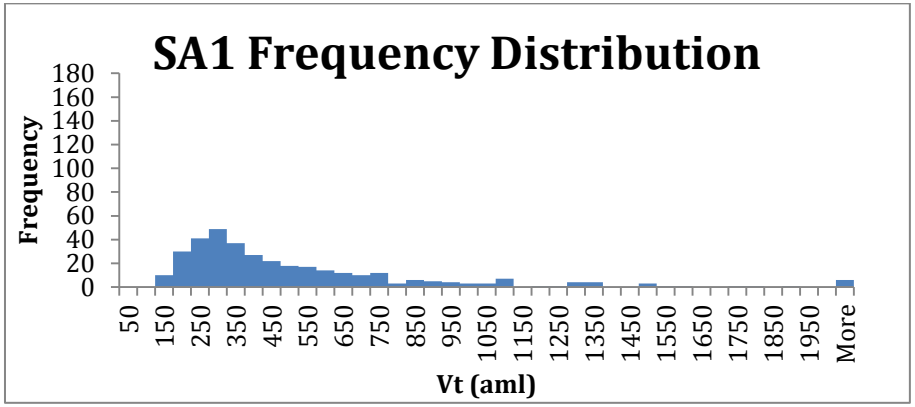

Figure 127 Frequency distribution of tidal volume for severe asthma participant 1.

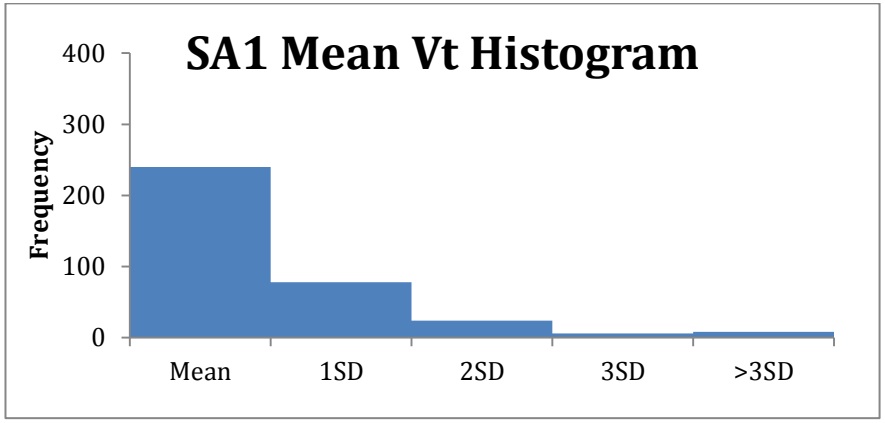

Figure 128 : Histogram of tidal volume for severe asthma participant 1.

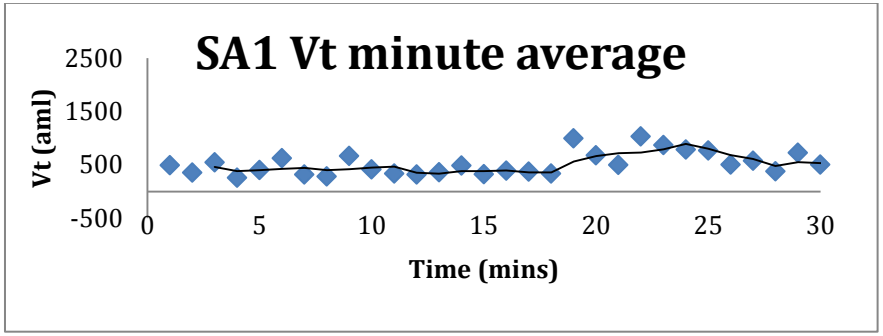

Figure 129: Tidal volume minute average for severe asthma participant 1.

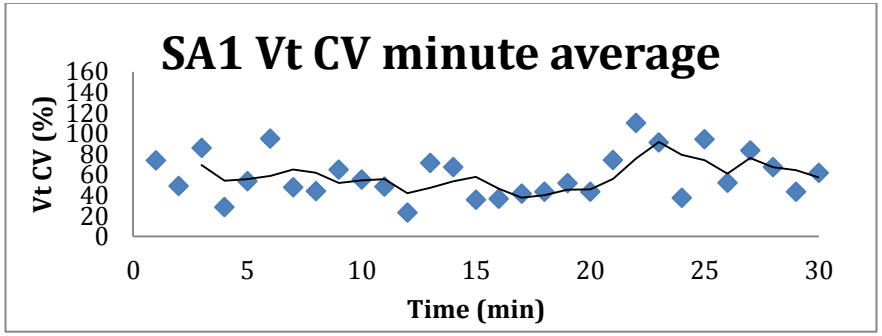

Figure 130: Tidal volume variability minute average for severe asthma participant 1.

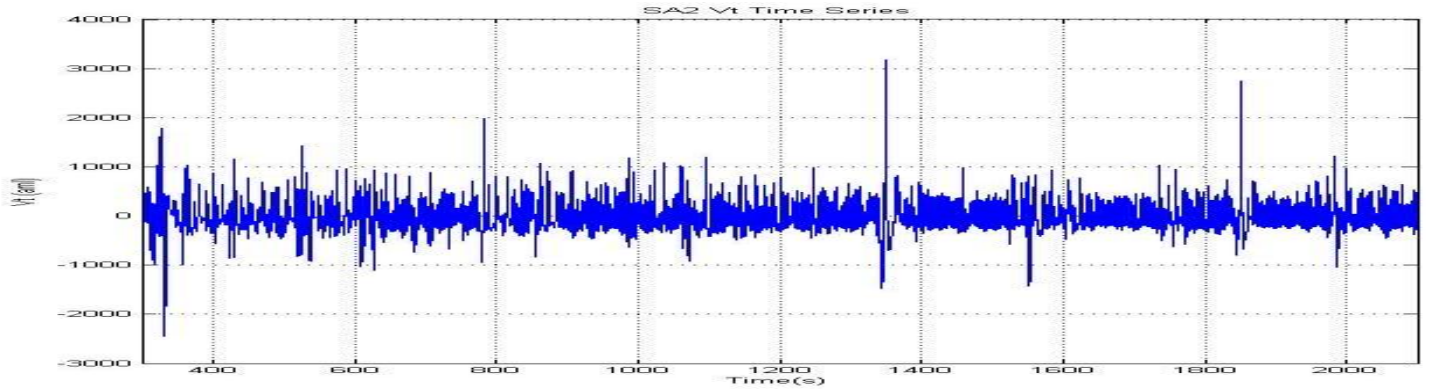

Figure 131: Time series for tidal volume for severe asthma participant 2.

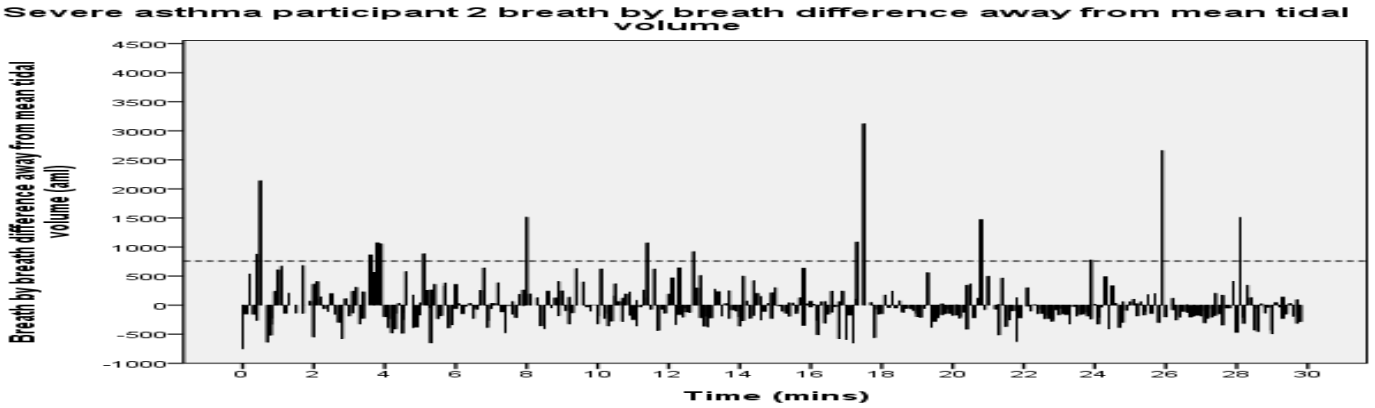

Figure 132: Breath by breath difference away from mean graph for severe asthma participant 2.

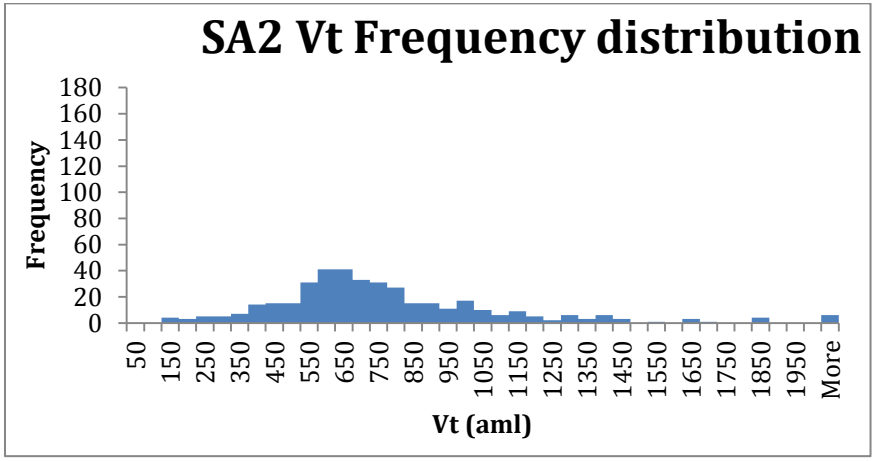

Figure 133: Frequency distribution of tidal volume for severe asthma participant 2.

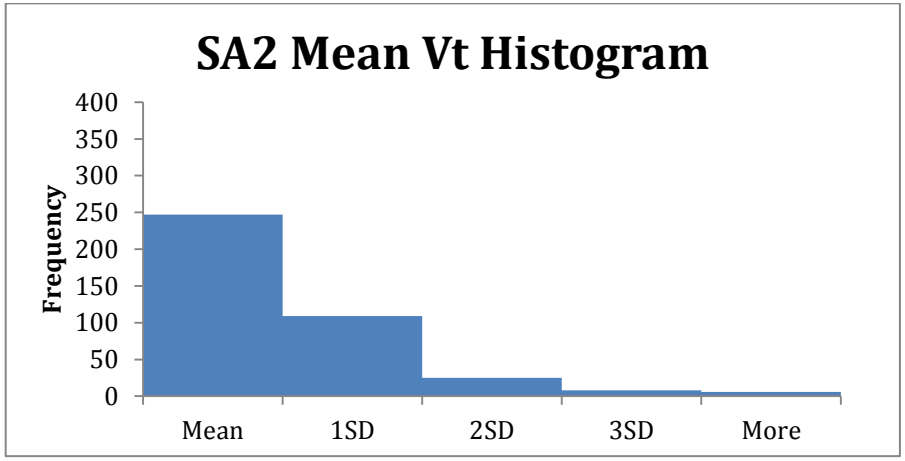

Figure 134: Histogram of tidal volume for severe asthma participant 2.

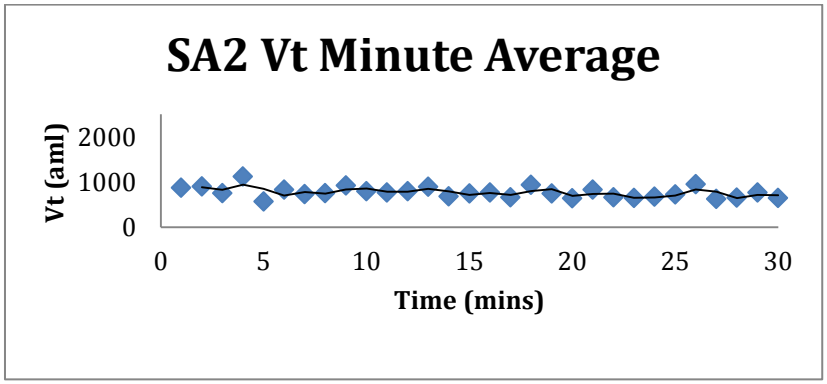

Figure 135: Tidal volume minute average for severe asthma participant 2.

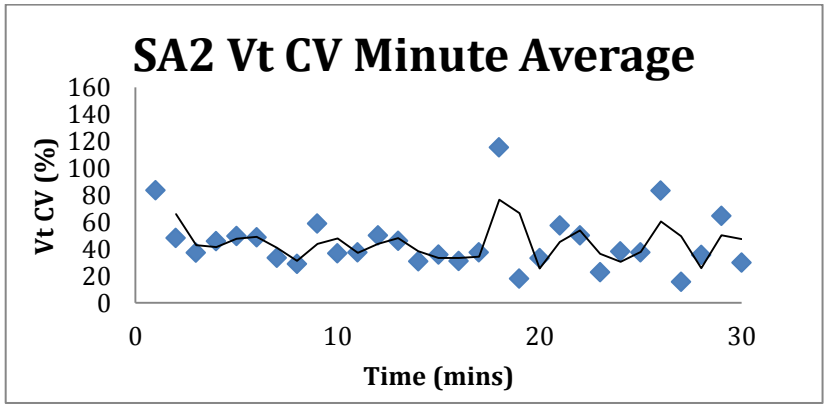

Figure 136: Tidal volume variability minute average for severe asthma participant 2.

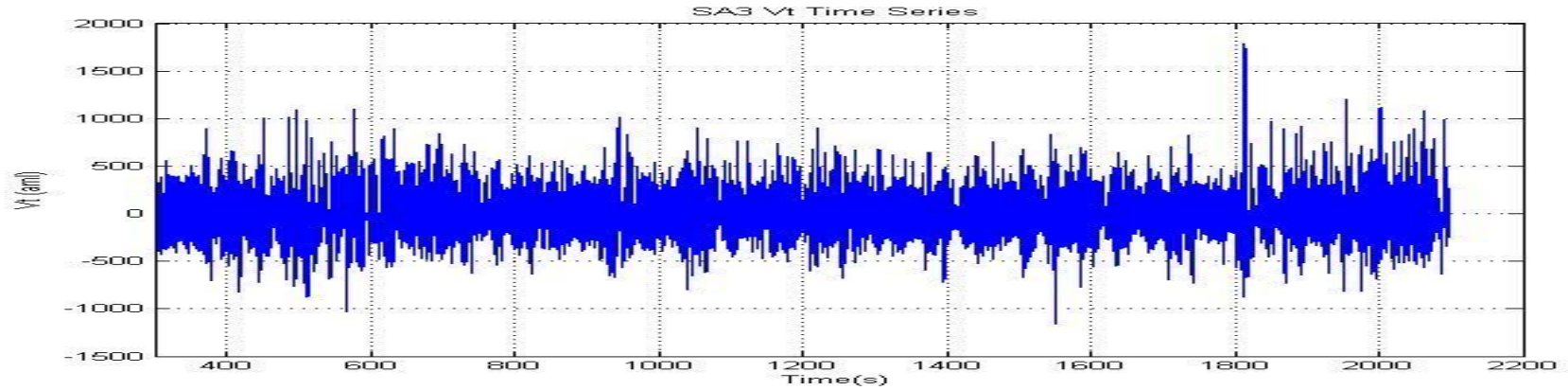

Figure 137: Time series for tidal volume for severe asthma participant 3

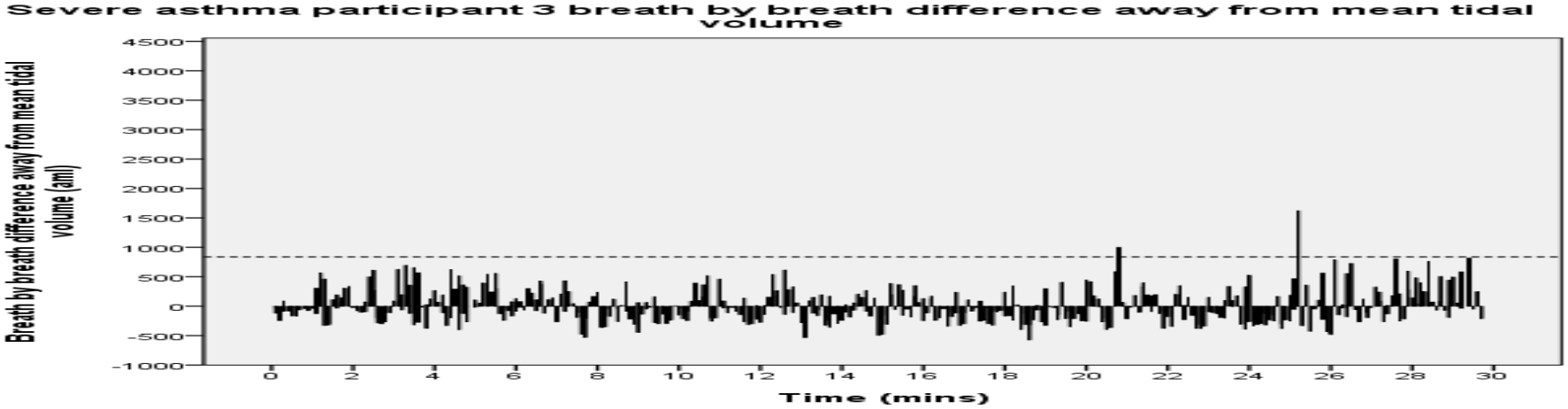

Figure 138: Breath by breath difference away from mean graph for severe asthma participant 3.

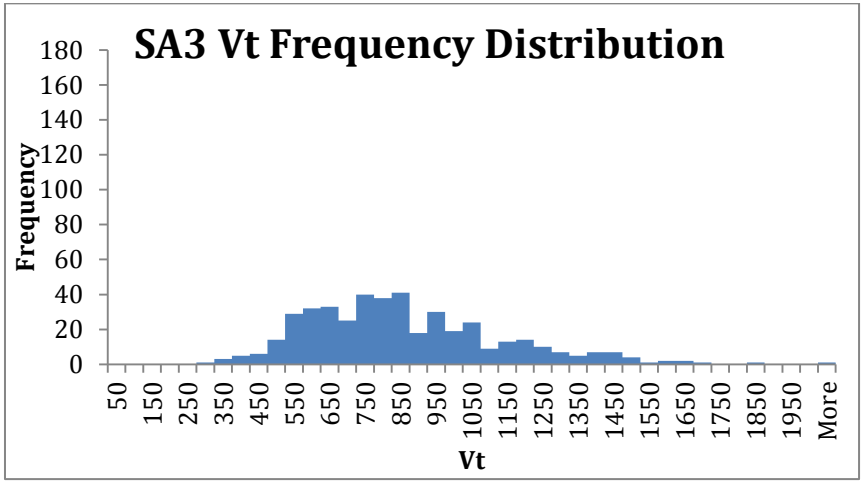

Figure 139: Frequency distribution of tidal volume for severe asthma participant 3.

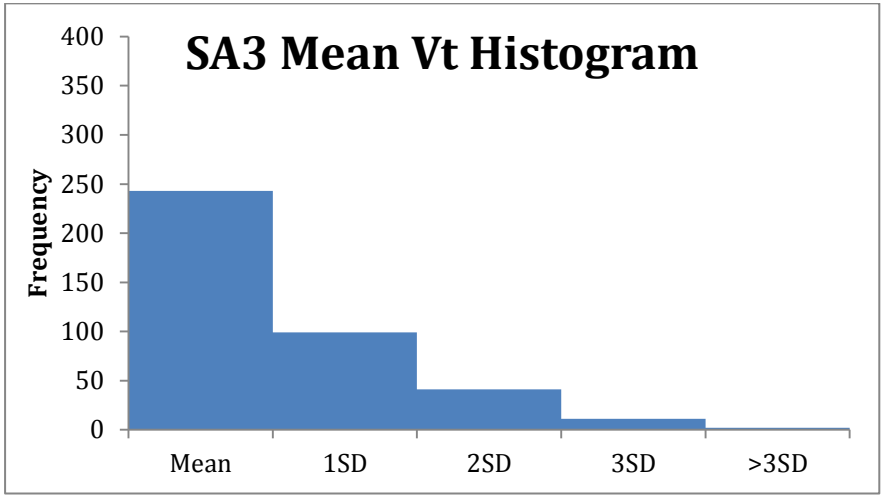

Figure 140: Histogram of tidal volume for severe asthma participant 3.

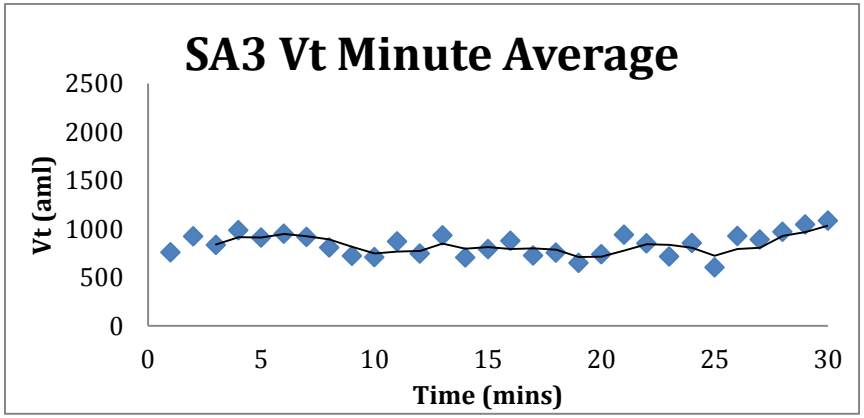

Figure 141: Tidal volume minute average for severe asthma participant 3.

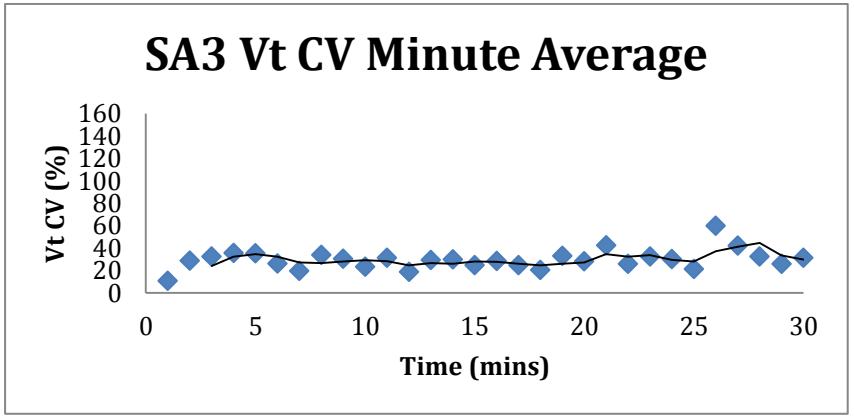

Figure 142: Tidal volume variability minute average for severe asthma

participant 3.

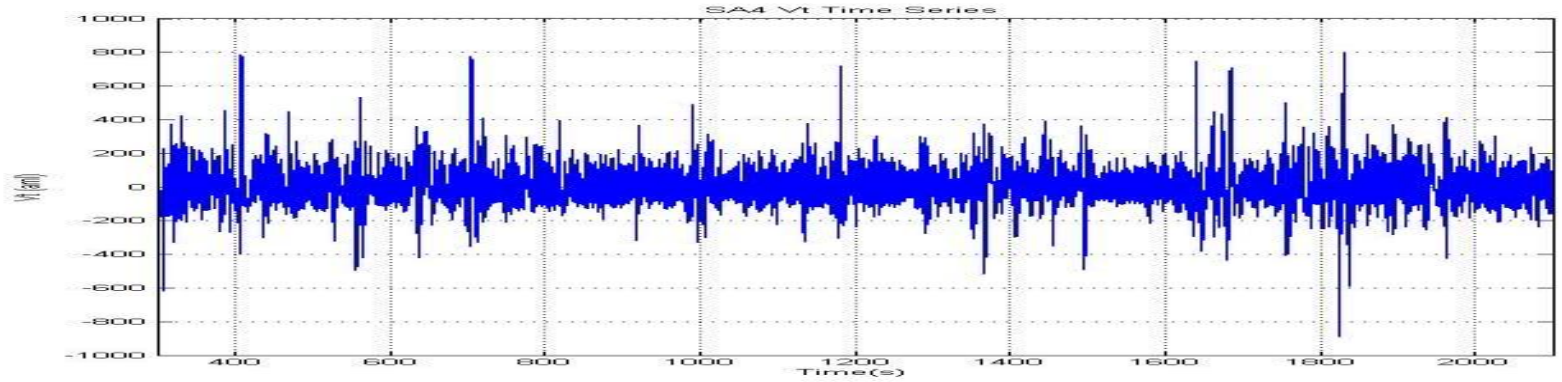

Figure 143: Time series for tidal volume for severe asthma participant 4.

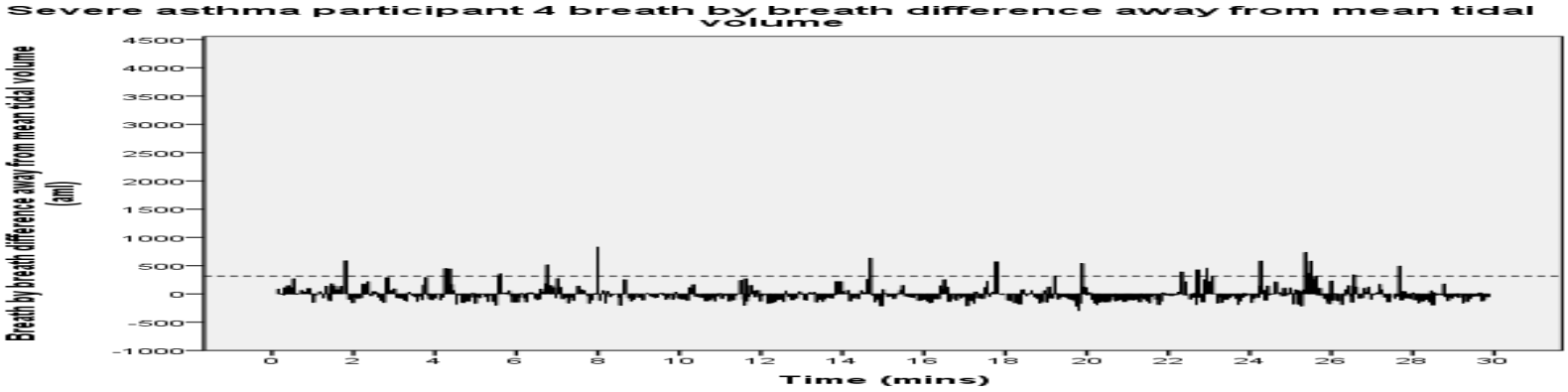

Figure 144: Breath by breath difference away from mean graph for severe asthma participant 1.

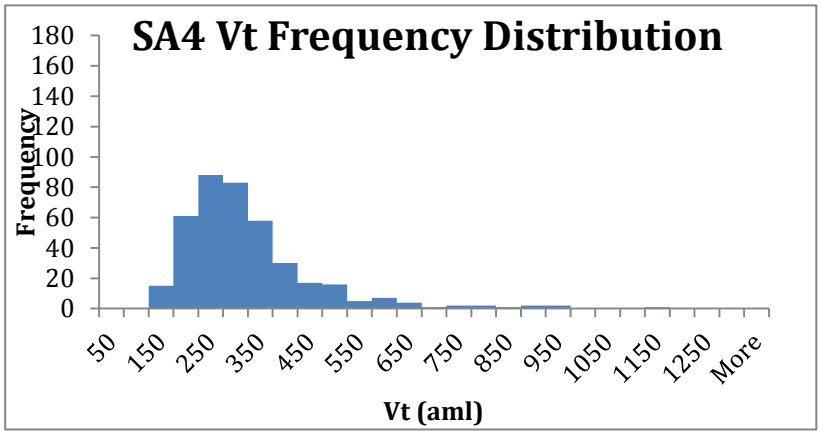

Figure 145: Frequency distribution of tidal volume for severe asthma participant 4.

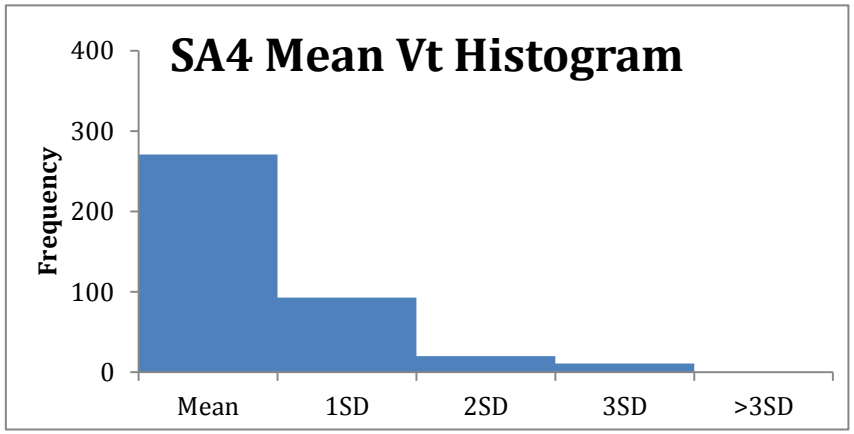

Figure 146: Histogram of tidal volume for severe asthma participant 4.

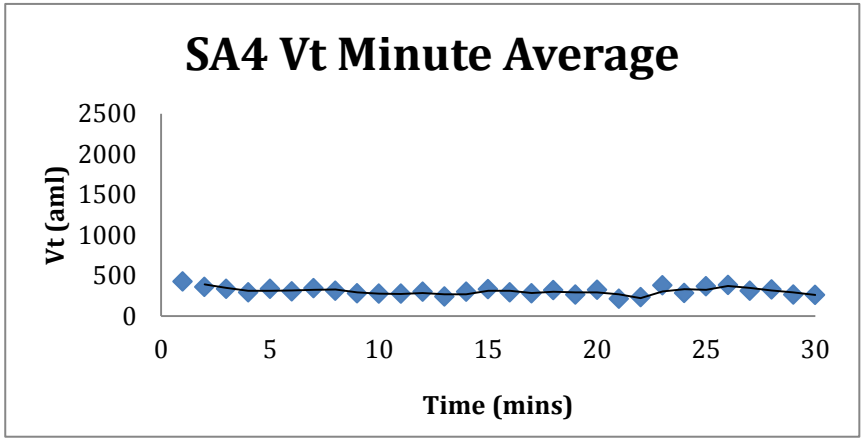

Figure 147: Tidal volume minute average for severe asthma participant 4.

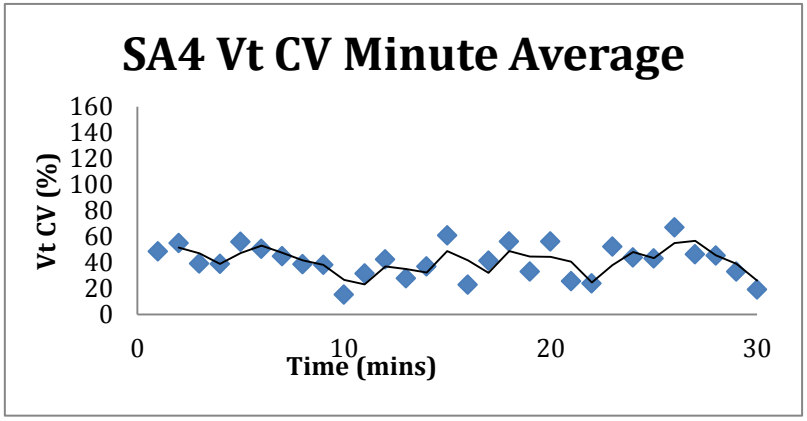

Figure 148: Tidal volume variability minute average for severe asthma participant 4.

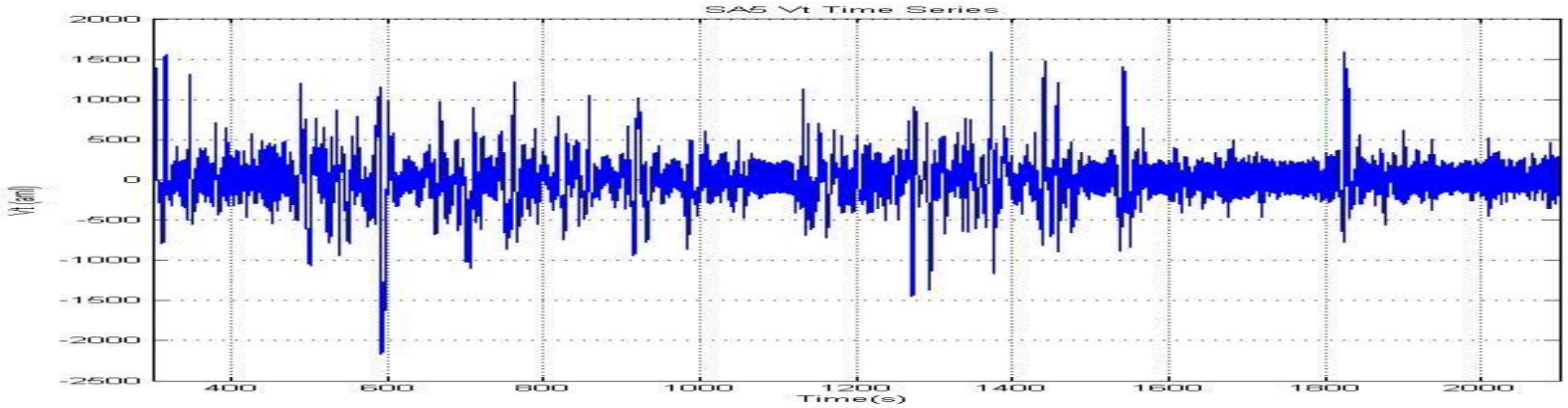

Figure 149: Time series for tidal volume for severe asthma participant 5.

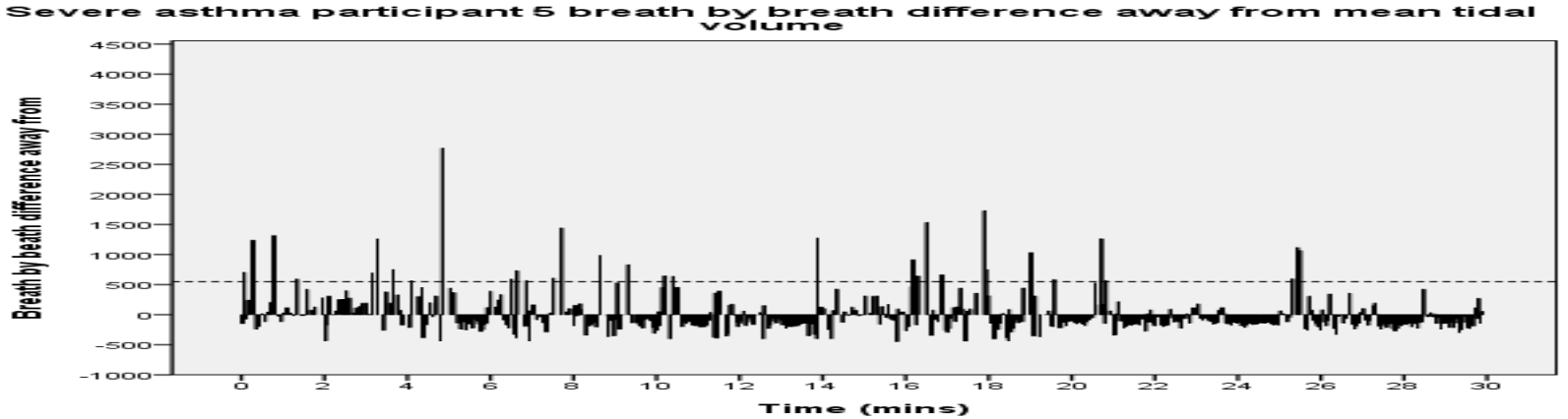

Figure 150: Breath by breath difference away from mean graph for severe asthma participant 5.

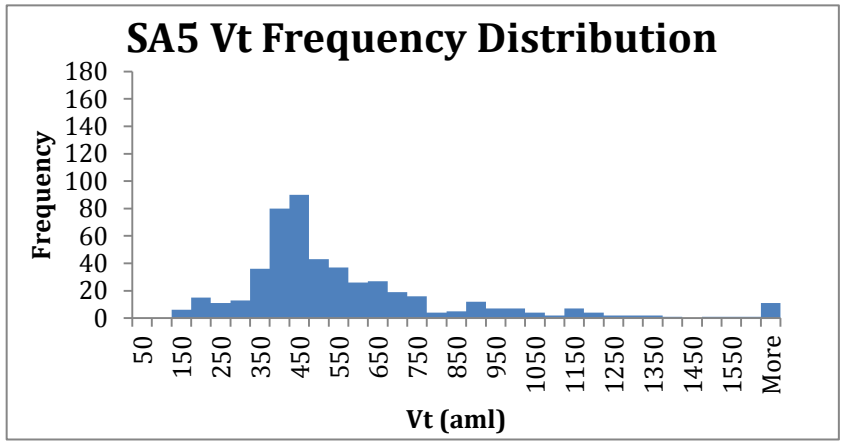

Figure 151: Frequency distribution of tidal volume for severe asthma participant 5.

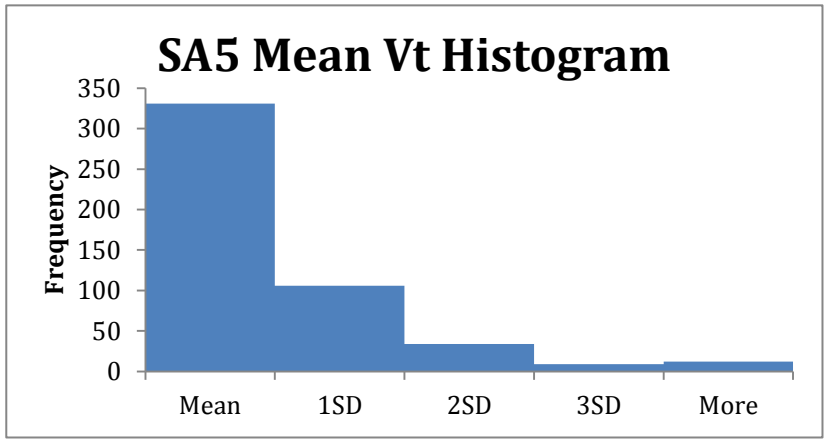

Figure 152: Histogram of tidal volume for severe asthma participant 5.

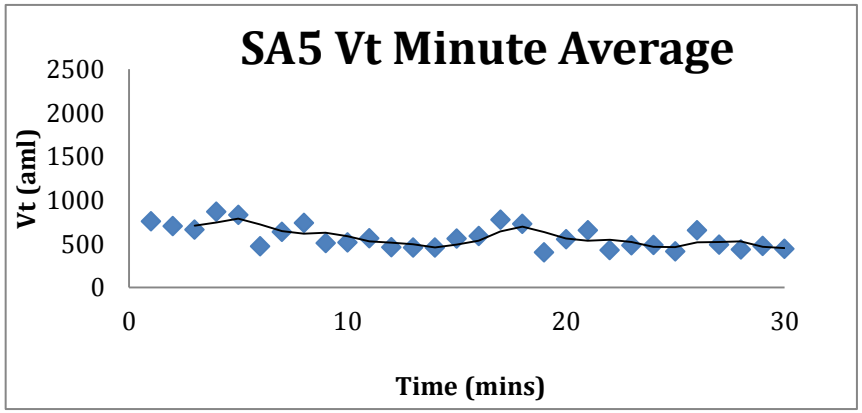

Figure 153: Tidal volume minute average for severe asthma participant 5.

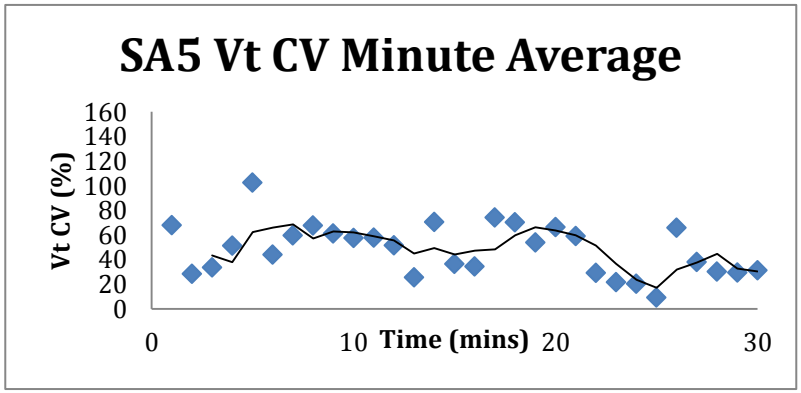

Figure 154: Tidal volume variability minute average for severe asthma participant 5.

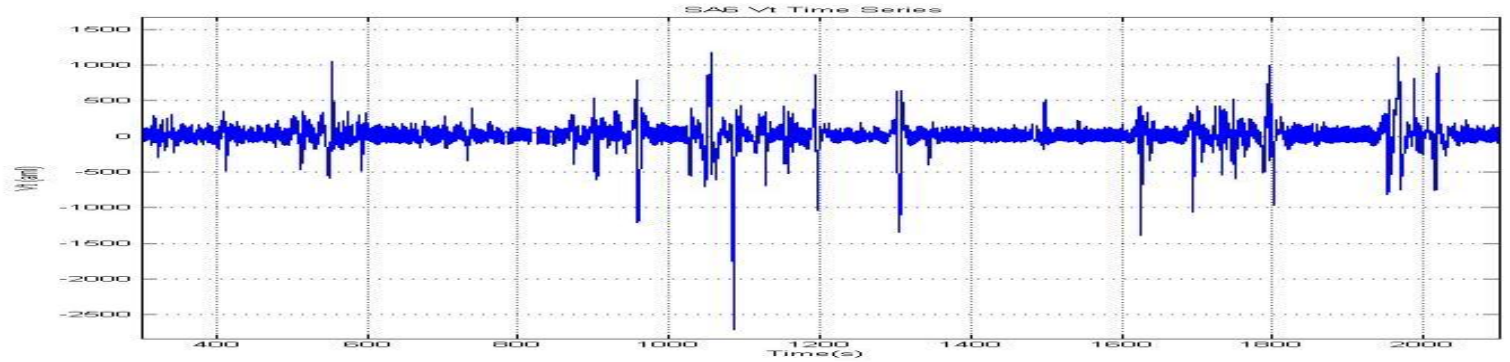

Figure 155: Time series for tidal volume for severe asthma participant 6.

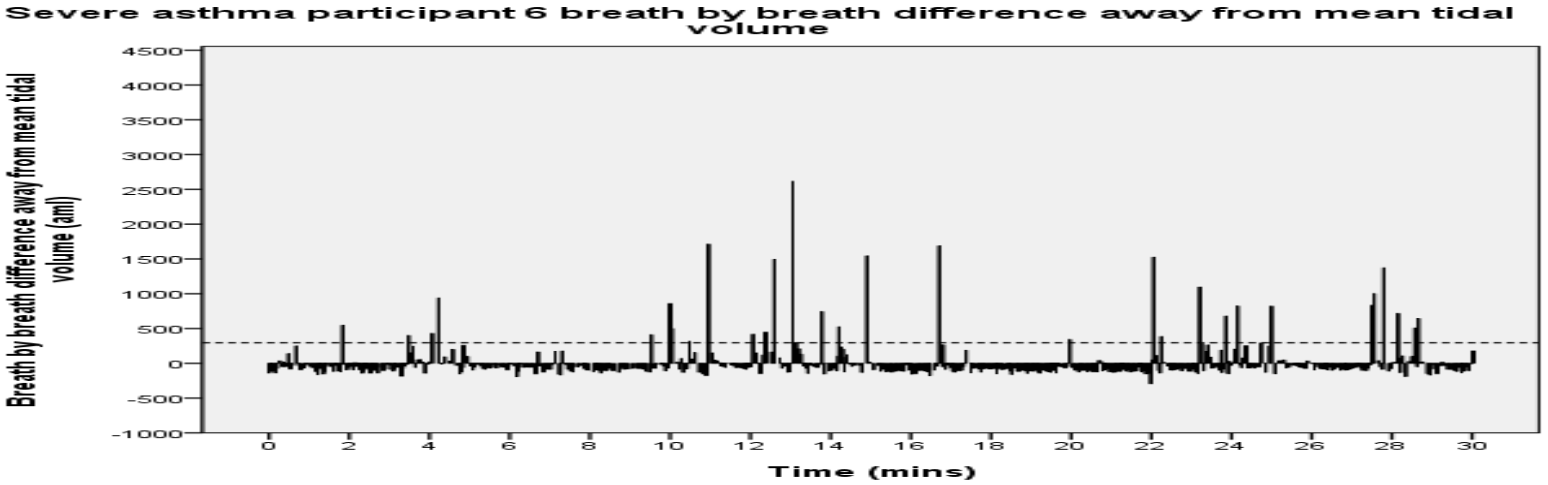

Figure 156: Breath by breath difference away from mean graph for severe asthma participant 6.

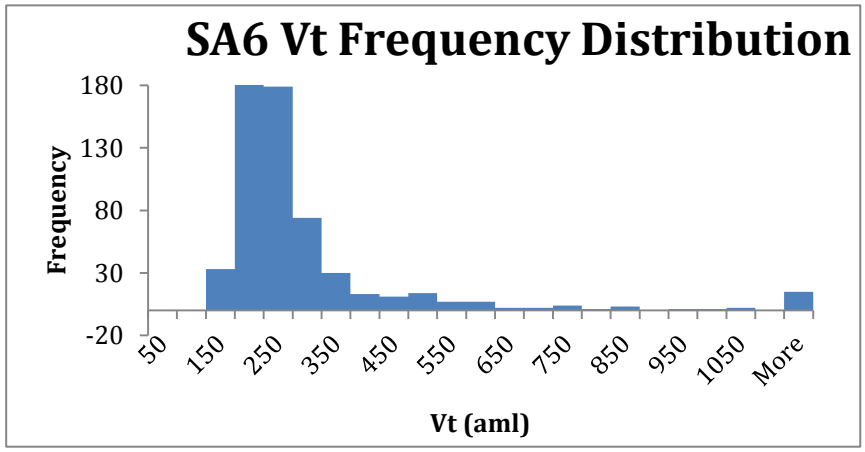

Figure 157: Frequency distribution of tidal volume for severe asthma participant 6.

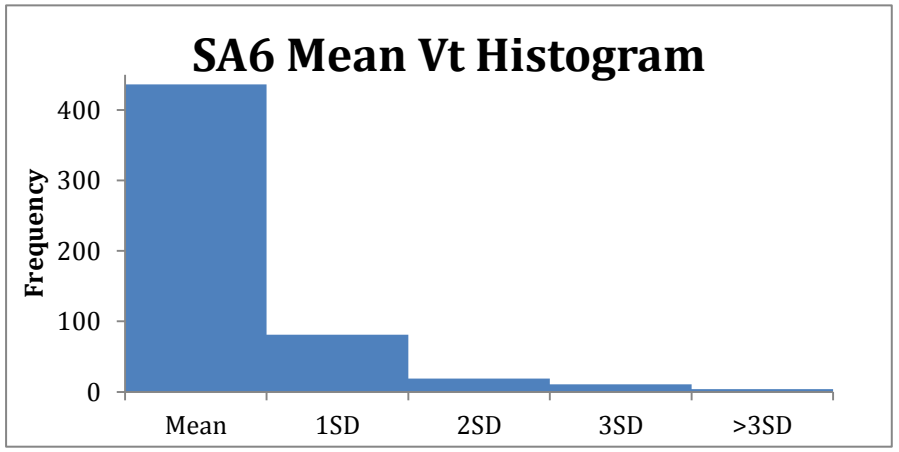

Figure 158: Histogram of tidal volume for severe asthma participant 6.

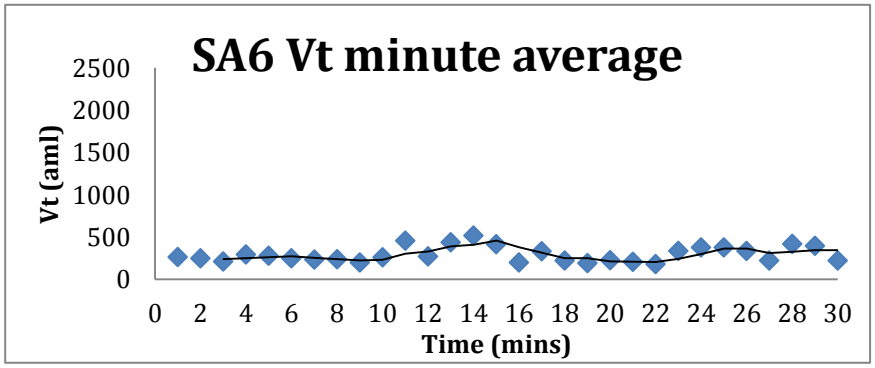

Figure 159: Tidal volume minute average for severe asthma participant 6.

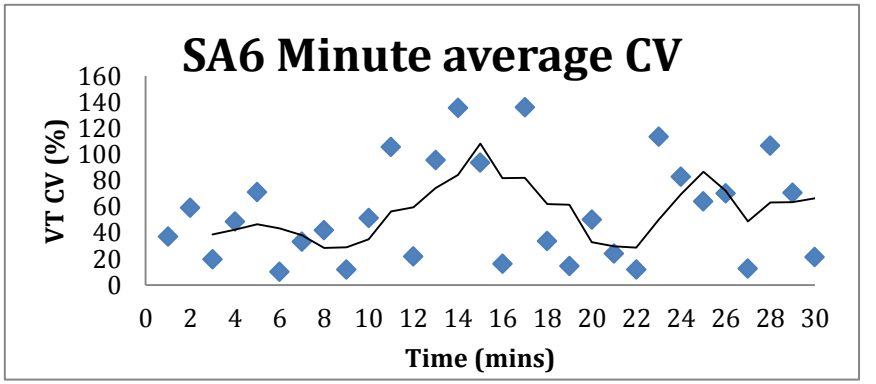

Figure 160: Tidal volume variability minute average for severe asthma participant 6.

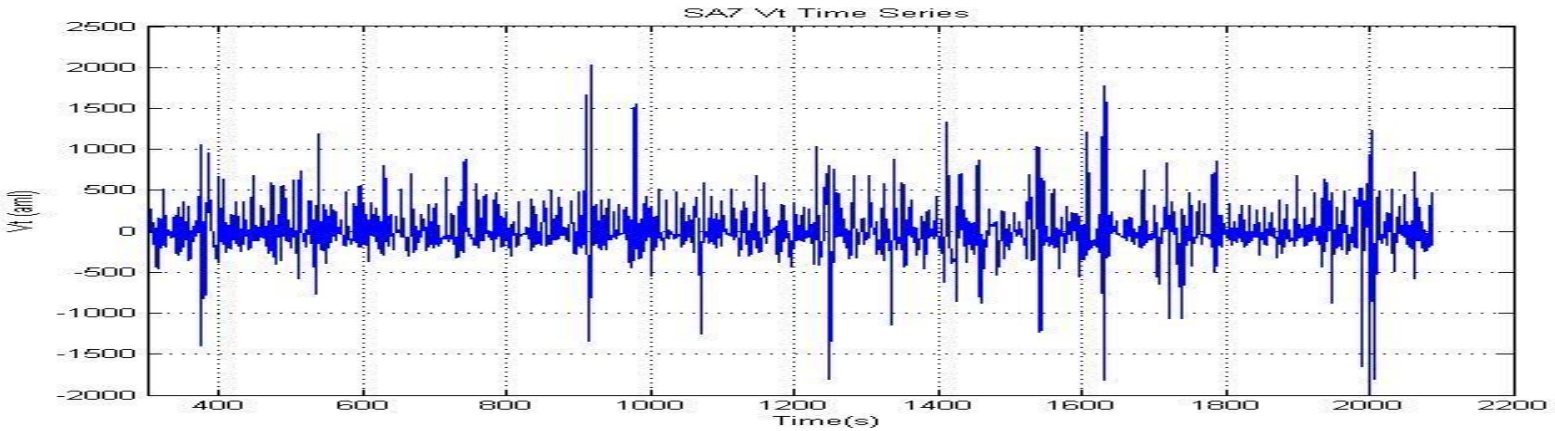

Figure 161: Time series for tidal volume for severe asthma participant 7.

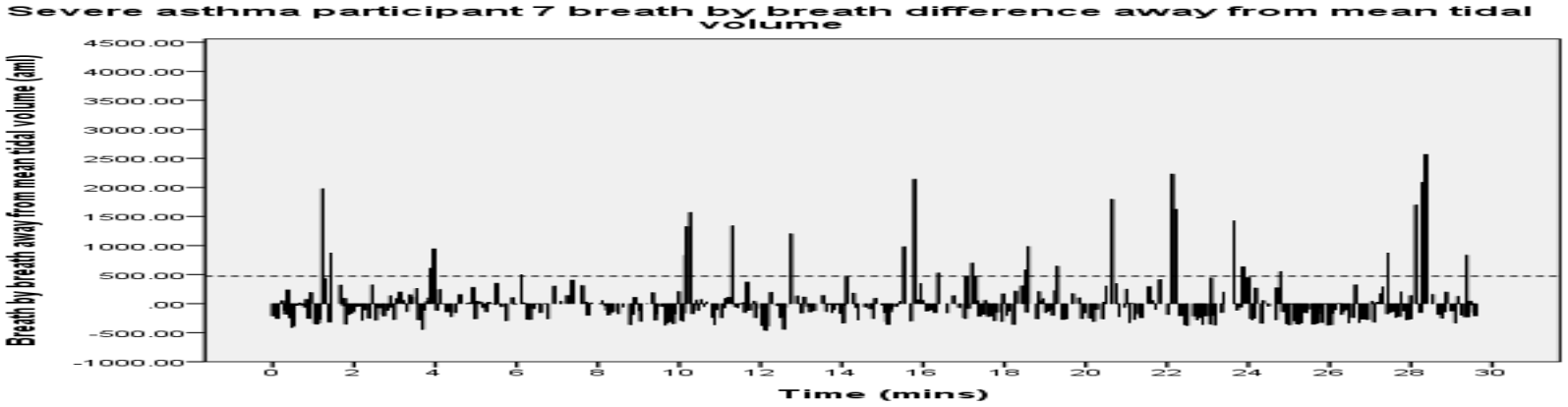

Figure 162: Breath by breath difference away from mean graph for severe asthma participant 7.

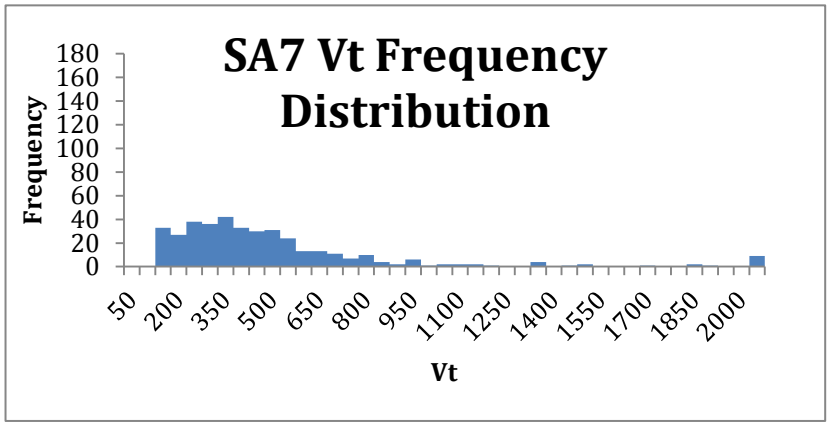

Figure 163: Frequency distribution of tidal volume for severe asthma participant 7.

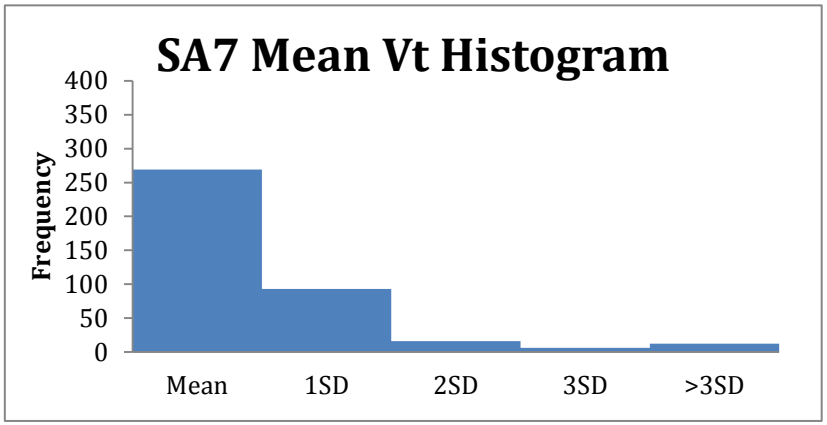

Figure 164: Histogram of tidal volume for severe asthma participant 7.

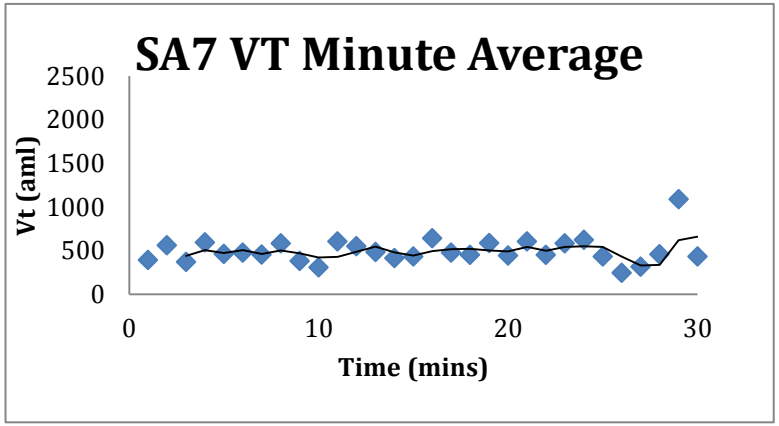

Figure 165: Tidal volume minute average for severe asthma participant 7.

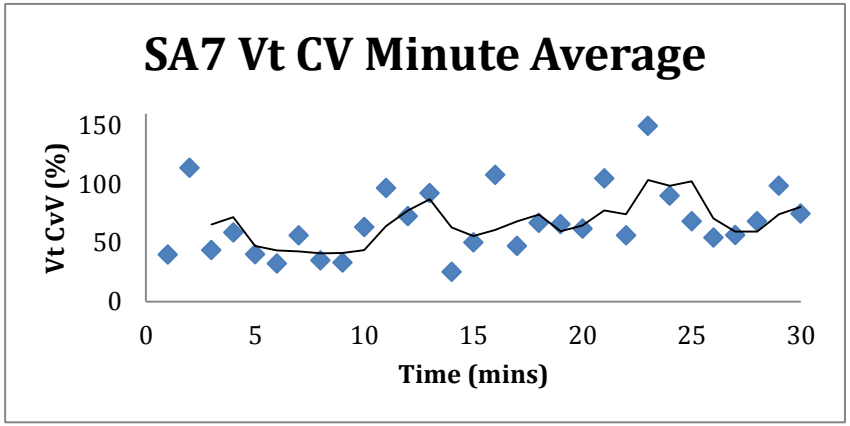

Figure 166: Tidal volume variability minute average for severe asthma participant 7.

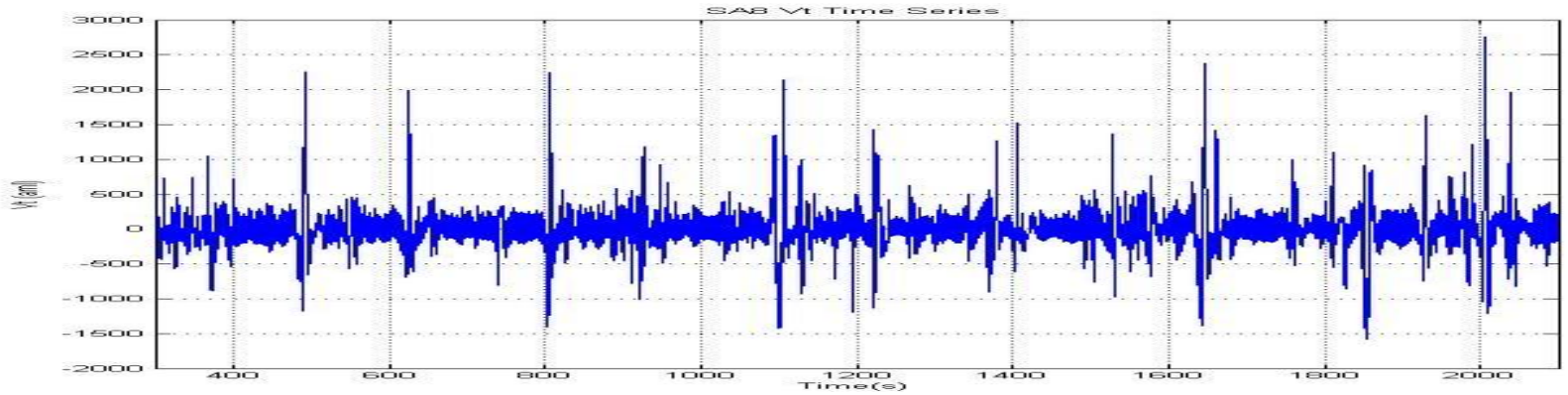

Figure 167: Time series for tidal volume for severe asthma participant 8.

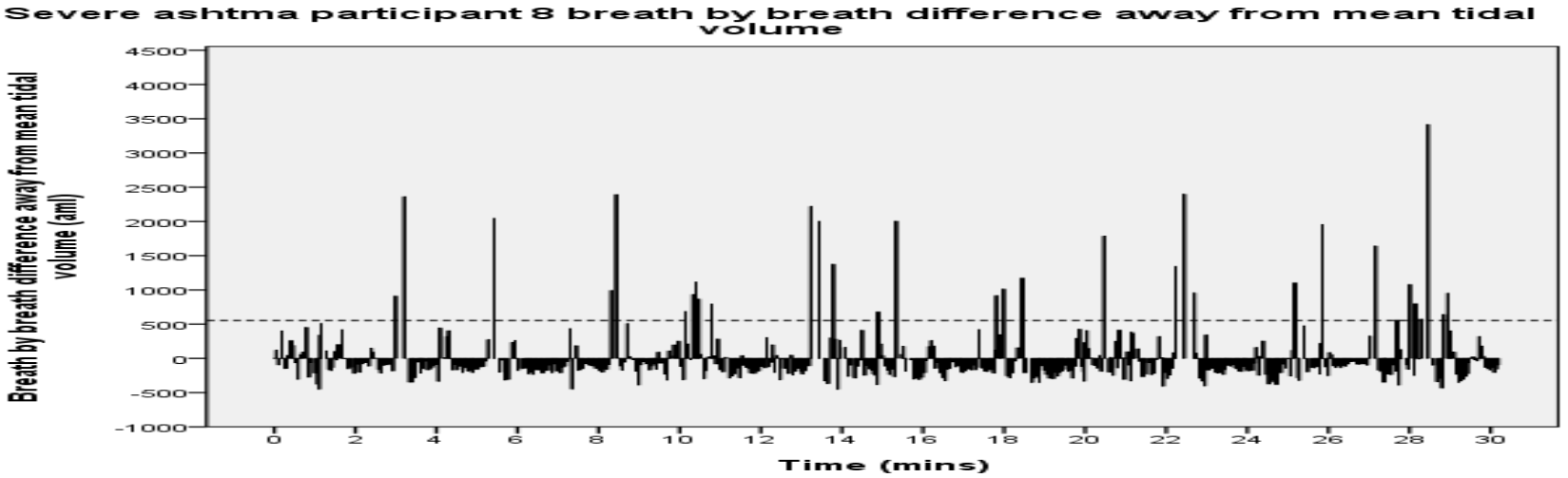

Figure 168: Breath by breath difference away from mean graph for severe asthma participant 8.

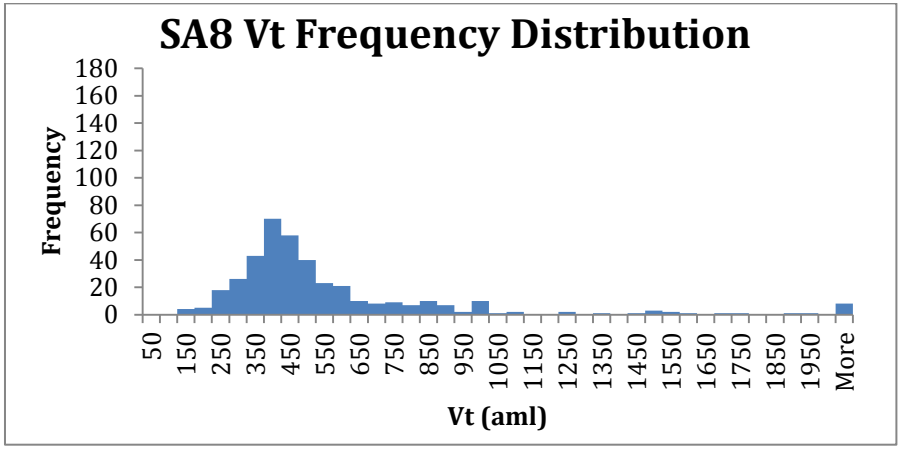

Figure 169: Frequency distribution of tidal volume for severe asthma participant 8.

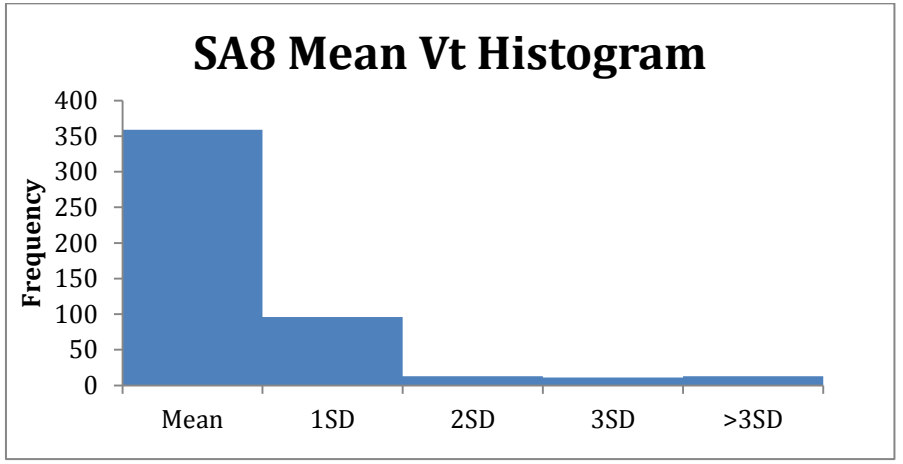

Figure 170: Histogram of tidal volume for severe asthma participant 8.

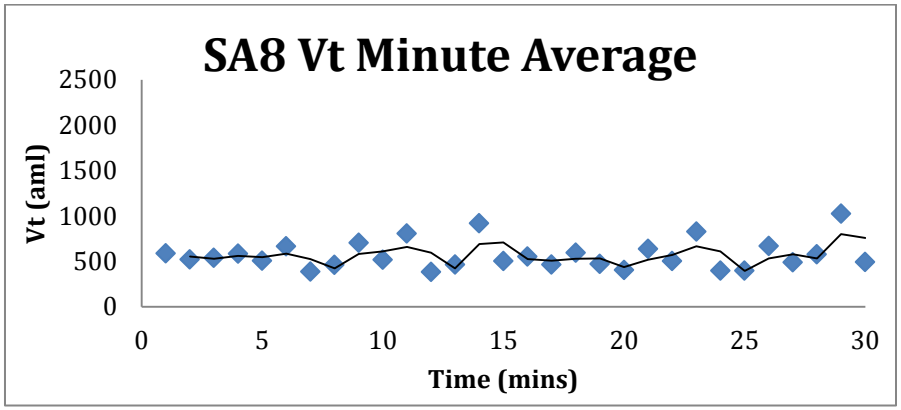

Figure 171: Tidal volume minute average for severe asthma participant 8.

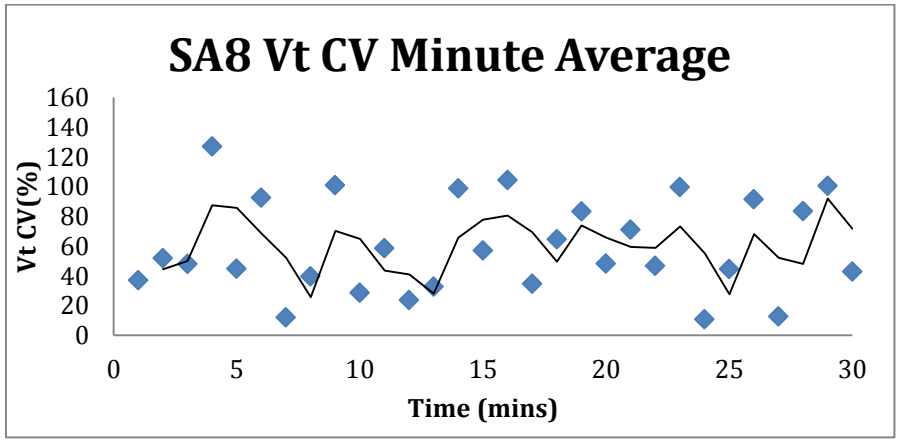

Figure 172: Tidal volume variability minute average for severe asthma participant 8.

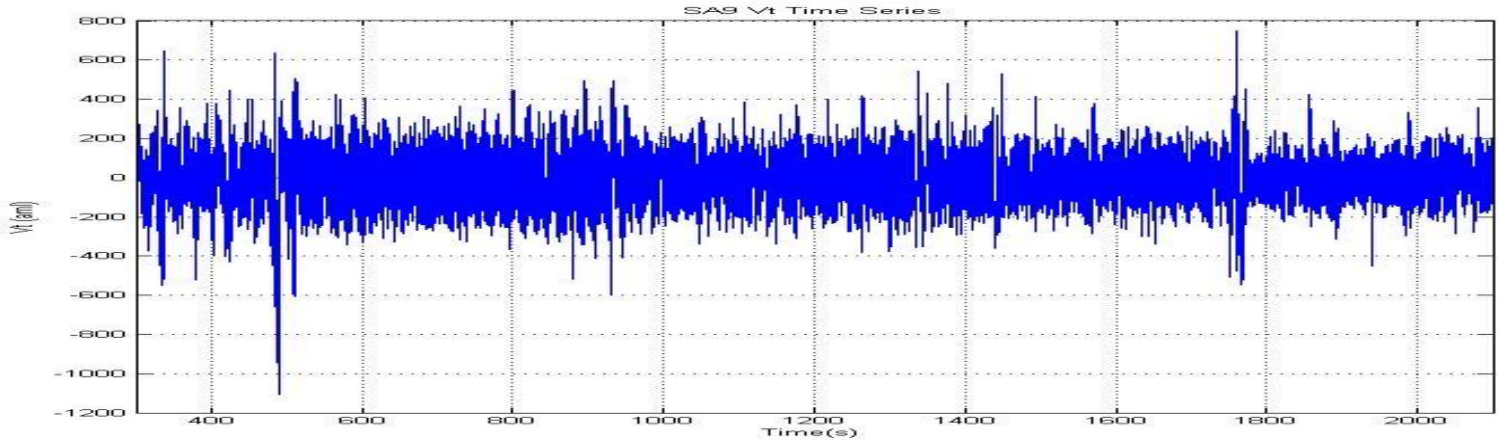

Figure 173: Time series for tidal volume for severe asthma participant 9.

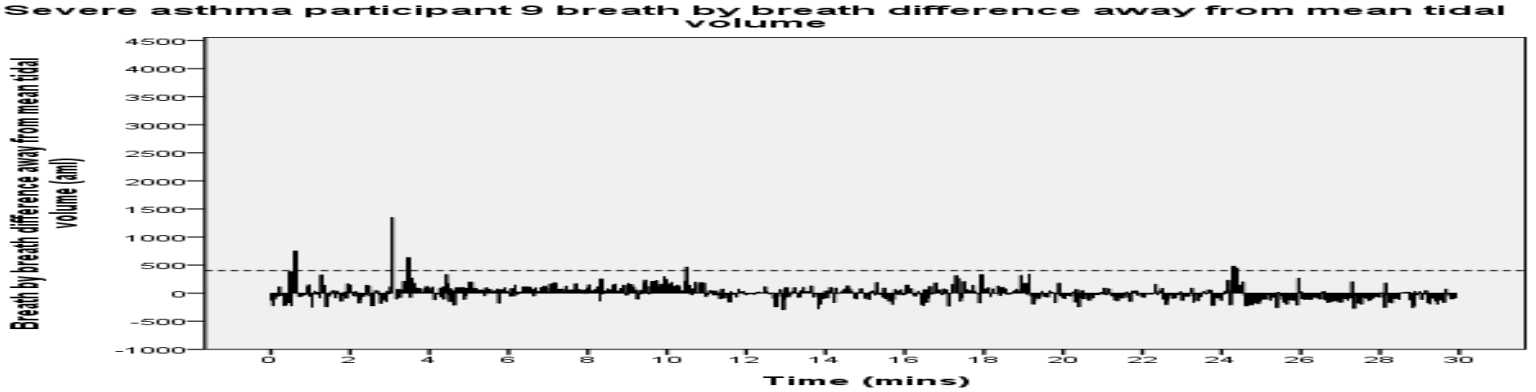

Figure 174: Breath by breath difference away from mean graph for severe asthma participant 9.

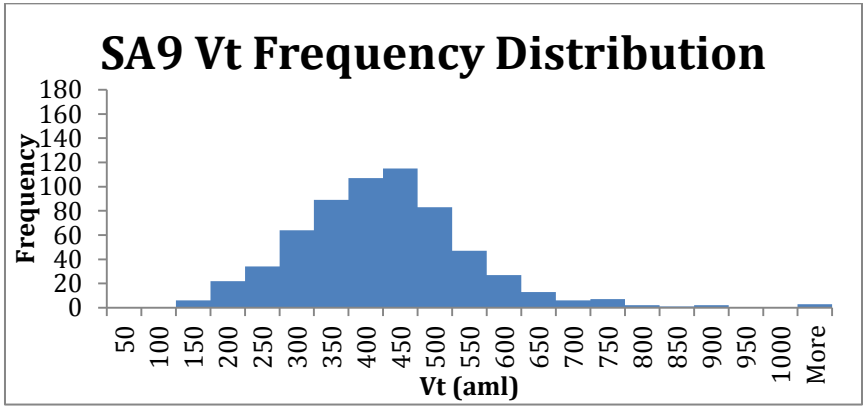

Figure 175: Frequency distribution of tidal volume for severe asthma participant 9.

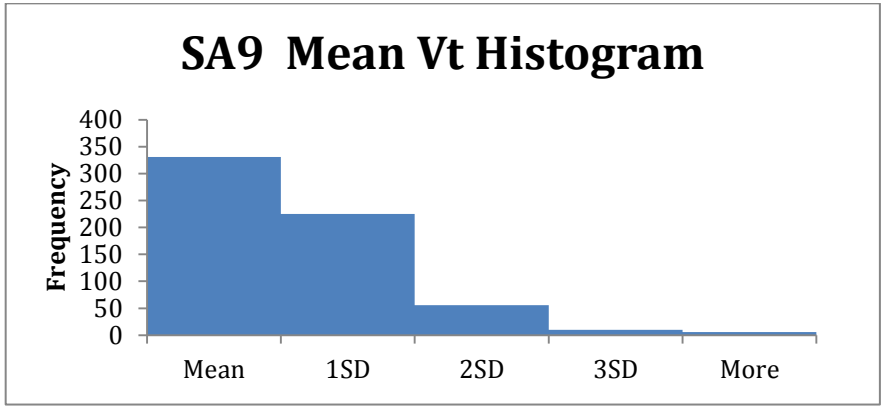

Figure 176: Histogram of tidal volume for severe asthma participant 9.

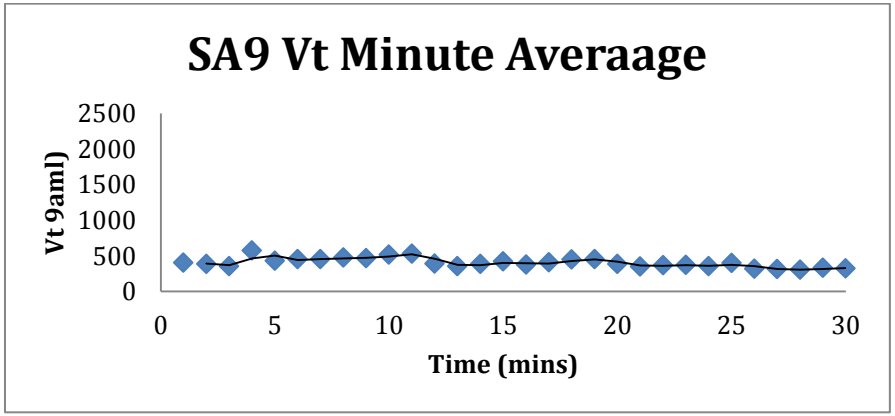

Figure 177: Tidal volume minute average for severe asthma participant 9.

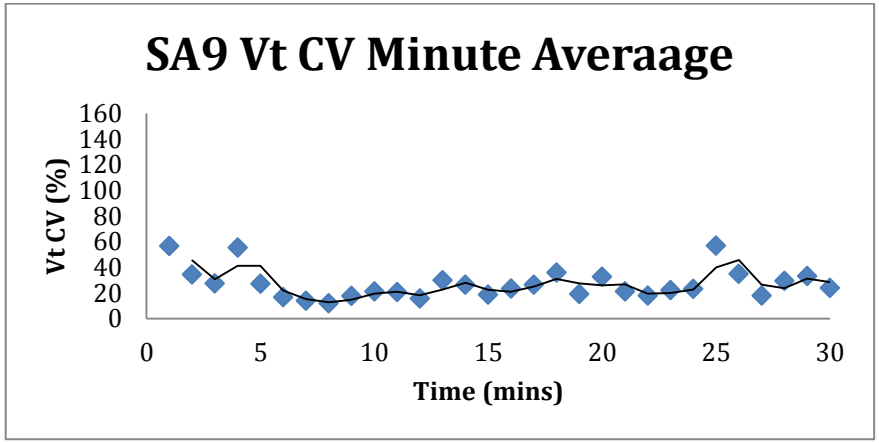

Figure 178: Tidal volume variability minute average for severe asthma participant 9

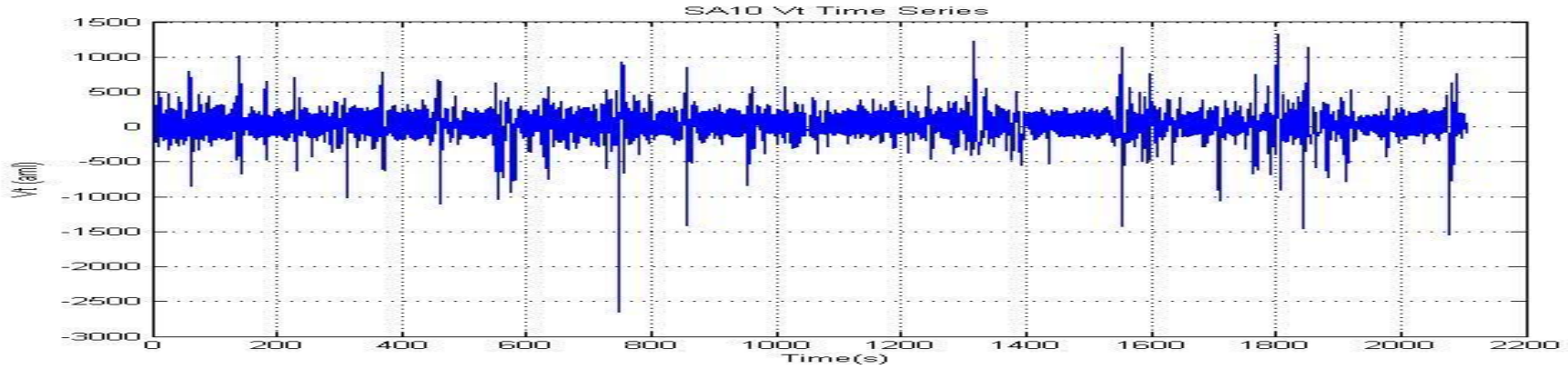

Figure 179: Time series for tidal volume for severe asthma participant 10.

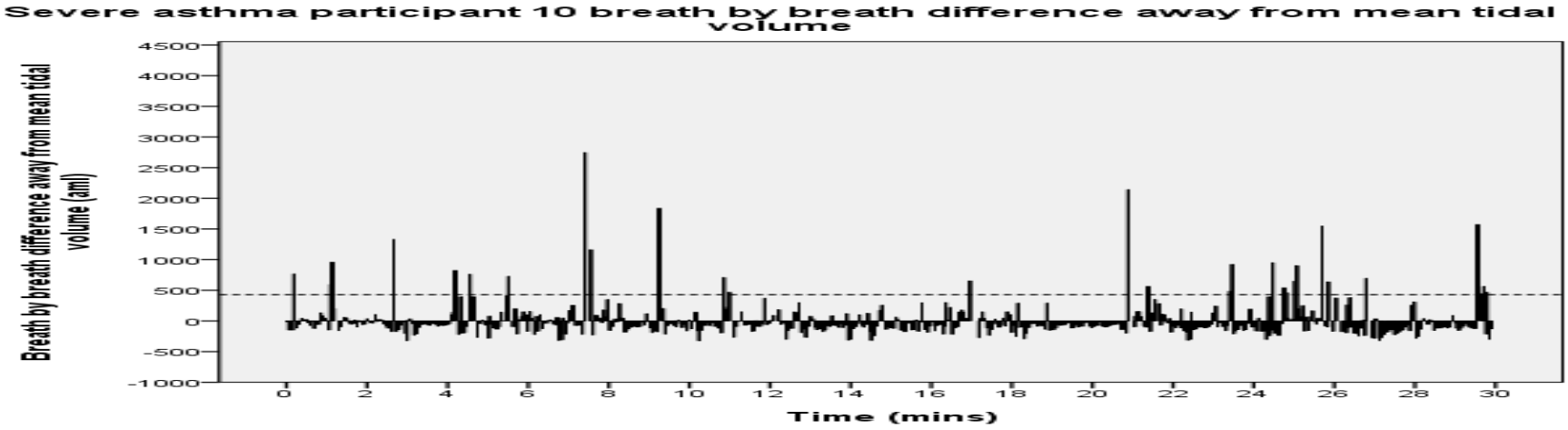

Figure 180: Breath by breath difference away from mean graph for severe asthma participant 10.

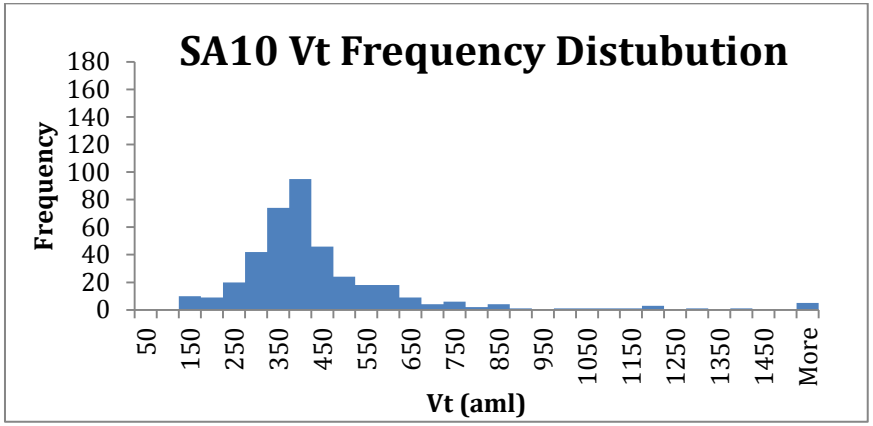

Figure 181: Frequency distribution of tidal volume for severe asthma participant 10.

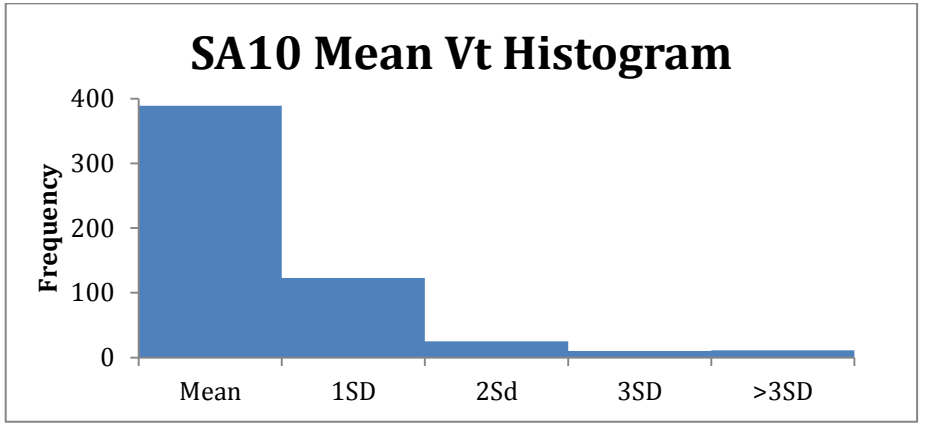

Figure 182: Histogram of tidal volume for severe asthma participant 10.

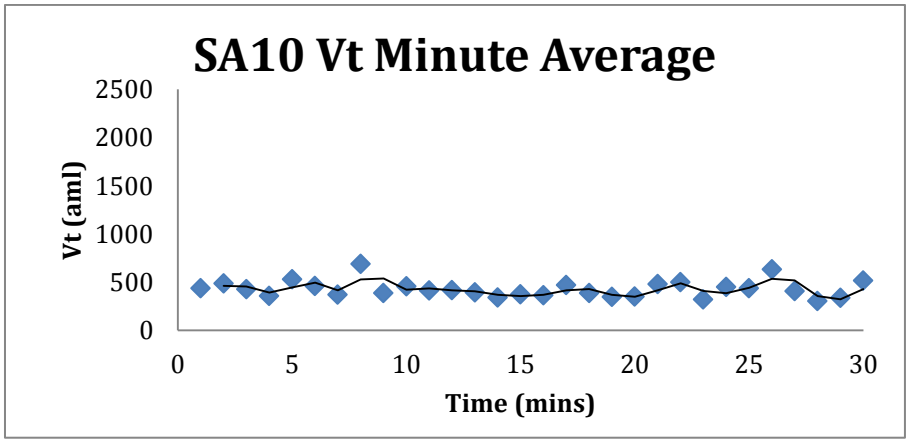

Figure 183: Tidal volume minute average for severe asthma participant 10.

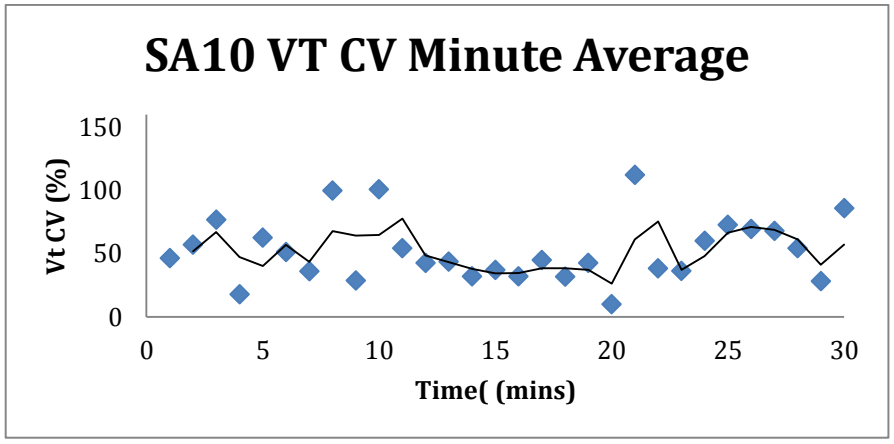

Figure 184: Tidal volume variability minute average for severe asthma participant 10.

Appendix XV – Non parametric test for respiratory parameters from healthy and severe asthma cohort.

The follow tables present the descriptive data of median, mode and quartiles of respiratory parameters recorded from healthy and severe asthma cohorts during rest.

Healthy	Vt (aml)	Ti (s)	Te (s)	$ETCO_2$ (kPa)	NQ	HADS
Median	524.62	1.45	2.32	5.10	5.50	9
Mode	308.26	1.02	1.82	5.10	4.00	1.00
Percentiles						
25	366.79	1.17	2.05	5.00	4.00	4
50	524.62	1.45	2.32	5.10	5.50	9
75	673.56	1.91	2.96	5.15	11.25	12

Table 53: Non-parametric descriptive statistics for healthy cohort.

Severe Asthma	Vt (aml)	Ti (s)	Te (s)	ETCO2 (kPa)	NQ	HADS
Median	380.57	1.32	1.96	5.00	23.00	9
Mode	221.58	1.16	1.63	5.40	14.00	1
Percentiles						
25	340.00	1.21	1.82	4.68	17.75	5
50	380.57	1.32	1.96	5.00	23.00	9
75	514.57	1.52	2.25	5.40	28.00	15

Table 54: Non-parametric descriptive statistics for severe asthma cohort.

The descriptive statistics for non-parametric test were similar to the results obtained in parametric tests. Severe asthma group had a lower median tidal volume, inspiration time, expiration time and end tidal carbon dioxide levels than the healthy group.

On average, the severe asthma group is positive for hyperventilation symptoms.

	NQ	$ETCO_2$	HADS	Vt	Breathing rate	Ti	Te	Sigh Rate
Mann-Whitney U	4.50	34.00	44.500	28.00	30.50	42.50	32.00	41.00
Wilcoxon W	59.50	89.00	99.500	83.00	85.50	97.50	87.00	96.00
Z	-3.45	-1.21	-0.42	-1.67	-1.49	-0.57	-1.36	-0.68
Asymp. Sig. (2-tailed)	0.00	0.23	0.68	0.10	0.14	0.57	0.17	0. 50
Exact Sig. [2*(1-tailed Sig.)]	0.00	0.25	0.68	0.11	0.14	0.58	0.19	0.53

Table 55: Results of Man-Whitney U test for respiratory parameters.

Mann-Whitney U-test demonstrates that there is no statistically significant difference in the recorded respiratory parameter between healthy and severe asthma cohorts, except for NQ score. This is the same findings as with the ANOVA test. These analyses again suggest that the severe asthma cohort has a similar breathing pattern in comparison with the healthy population.

	Vt CV	Te CV	$ETCO_2$ CV
Mann-Whitney U	50.00	49.00	39.50
Wilcoxon W	105.00	104.00	94.50
Z	0.00	-0.08	-0.81
Asymp. Sig. (2-tailed)	1.00	0.94	0.42
Exact Sig. [2*(1-tailed Sig.)]	1.00	0.97	0.44

Table 56: Results of Man-Whitney U test for variability between severe asthma and healthy group.

Man-Whitney U-test demonstrates no difference in the variability of tidal volume, expiratory time and end tidal volume.