Mechanisms and Management of Breathlessness in Chronic Heart Failure

A thesis submitted for the degree of Doctor of Philosophy at Oxford Brookes University

By

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STATEMENT

I have been the principal investigator in the work presented in this thesis. I have been involved in the planning, experimentation and analysis of all of the studies. The experiments were conceived and executed in collaboration with Dr Shakeeb Moosavi (Senior Lecturer in Clinical Physiology, Faculty of Health and Life Sciences, Oxford Brookes University). Oxford Brookes University Undergraduate students; Jonathan Morris, Fiona Moore and Roisin Mckenna-Favier assisted in data collection during the inhaled furosemide studies, and Fatemah Hegab assisted with administering the survey in the community. Nurses Clare Butler and Francisca Mada maintained blinding, and Hooshang Izadi assisted me in the statistical analysis of the healthy volunteer study of inhaled furosemide. I am very grateful to the support they have given me.

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ABSTRACT

Background: The pathophysiology of dyspnoea ('breathlessness') is poorly understood and treatment options limited. This is particularly true for heart failure in which dyspnoea is a cardinal symptom, even when the heart failure is optimally managed. This thesis aims to untangle mechanisms and utilise this knowledge to optimise heart failure management. It focuses on the potential of nebulised furosemide as an adjunct treatment, given its excellent safety record and existing evidence that it modulates dyspnoea via direct action on lungs.

Methods: A multi-dimensional questionnaire was used to survey the prevalence of dyspnoea in the heart failure community. A randomised, double blind, placebo-controlled crossover trial (RCT) was then performed in healthy participants to determine the specific components of dyspnoea that are relieved by the action of furosemide on the lungs. This study led to the design of a feasibility RCT in patients with heart failure using the visual analogue scale (VAS) ratings of the 'air hunger' (AH) component of dyspnoea as the primary outcome measure. The RCT itself; i) addressed other issues that could account for variability in relief seen in previous studies, ii) explored blood biomarkers of heart failure in relation to dyspnoea and iii) provided guidance for future definitive clinical trials.

Results: 1) 47% of patients experienced dyspnoea in the community. Dyspnoea-12 scores correlated with New York Heart Association class, with many in class III experiencing dyspnoea at rest. 2) Nebulised furosemide specifically relieved AH induced in healthy participants but did not affect the 'work/effort' component. Relief was only with nebulised, not intravenous furosemide. 3) Breathing furosemide quickly or slowly did not alter dyspnoea relief, but ventilation was not matched. 4) Cardiopulmonary exercise testing (CPET) produced an average VO_2peak of 54±15% predicted, with a measurable anaerobic threshold in 73% of tests and raised dyspnoea to 42±19%VAS. 5) Nebulised furosemide resulted in no significant improvements in exercise capacity. 6) Cardiac biomarkers increased appropriately and returned to baseline within 1 hour of exercise. The maximal absorption efficiency of nebulised furosemide was 2%.

Conclusion: 1) Dyspnoea is a prevalent symptom in heart failure, comparable to chronic obstructive pulmonary disease. The NYHA classification may require clarification regarding presence of breathlessness at rest. 2) Relief of dyspnoea with nebulised furosemide occurs via a mechanism within the lungs and should be targeted at those in whom 'air hunger' predominates. 3) CPET is a feasible method for dyspnoea assessment in heart failure. 4) Fully powered RCT of nebulised furosemide in heart failure are warranted taking on board the preliminary information gathered in this thesis to optimise treatment effect.

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ABBREVIATIONS

AH	Air hunger
BP	Blood pressure
BNP	Brain Naturetic Peptide
CDS	Cancer Dyspnoea Scale
CHF	Chronic Heart Failure
COPD	Chronic obstructive pulmonary diseases

CPET	Cardiopulmonary exercise testing
CVa	Analytical variation
CVi	Within-person variation
CVg	Between person variation
D12	Dyspnoea 12
DLCO	Diffusing capacity of the lung for carbon monoxide
ECG	Electrocardiogram
EF	Ejection fraction
EOV	Exercise oscillatory ventilation
ESC	European Society of Cardiology
fR	Respiratory rate/Breathing frequency (per minute)
FVC	Forced Vital capacity
FEV	Forced expiratory volume
HF	Heart Failure
HRQoL	Health-related quality of life
hsTn	High sensitivity troponin
II	Index of individuality
LoQ	Limit of quantification
LV	Left ventricle
MBS	Modified Borg Scale
MCID	Minimally clinically important difference
MDP	Multidimensional dyspnoea profile
MLHFQ	Minnesota Living with Heart Failure Questionnaire
MRI	Magnetic Resonance Imaging
NRS	Numerical Rating Scale

NT-proBNP	N-terminal pro brain naturetic peptide
NYHA	New York Heart Association
O_2 pulse	Ratio of oxygen consumption to heart rate
OUES	Oxygen uptake efficiency slop
PEF	Peak expiratory flow
PAW	Airways pressure
$PaCO_2$	Partial pressure of carbon dioxide
PaO_2	Partial pressure of oxygen
$P_{ET}CO_2$	End-tidal carbon dioxide partial pressure
$P_{ET}O_2$	End-tidal oxygen partial pressure
PSR	Pulmonary stretch receptors
PAWP	Pulmonary arterial wedge pressure
PCWP	Pulmonary capillary wedge pressure
raPSR	Rapidly adapting pulmonary stretch receptors
RCT	Randomised controlled trial
RCV	Reference change value
saPSR	Slowly adapting pulmonary stretch receptors
sd	Standard deviation
se	Standard error
sem	Standard error of the mean
SpO_2	Oxygen saturation
Tn	Troponin
Te	Expiratory time of *respiratory* cycle
Ti	Inspiratory time of *respiratory* cycle
Ttot	Total time of *respiratory* cycle

TV	Tidal volume
VAS	Visual analogue scale
VE	Minute Ventilation
VE/VCO2	Minute ventilation/carbon dioxide production ratio
VO_2	Oxygen consumption
VO2 max	Maximal oxygen uptake
VCO_2	Expired carbon dioxide
VT	Tidal volume
WE	Work and effort
6MWT	Six-minute walk test

INTRODUCTION

Severe breathlessness or dyspnoea is a frequent and debilitating symptom observed in patients with broad ranging pathologies including pulmonary disease, heart disease, neuromuscular disorders and cancers. Dyspnoea is a cardinal symptom of heart failure irrespective of the underlying aetiology and progresses as the disease advances. Heart failure is a major global disease, affecting around 26 million people worldwide[1] and accounting for 8.5% of deaths in the US[2]. Dyspnoea occurs both in patients with preserved and reduced ejection fraction, and can be acute, such as in the context of acute pulmonary oedema, or chronic. The work of this thesis begins with a survey of dyspnoea within the community to better understand the scope of the problem in heart failure. Dyspnoea is described as one of the most unpleasant/distressing symptoms experienced by the patient and yet the mechanism behind dyspnoea in heart failure is not fully understood. Assessment can therefore be difficult and treatment options are often limited. Despite proven therapies in heart failure for relief of symptoms and mortality benefit seen from beta-blockers, angiotensin-converting-enzyme inhibitors or angiotensin receptor blockers, mineralocorticoid receptor antagonists, diuretics and device therapy such as cardiac resynchronisation, many patients remain dyspnoeic[3].

A better understanding of the mechanisms of dyspnoea is needed to be able to identify and implement much needed new treatment options. This thesis continues by exploring the mechanisms of dyspnoea. This initially involves studying healthy

volunteers and then focuses on the potential application of nebulised furosemide for dyspnoea relief in chronic heart failure (CHF).

This introduction covers the different types of dyspnoea, the current theories regarding the potential underlying mechanisms, its assessment, and the treatment options available; whilst focusing on nebulised furosemide as a putative future therapy.

1.1. CURRENT UNDERSTANDING OF DYSPNOEA

For a long time dyspnoea was thought to be a single sensation arising from a single source (respiratory muscles). The current consensus for the definition of dyspnoea is that it is a subjective experience of breathing discomfort that is comprised of qualitatively distinct sensations that may vary in intensity[4]. It is modulated by multiple interactions involving physiological, psychological, social and environmental factors[5]. Advances have also been made with regard to assessment of dyspnoea with the advent of multi-dimensional dyspnoea questionnaires[6, 7] and establishment of experimental models capable of inducing the individual components of clinical dyspnoea.

At least three distinguishable sensations of dyspnoeic discomfort have been identified; air hunger, work/effort, and chest tightness;[8-10] thus the sensation of breathlessness due to excessive exertion feels different to that caused by asphyxiation. Individual component sensations can be separately manipulated and therefore are thought to arise from different neural pathways[11-14]. These different types of dyspnoea may open up the possibility for different types of targeted treatment.

1.1.1. Mismatch theory of dyspnoea

In its simplest form, dyspnoea is thought to be both related to an awareness of increased breathing and the perception that the drive to breathe has not been matched by adequate pulmonary ventilation[4, 15]. The size of the resulting mismatch between the voluntary motor command and the amount of chest wall expansion or lung stretch, gives the severity/intensity of the sensation perceived, Fig. 1.1. This is known by a variety of terms such as efferent-reafferent mismatch, length-tension inappropriateness, or neuromechanical dissociation[16-18].

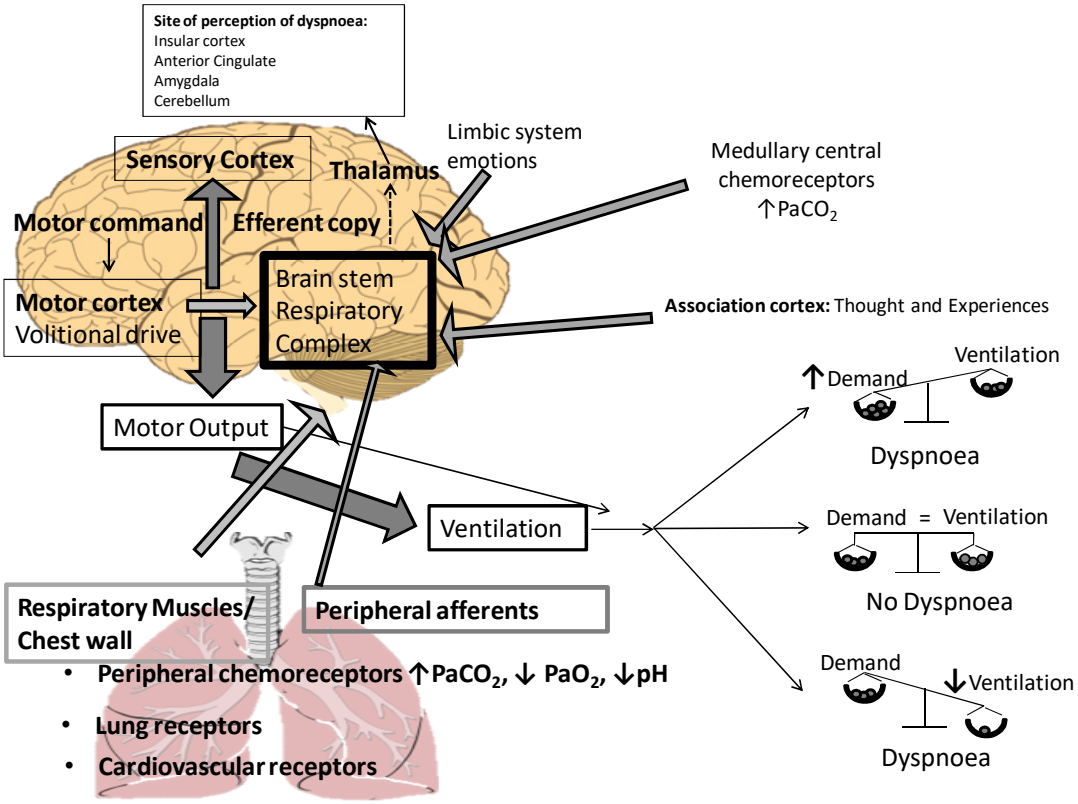


Figure 0.1. Mismatch theory of dyspnoea

There are multiple central respiratory controllers in the brain stem, mechanoreceptors in the lung and chemoreceptors in the peripheral circulation and medulla that control breathing. A mismatch between the efferent copy (need to breathe) and feedback from peripheral afferent receptors (how much you are breathing) results in dyspnoea. $PaCO_2$ = partial pressure of carbon dioxide, PaO_2 = partial pressure of oxygen.

Sense of air hunger

When the spontaneous respiratory motor drive of the brainstem[15] is increased due to hypoxia, hypercapnia, acidosis or exercise, this information is relayed as a 'corollary' copy to the cerebral cortex[19, 20] leading to the air hunger[21, 22]. However, when subjects are free and able to respond with larger tidal volumes, increased vagal afferent discharge from lung mechanoreceptors inhibit the 'corollary' copy as it ascends to the midbrain[19]. Four different lung mechanoreceptors have been identified; slowly adapting pulmonary stretch receptors (saPSR), rapidly adapting pulmonary stretch receptors (raPSR), pulmonary C-fibre receptors and bronchial C-fibre receptors. All of these transmit information to the central nervous system[18, 23] and information from these receptors may modulate the intensity of air hunger[24]. If the reflex increase in ventilation is prevented due to clinical pathology or due to experimental intervention, the sensation of air hunger occurs. Neuroanatomical studies in humans are challenging and therefore little is known about how afferent pulmonary information is processed centrally. Animal studies have proposed that the site of comparison of the signals reporting need to breathe and signals reporting the prevailing ventilation for air hunger resides within the midbrain/thalamus region[25]. The nucleus tractus solitarius (NTS) in the medulla receives afferent information from peripheral sensors via the vagal C fibres[26]. The NTS processes the signals and determines the output of sensory information from the lungs to downstream reflex pathways and to areas within the higher brain[26]. A study in decerebrate, paralyzed and ventilated cats with the spinal cord transected at C7-T1 and carotid sinus nerves cut but vagus nerve left intact found neurons with respiratory-associated rhythmic activity. Changing the pulmonary vagal input via a variety of methods showed that the vagal input (probably from PSRs)

inhibited the respiratory-associated firing of neurons by a direct mechanism which was independent of a vagal effect on medullary respiratory drive[25]. This suggested that these neurons are involved in relaying information to the cortex where it could be interpreted as the sensation of dyspnoea and if the lungs expand this would be expected to lessen the sensation. This occurs in automated reflex breathing. This is in contrast to the conscious awareness of the outgoing respiratory motor command to the ventilatory muscles. Air hunger is thought to increase when information from saPSR decrease[24] for a given respiratory motor drive. This suggests an inhibitory effect of saPSR activity on the sensation of air hunger[27-30]. Conversely, an increased drive to breathe from arterial blood gas disturbance in the absence of changes in saPSR activity associated with fixed tidal volumes will accentuate air hunger[24, 25, 31-37].

Sense of breathing effort

Excessive respiratory muscle activity (e.g. due to increased impedance to inspiration[12, 13, 38, 39] such as from weak breathing muscles, interstitial lung disease and chronic obstructive pulmonary disease (COPD)), often results in the work/effort type of dyspnoea [9]. When there is an increase in *voluntary* motor drive from the cerebral cortex[40, 41] to the respiratory muscles; this triggers a sensation of respiratory effort[12, 13]. If the brain sends an efferent voluntary motor command to the respiratory muscles to increase tidal volume, a corollary discharge of this signal reports the demand for breathing to the brain. If the returning afferent signals (reporting the prevailing level of ventilation) from the mechanical receptors in the airways and chest wall, show that expansion is less than expected, then the cortex registers that the inspiratory muscles are weak or not working properly and that the system is 'loaded'[5, 42, 43].

1.1.2. Psychological influences on dyspnoea

It is well documented that there is a strong psychological component to dyspnoea[44]. For example, breathlessness during light exercise is not unpleasant whereas breathlessness present to the same degree in certain diseases can be intolerable. It is therefore not just the physical sensory input but the context in which it arises that culminates in the symptom of dyspnoea. The areas of the brain that have been identified in functional brain imaging studies of dyspnoea perception are also associated with psychological factors (e.g. fear and anxiety) or have great connectivity to areas known to be involved in manifesting these psychological states. For example, the amygdala which is involved with emotion processing and sensory modulation has been shown to be activated in several (but not all) functional MRI dyspnoea studies[45, 46]. Another area likely to be involved in the psychological processing of dyspnoea is the pre-frontal cortex. This is central to emotional processing, including threat/fear awareness associated with unpleasant sensations (e.g. pain), and has recently been identified during breathlessness-related anxiety processing in COPD patients[47-49]. It is likely that the activation of brain regions depends on the type of breathlessness or breathing stimulus employed, and that these networks may be different between patients and healthy controls. This is particularly salient for 'emotional' breathlessness processing, which is highly subjective and dependent upon context.

1.2. CLINICAL DYSPNOEA

The main causes of dyspnoea are increased chemical or neurological drive to breathe such as by stimulation of chemoreceptors, increased work of breathing such as in pleural effusions and decreased neuromuscular power as found in cachexia. The

chemical drive to breathe is controlled by hypercapnia and hypoxia, detected by chemoreceptors in the medulla and carotid bodies. Peripheral receptors include mechanical stretch receptors in the chest wall and diaphragm, stretch receptors in the airways and pulmonary C-fibres in the lung parenchyma. These all send afferent signals to the brain about the current level of ventilation. In heart failure the increased pulmonary venous pressure and presence of fluid stimulates the pulmonary C fibres[50].

In clinical practice it would be useful to be able to determine the underlying cause of dyspnoea by the descriptors patients use to describe their breathlessness. One study comparing lung cancer patients to those with cardiorespiratory disease found that all patient groups were characterised by more than one cluster of descriptors, with several clusters being shared between groups. There were some specific sets of clusters, such as in heart failure the most common descriptors were 'I feel out of breath (57%)', 'my chest feels tight (33%)', 'I cannot get enough air (33%).' However, the relationship was not sufficient to aid differential diagnosis[51]. It is therefore not possible to diagnose someone with heart failure based on their description of dyspnoea. Another study found that the most common terms volunteered by patients with chronic heart failure were 'hard to breathe', 'shortness of breath' and 'gasping'. These overlapped with some of the descriptors of dyspnoea used in patients with COPD which were; 'scary', 'hard to breathe', 'shortness of breath', and 'cannot get enough air'. The only term that was significantly different between COPD and CHF was 'my breath does not go all the way out' which was more specific to COPD[52]. Identifying the different components of patients dyspnoea could be useful as it implies that the dyspnoea is being generated by different underlying pathways/mechanisms and this could aid treatment. The multi-dimensional dyspnoea questionnaire (MDP) was

designed to identify the different components and has been shown to correlate well with the D12[53].

1.2.1. Heart Failure

Dyspnoea in patients with stable CHF has a different presentation and quality to the dyspnoea resulting from acute heart failure or decompensated chronic heart failure resulting in pulmonary oedema. In pulmonary oedema, pulmonary congestion reduces the compliance of the lung and increases the work/effort of breathing[54]. Conversely, in stable CHF, patients tend to report their dyspnoea as smothering, suffocating at rest, not enough air, an inability to breathe or they express a sensation of rapid breathing rather than describe an increase in work/effort[4]. Dyspnoea in CHF remains poorly understood and poorly investigated despite dyspnoea severely limiting quality of life for these patients. One approach to managing heart failure is to resolve the peripheral pathophysiological issues giving rise to breathlessness, Fig. 1.2. However even when heart failure is optimally managed the debilitating breathlessness persists; the limited literature available on dyspnoea in patients with heart failure suggests that over 50% experience daily dyspnoea or fatigue that affects their quality of life, restricts their activities and undermines their will to live[55, 56]. It has been shown that dyspnoea is a better predictor of cardiac mortality than the presence of angina[57]. An alternative approach is to alter the perception of dyspnoea utilising advances in the understanding of brain mechanisms as shown in Fig. 1.1 above. This could help identify new therapy targets in CHF[48, 58].

Potential Mechanisms of Dyspnoea in Chronic Heart Failure

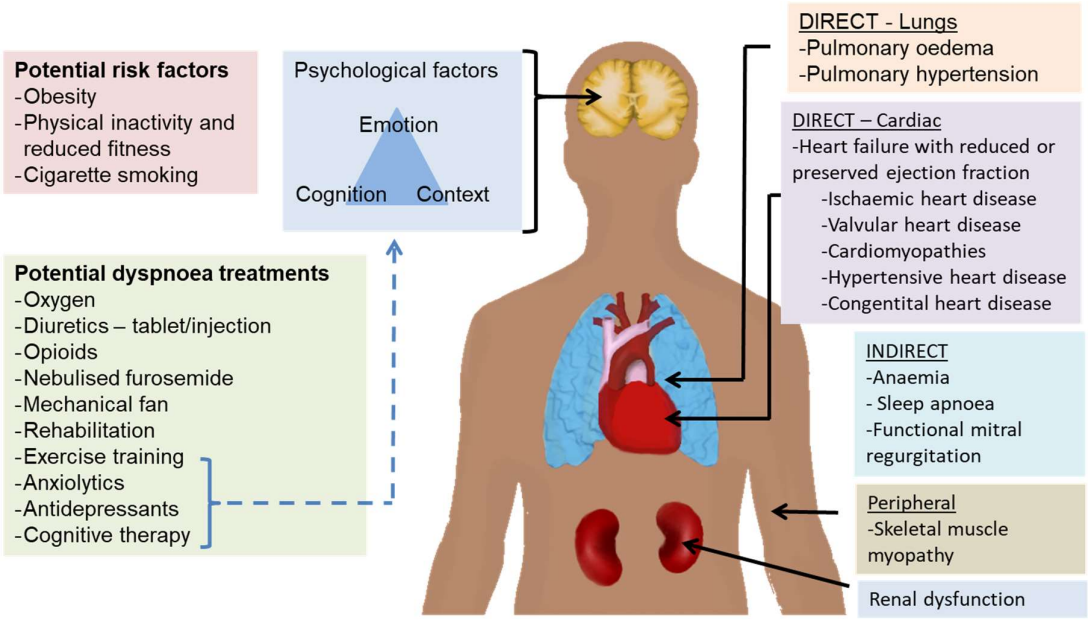


Figure 0.2. Potential causes, risk factors and treatments of dyspnoea in CHF

Original teaching initially explained the mechanisms of dyspnoea in heart failure through the haemodynamic theory of poor left ventricular (LV) function requiring high left filling pressures to try to maintain cardiac output. This increased pressure is transmitted back to the pulmonary vasculature resulting in vascular distension and interstitial oedema. The oedema reduces pulmonary diffusion and may stimulate pulmonary vascular nerve endings/receptors (pulmonary C-fibre receptors, previously known as J-receptors) and play a role triggering dyspnoea[4, 50, 59-61]. However, even when the pulmonary oedema is treated and resolved patients often still report dyspnoea. This therefore suggests that pulmonary oedema is not the sole cause of dyspnoea in heart failure. While increased LV filling pressures may reduce pulmonary diffusion and result in interstitial oedema, haemodynamic changes alone cannot fully explain the cause of dyspnoea in chronic heart failure. No studies have directly compared ventricular function to ratings of dyspnoea. If exercise is used as a surrogate

marker for dyspnoea, there is only a weak correlation with LV dysfunction, Fig. 1.3[62, 63]. Again, if using exercise as a surrogate for dyspnoea, measurement of pulmonary venous pressures also does not show any correlation[64, 65], nor does the use of vasodilators[66]. In those studies that have used exercise tolerance as a marker of dyspnoea there does not appear to be a direct link to a reduced cardiac output. Therefore, one needs to look beyond LV dysfunction to gain a full understanding of dyspnoea in heart failure.

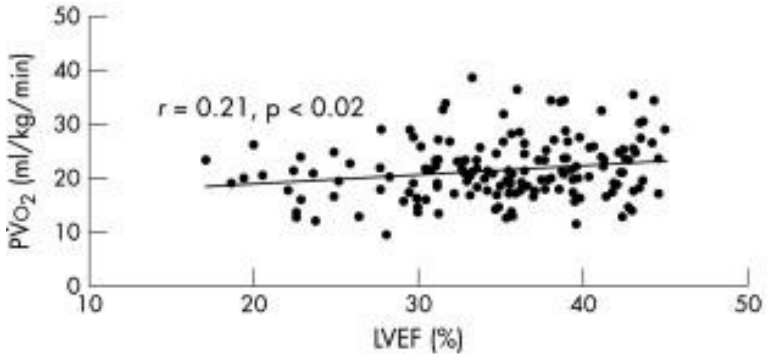


Figure 0.3. Relationship between heart function and exercise capacity

There is a relatively poor correlation between LV ejection fraction (LVEF) and exercise capacity[63].

Evidence suggests there is a reflex network that becomes hyperactive in heart failure due to changes in the musculoskeletal system, and that this is detected by mechanoreceptors/metaboreceptors in skeletal muscles[67]. It is known that in heart failure the sympatho-inhibitory reflexes are suppressed. A recent theory thus proposes that fatigue and dyspnoea in heart failure are due to hyperactivation of signals originating from receptors located in the skeletal muscles[68]. Metaboreceptors within the muscle detect changes in the metabolic milieu of the muscle tissue, such as poor oxygen delivery compared to oxygen demand. Activation of these afferent receptors

can affect the heart rate, blood pressure, cardiac output, systolic volume, ventilation and sympathetic tone[67, 69] and could generate the feeling of dyspnoea.

Another theory implicates ergo receptors (mechanoreceptors sensitive to movement) in the respiratory skeletal muscles which, when activated, signal dyspnoea[70]. These have been shown to be overactive in patients with CHF. Their activity is linked to the ventilatory response to exercise resulting in dyspnoea and contributing to the sympathetic overactivity of CHF. Diaphragmatic weakness is present in heart failure and increases progressively during pressure overload, irrespective of the presence of pulmonary oedema. This has been shown to be associated with signalling changes in angiotensin II and adrenoceptors resulting in centrally controlled ventilatory overdrive[71]. However, evidence suggests that dyspnoea remains, despite paralysis of these respiratory muscles[24], which indicates that another signalling pathway is involved; most likely arising from receptors in the lung.

A further potential mechanism is related to pulmonary pressures. Chronic LV failure can result in pulmonary hypertension, with an increased trans-pulmonary gradient and increased pulmonary vascular resistance. Dyspnoea is often found with pulmonary hypertension regardless of the underlying cause. In patients with heart failure, pulmonary vascular resistance is an important determination of exercise capacity, which contributes to dyspnoea on exertion[72, 73]. Malfatto et al (2015) showed that increased pulmonary capillary wedge pressure (PCWP, measured directly and estimated non-invasively) significantly contributes to abnormal ventilation in chronic advanced heart failure[74]. The authors suggest that pulmonary congestion may be the crucial component eventually leading to exertional dyspnoea, rather than low cardiac output or abnormal autonomic balance. However, other studies have shown a

dissociation between PCWP and dyspnoea severity, such that patients with a high PCWP are minimally dyspnoeic and patients with low PCWP may experience severe dyspnoea[75]. Stable CHF patients with high PCWP typically do not develop pulmonary oedema. This is partly due to the protective mechanisms[76, 77], but also the development of alveolar fibrosis. This alveolar remodelling process may be protective against further high pressure damage and may increase resistance of the lung to the development of pulmonary oedema in stable CHF patients[78]. These patients also have reduced pulmonary microvascular permeability[79], which may be a defence against pulmonary oedema in patients with chronic pulmonary venous hypertension. However, it also causes a decrease in alveolar diffusion capacity, impairs gas transfer and reduces exercise capacity.

Patients with stable CHF often exhibit a restrictive ventilatory flow loop. They also present with reduced diffusing capacity of the lung for carbon monoxide (DLCO)[80] which are typically associated with elevated pulmonary vascular pressures. It is possible that the simple displacement of the lung by the enlarged heart accounts for a substantial portion of the restrictive pattern seen, while the rest is due to interstitial oedema, pleural effusions, vascular engorgement and respiratory muscle weakness[54]. This may all contribute to the development of dyspnoea.

The emergence of dyspnoea in CHF thus appears to be due to activation of mechanoreceptors (including ergoreceptors) and metoboreceptors in skeletal and respiratory muscles such as the intercostal and diaphragm muscles. While a role for pulmonary congestion (interstitial oedema) and reduced pulmonary diffusion is appearing increasingly unlikely in many patients with chronic heart failure, one cannot rule out a contribution of this factor. More research is needed to fully elucidate the

origins of dyspnoea in CHF, for example: magnetic resonance imaging of the lungs to assess for presence of interstitial oedema and correlate with dyspnoea ratings. It should also be considered that there may be different aetiologies depending on individual pathophysiology and psychology. Given current understanding of the mechanism of air hunger involving comparison of signals reporting the need to breathe with those reporting the prevailing ventilation, it is interesting to speculate whether the air hunger experienced by heart failure patients arises from diffusion impairment combined with respiratory muscle dysfunction. Patients with both COPD and HF have an increased neural drive, with a ventilator response above that required to overcome an increase in 'wasted' ventilation resulting in hypocapnia and reduced exercise capacity[81].

While the development of dyspnoea in CHF is not yet fully understood, it is clear that the dyspnoea in these patients is having a real and measurable impact on their day to day life.

1.2.2. The impact of physical exertion in CHF

Reduced exercise tolerance is a key symptom of patients with heart failure. This has been characterised by a decrease in maximal oxygen uptake (VO_2 max), which is the most powerful predictor of dyspnoea during exercise[82] and has prognostic value[83]. Impaired systolic function is not the only factor involved in reduced exercise tolerance in CHF and the determinants of VO_2 max are more complex than in the normal subjects. The majority of haemodynamic parameters assessed at rest do not correlate with VO_2max and this is likely due to the fact that these do not take into consideration the reserve of the heart, which can only be assessed during exercise or with

pharmacological stress, and the key role played by circulatory and muscular peripheral factors.

In patients with CHF the maximum heart rate is reduced with a blunted chronotropic response. This is due to the desensitization of beta adrenergic receptors[84] . Stroke volume (end-diastolic minus end-systolic volume) is reduced at rest in CHF and it often reduces further during exercise[85]. Patients with CHF are not able to increase their contractile reserve (and reduce end-systolic volume) to meet the increased afterload that occurs during exercise. Therefore the only way to alter the stroke volume is to increase the end-diastolic volume but this is varies among patients, and is accompanied by significant increases in filling pressures. Increased compliance of the ventricles can increase the end-diastolic volume but in advanced heart failure this is often exhausted. There is also diastolic dysfunction with impaired relaxation that reduces LV filling during exercise. There is a blunted blood pressure response during exercise related to the reduction in cardiac output and also has prognostic value[86]. Mitral regurgitation can increase significantly during exercise and in some patients can explain the reduced exercise tolerance and associated dyspnoea[87].

Exercise limitation in CHF is often not only related to the heart but to extra-cardiac/peripheral abnormalities. Increasing cardiac output during exercise with dobutamine results in a minimal increase in oxygen consumption due to a parallel reduction in arteriovenous oxygen difference[88]. This reduced oxygen extraction is thought be due to a redistribution of cardiac output away from the active muscles or due to altered muscular use of oxygen.

There is also a blunted vasodilation in response to exercise in patients with CHF due to abnormalities in the endothelium and profound alterations in flow-dependent vasodilatation[88]. Atrophy of muscle is common in patients with heart failure and is associated with cachexia, deconditioning and malnutrition. Cross sectional area of a muscle group is closely correlated to VO2max. There is also a change in the muscle fibre distribution and skeletal muscle composition that correlate with reduced exercise tolerance in CHF[89, 90]. Reductions in mitochrondial density and enzymes involved in the oxidative pathway are also reduced and explain the rapid fall in intracellular pH indicating the preferential use of anaerobic glycolysis compared to normal subjects[91]. It is unknown whether abnormalities in the vasculature or muscles are more important in determining exercise limitation.

Patients with CHF experience dyspnoea during physical exertion[92]. These patients have a higher degree of ventilation for any given workload compared to normal subjects[93]. The respiratory pattern is also different with an increased respiratory rate and decreased tidal volume[94]. Dynamic hyperinflation is known to contribute to dyspnoea in patients with COPD[95]. It results in an increase in end-expiratory lung volume and subsequent reduction in inspiratory capacity. Unlike in COPD dynamic hyperinflation is not known to occur in patients with chronic heart failure and is therefore not thought to cause dyspnoea. However, there is a significant overlap of patients with COPD and HF, with approximately 30% suffering from both conditions[96] and therefore many patients with HF may show signs of hyperventilation if they have co-existing COPD.

In patients with stable chronic heart failure there is an elevated exercise ventilation response compared to normal subjects at all levels of exertion. Exercise studies in

patients with heart failure show an increased minute ventilation/carbon dioxide production ratio (VE/VCO_2). This is mainly attributed to alveolar hyperventilation due to augmented peripheral chemosensitivity due to the abnormal cardiorespiratory reflex control in CHF (increase in sympathetic tone)[97]. This alone will not reduce the $PaCO_2$ unless the set point about which the $PaCO_2$ is controlled is depressed or the hypoxic or ergoreceptor stimulus is high. However, there is also an increase in physiological dead space (calculated from measurements of arterial CO_2 and mixed expired CO_2) during exercise in patients with severe CHF[98-100]. It is thought that this occurs due to exercise hyperventilation and impaired cardiac output with most severe patients unable to double their cardiac output from rest to maximal effort. The disproportionate increase in ventilation associated with reduced cardiac output and lead to high alveolar ventilation/perfusion ratios during exercise. This shift leads to an increased physiological dead space measurement for CO_2 (as solubility of CO_2 remains unchanged), and is not due to the lack of perfusion to some alveolar regions as previously thought[101].

Exercise oscillatory ventilation (EOV) is a parameter seen during cardiopulmonary exercise testing of patients with severe CHF. It is characterised by the cyclic waxing (hyperpnoea) and waning (hypopnoea) periods of ventilation. It can be present throughout exercise or terminate before peak exercise. It is defined by the American Heart Association as the persistence of a periodic breathing pattern for at least 60% of exercise with an amplitude of ventilatory oscillation $\geq$15% of the average resting ventilation value. The cause of EOV is debated but thought to be related to circulatory delay (due to reduced cardiac output), deranged chemoreflex response (increased

chemo-sensitivity to $PaCO_2$ and PaO_2) and/or baroreflex impairment[102]. It has been shown to have good prognostic value for morbidity and mortality[103].

1.2.3. Measurement of dyspnoea in CHF

It is clear that dyspnoea is a major problem in CHF, and there are several ways to measure this symptom, some of which remain open for debate. The purpose, setting and patient population can assist in deciding the choice of scale. While it is recommended that the intensity of dyspnoea should be regularly documented in the notes of patients with advanced cardiac disease[104], there is a lack of consensus on which tools should be used to measure it both qualitatively and quantitatively. Over 30 ways of recording dyspnoea have been used in clinical trials[105, 106], each with a different focus (e.g. sensory perception versus distress)[107, 108] and addressing different scenarios (e.g. rest versus exercise). Many of these scoring systems have not been validated in heart failure and dyspnoea evaluation is therefore often included in generic instruments (e.g. New York Heart Association Classification) or general quality of life measurements (e.g. Chronic Heart Failure Questionnaire[109]). The Chronic Heart Failure Questionnaire is well validated in patients with CHF and includes other related symptoms such as fatigue, and there are specific dyspnoea questionnaires that may also be developed for use in CHF[6]. Simpler and faster rating scales may also be used. The Borg scale is often used to assess dyspnoea in cardiovascular disease, although it is considered less sensitive for dyspnoea ratings and reproducibility than visual analogue scales (VAS)[110]. VAS, numeric rating scales (NRS) and Likert scales (Fig. 1.4) are all quick and easy, allow for repeated measures at a variety of time points, and have been widely used in multiple heart failure trials[111-113] as well as in studies of dyspnoea[114-116]. The flexibility of these scales, however, also results in inconsistencies in the type of

dyspnoea measured between studies, making comparisons difficult. Multi-dimensional scales may be preferable when precision and the ability to detect a change are required. Scales that select a single number (VAS, NRS, MBS) may be preferable when they are completed at regular short intervals, or included in a larger group of patient reported outcomes. There has been no standardised adoption of any single breathlessness tool and therefore it can be difficult to transfer information from clinical trials into clinical practice. NRS has been shown to be more practical than the VAS for repeated measures in cancer patients[117]. The ordered categorical Verbal Descriptor Scale (VDS = none, mild, moderate, severe) is a four level scale has been shown to strongly correlate with the NRS[118]. Repeatability of NRS and VAS often require large numbers of patients to achieve sufficient power in clinical trials. Reading numbers aloud and assessing the maximum number of numbers that can be read aloud in 1 minute and the number of numbers read per breath has been proposed as a potentially useful measure of assessing breathlessness in patients with pleural effusions. It showed good repeatability and was sensitive to detecting an improvement with pleural drainage[119]. This has not yet been tested in patients with heart failure. As a rule of thumb, measurements of dyspnoea in a clinical population should be sensitive to the minimally clinically important difference (MCID), which is the smallest change in score which patients perceive as beneficial and which would mandate, in the absence of side effects and excessive cost, a change in the patient's management [120]. MCID has been suggested as a 1 point improvement in the Borg score and 10mm on a VAS in all aetiologies [121], although it has not been fully explored in heart failure.

In summary the VAS, NRS and MBS are all quick methods of measuring and detecting changes in dyspnoea. They are very simple to use, easily understood by the patient, have good reproducibility (completed by the patient) and do not matter if it is being administered by different healthcare professionals. In this thesis the VAS and MBS are utilised. These represents a simplistic view of dyspnoea and neglects the different physical and affective components that are now known to constitute dyspnoea[122]. Some studies will require more complex dyspnoea specific questionnaires.

The Dysnpoea-12 questionnaire (D12) has also been used throughout this thesis as a method for assessing dyspnoea. This is a multi-dimensional questionnaire that was designed to give a single score of dyspnoea that comprises of both the physical and the emotional components. The D12 was developed with the aim to select the minimum number of descriptors that covers all components of dyspnoea, irrespective of the underlying pathology. The descriptors were identified as terms used by the patients. Therefore it is a quick and easy tool for patients and researchers to use to assess both the physical and emotional aspects of dyspnoea. The questionnaire was designed by collecting a pool of 88 common phrases that patients use to describe breathlessness from published primary data studies. It has been validated in many different cohorts of patients, including heart failure. The total dyspnoea score is independent of underlying pathology. It is a short form and consists of 12 descriptors, divided into 7 physical and 5 emotional aspects. Subjects can answer 'none', 'mild', 'moderate' or 'severe' with an associated score of 0, 1, 2 and 3, giving an overall maximal score of 36. It provides an overall total score of dyspnoea and while not designed to measure the physical and emotional individual components, it is possible to assess these separately. The D12 in this thesis is often used to assess dyspnoea at a specific point in time. The

original D12 validation paper tested the D12 using the reference frame 'these days' i.e non temporally specific. This paper does not state it cannot be used in a temporally specific way and a recent paper by Ekstom et al (2020) has shown the minimally important clinical difference (MCID) does not change if you use various timeframes (average, best, worst and current)[123]. The MCID for the Dyspnoea-12 questionnaire is 3 points[124]. The questionnaire is included in the methods chapter. The D12 has not previously been used in clinical trials to test an intervention or used in patients with heart failure. To date it has mainly been used as a valid and reliable instrument to measure dyspnoea in different cohorts of patients with dyspnoea[125]. It has been shown to be well correlated with the multi-dimensional dyspnoea questionnaire (MDP)[53]. This is a longer questionnaire and needs health professional input for completion. The MDP and D12 have been designed for different purposes. The MDP is designed to specifically to measure different dimension, whereas the D12 includes them in a single score[6].

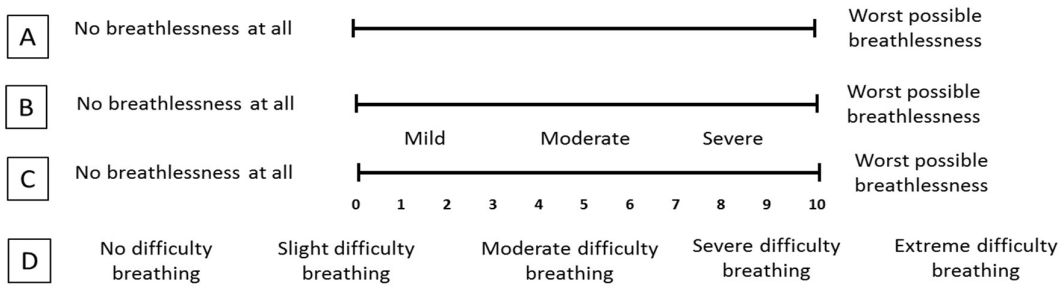


Figure 0.4 Varieties of rating scales

A. The visual analogue scale (VAS), which consists of a bland line usually 100mm long whose ends are labelled as the extremes (no dyspnoea at all; worst possible dyspnoea). The patient is asked to put a line indicating their severity of dyspnoea (at that time or another time point). B. Word-labelled/graphic VAS with descriptive terms added. C. Graphic/numeric rating scale G/NRS with numbers added. D. The Likert scale, which uses descriptive terms to describe severity/intensity of dyspnoea.

In addition to questionnaire-based measurements, cardiopulmonary exercise testing (CPET) is increasingly used to assess dyspnoea. This method is often useful in assessing dyspnoea of unknown aetiology or used to work out which system is contributing the most to the presence of dyspnoea in patients with multiple co-morbidities. It is recommended to use CPET for assessing the cause of dyspnoea when the above measurements do not correlate with the clinical picture (often patients reduce their activity levels to avoid experiencing breathlessness without realising it), or where there is co-existent lung and heart disease and it is necessary to determine which one is contributing the most to the sensation of dyspnoea.

The few studies of dyspnoea that have been performed in CHF have rarely addressed all 3 aspects of dyspnoea; the intensity, quality (individual components of dyspnoea) and the emotional domain. The recent development and validation of multi-dimensional dyspnoea questionnaires[6] provides new opportunities to better understand dyspnoea in CHF.

1.3. TREATMENT OF DYSPNOEA IN CHF

Efforts to relieve dyspnoea initially targeted improving the mechanics of breathing and reducing demand of breathing. A newer focus is to alter the perception of dyspnoea via altering the activity of neural signals sent to the brain reporting the level of breathing. This new approach has led to the exploration of novel interventions, including the use of nebulised furosemide.

The ideal approach is to determine the underlying aetiology for the individual patient, and treat this accordingly, but unfortunately this is not usually feasible in patients with chronic heart failure where reversible causes have already been excluded. In the

absence of a simple solution (e.g. correction of dyspnoea caused by anaemia), several options have been suggested for the treatment of dyspnoea in CHF. Therefore after optimisation of the underlying cause (such as with diuretics and afterload reduction) options include oxygen, opioids (such as morphine), nebulised furosemide, sildenafil, serelaxin, nesiritide, or with non-pharmacological therapies such as the hand-held fan, pulmonary rehabilitation, cardiac rehabilitation exercise training and cognitive therapy[126].

The impact of oxygen treatment on dyspnoea in chronic heart failure remains uncertain. Patients who are hypoxic at rest or on minimal exertion may benefit[104, 127] but those without hypoxia have not been shown to benefit[128].

Opioids have long been used to treat dyspnoea within palliative scenarios or in acute heart failure, but the current heart failure guidelines by the European Society of Cardiology do not recommend routine use of opioids and advise cautious use in patients with severe dyspnoea, such as in pulmonary oedema[3]. Morphine use is only currently recommended in the American Heart Association for patients in the end-stages of heart failure[129]. Imaging studies have shown that opioids dampen the brains response to breath-holding[130]. It is thought to act by depressing spontaneous respiratory drive (thereby reducing corollary discharge) and by modulating cortical activity. One pilot study showed that short term opioid administration reduced dyspnoea in CHF[131] but the effect diminishes over 6 weeks to 3 months[132] and long term studies are lacking[133]. Whilst opioids remain a potential treatment option; they are associated with side effects including constipation, sedation, addiction and importantly respiratory depression. Despite this, their use is recommended on a case-by-case basis for patients with advanced cardiopulmonary disease and unrelieved

dyspnoea, taking into account the patient's history, comorbidities and risk of respiratory depression[104]. A recent RCT in CHF suggested a medium-term benefit, although underpowered, and proposed that morphine should only be prescribed when other measures are unhelpful ensuring early management of side effects[134].

Furosemide is commonly prescribed as a tablet or injection for fluid overload in patients with heart failure. It is a loop diuretic which acts through inhibition of the Na^+-K^+-$2Cl^-$ co-transporter in the thick ascending limb of the loop of Henle to inhibit sodium and chloride reabsorption to cause a diuresis (excretion of water in the urine). It can lead to symptomatic relief of breathlessness due to pulmonary oedema and relief of peripheral oedema. It acts by reducing filling pressures and slowing ventricular dysfunction[135, 136]. However, systemic furosemide has not been shown to improve survival and prolonged use can be harmful by activating neuro-hormonal responses. Furosemide can cause a reduction of intravascular volume resulting in renin-angiotensin-aldosterone activation and a reduction in natriuretic peptides[137]. Plasma renin was found to be increased in patients receiving a diuretic[138]. Activation of this system may impair renal blood flow and reduce glomerular filtration rate. One study showed that by reducing the dose of furosemide there was an increase in glomerular filtration rate[139]. Over a third of patients develop intra-renal resistance to furosemide requiring progressively increasing doses for adequate relief of circulatory congestion[140-142]. Subcutaneous administration of furosemide has also been studied in acute decompensated heart failure but not for dyspnoea relief in stable chronic heart failure[143]. Na^+-K^+-$2Cl^-$ channels are found in the airways and therefore another theoretical mechanism is the local easing of pulmonary congestion within the lungs. This, however, would only apply to those patients with pulmonary congestion and as

discussed earlier, patients with chronic heart failure can develop protective mechanisms to prevent pulmonary oedema.

Sildenafil is a selective inhibitor of type 5 phosphodiesterase and has, in a single study, been shown to improve ventilatory efficiencies and relieve breathlessness after both 3 and 6 months of treatment in patients with heart failure with reduced ejection fraction, especially if there is secondary pulmonary hypertension[144]. Therefore, this may be a novel treatment for dyspnoea relief in the management of heart failure. However, a meta-analysis in pulmonary hypertension showed that while it improved haemodynamic parameters, when compared to placebo, it did not have any clinically meaningful effect on breathlessness[145].

Non-pharmacological therapeutic options such as the hand-held fan, pulmonary rehabilitation, cardiac rehabilitation exercise programmes and cognitive therapy are all potentially beneficial. The hand-held fan is an attractive option as it has minimal side effects and the user has full control but there is conflicting evidence as to its efficacy[146-149].

Other drugs that may be of use include anxiolytics and antidepressants. The use of these have not been supported by experimental evidence, with results showing either no effect; or data on their efficacy being insufficient or lacking. Similarly, other potential targets, such as brain natriuretic peptide (BNP) or its N-Terminal prohormone (NT-proBNP), have not yielded treatments for dyspnoea or dyspnoea relief[150-152]. Treatment programmes such as pulmonary rehabilitation may be of use to CHF patients as they are to COPD patients, particularly as rehabilitation appears to act in

part on the processing of dyspnoea rather than its origin, although their impact on dyspnoea remains unclear[4, 48, 153-155].

In summary, several treatment options exist. Some have greater support in the literature than others and some come with concerns of side effects. Oxygen therapy, opioids, nebulised furosemide (discussed in more detail below), sildenafil, alongside non-pharmacological therapies might all be good options, but this should be determined on an individual basis. It has become clear, however, that dyspnoea is not a symptom that can be ignored, and it is imperative that one tries to alleviate dyspnoea to impart further symptom relief, even when the heart failure is optimally managed.

1.4. NEBULISED FUROSEMIDE

Although furosemide is commonly prescribed in tablet form for the relief of breathlessness in heart failure, nebulised furosemide has not yet been assessed with respect to its relief of breathlessness in this patient population. Nebulised furosemide for treatment of dyspnoea in CHF remains unexplored and this thesis aims to address this. This novel approach circumvents some of the side effects of systemic furosemide and is not known to have any significant side effects of its own. Nebulised furosemide aims to improve the quality of life of patients with CHF by relieving their breathlessness on exertion and enabling them to increase their physical activity. It also may offer dyspnoea relief to those who remain breathlessness at rest despite optimal medical management.

Nebulised furosemide has been shown to relieve cough via modulation of C-fibre receptor activity[156]. It has also been shown to prevent bronchoconstriction in patients

with asthma[157, 158]. The mechanism of action of nebulised furosemide for dyspnoea relief is thought to involve a different set of receptors; it appears to have a local effect on the airway mucosa and acts via altering the activity of pulmonary stretch receptors[27, 30]. Modulation of vagal afferent fibres from these receptors is known to modulate the sensation of dyspnoea[18, 23].

In rats, nebulised furosemide has been shown to increase the activity of pulmonary stretch receptors, and may result from an influx of sodium ions[30]. Furosemide inhibits the sodium-potassium-chloride cotransporter thereby increasing the sodium ions and activating the stretch receptors. Nebulised furosemide has been shown to activate the slowly adapting pulmonary stretch receptors and suppress the pulmonary irritant receptors in anaesthetised rats[27, 30]. It has also been shown to increased pulmonary stretch receptor activity in quadriplegic humans[24] and relieve 'air hunger.' Therefore, nebulised furosemide may relieve breathlessness by replicating the sensation of an increase in tidal volume.

Nebulised furosemide is not currently licensed for use in routine clinical practice, although it has been studied for over 20 years in research, Table 1.1. There are a few case reports and several small randomised clinical trials assessing the use of nebulised furosemide for dyspnoea relief in a wide spectrum of medical conditions such as, asthma[157 157-172], COPD[28, 173, 174] and cancer[175-178]. It has also been trialled in infants, including pre-term babies with breathing difficulties[179-181]. It has also been used in adult healthy volunteers[27, 29]. The results of these trials has been variable and in randomised controlled trials nebulised furosemide has only been shown to relieve dyspnoea in patients with COPD. There has been one case report of using nebulised furosemide in a patient with heart failure with no intravenous access[182] and one

randomised control trial. This study assessed the haemodynamic response of nebulised furosemide with a right heart catheter in patients with heart failure[183]. It was not designed to assess dyspnoea. The dyspnoea recording were taken at rest/baseline and were similar, and at low levels, in both the nebulised furosemide and placebo group. It did not assess the change in dyspnoea ratings during the study. No study has yet to formally assess the effect of nebulised furosemide on relief of breathlessness in patients with heart failure. Systemic absorption of nebulised furosemide into the circulation is a potential alternative mechanism for the action of furosemide in relieving breathlessness. Furosemide causes vasodilation in the lungs[184] however the amount absorbed from a nebulised dose is unlikely to have a significant effect on lung vasculature as the absorption efficiency has been shown to be up to 30% of the nebulised dose[185], equivalent to approximately 5mg for a 40mg nebuliser. It is unlikely that this small amount of furosemide causes any effect on dyspnoea. Further support that nebulised furosemide acts via a direct action in the lungs is provided by: i) direct exposure of the lung tissue to furosemide in rat preparations resulted in modulation of PSR afferent activity but not when administered intravenously[30] ii) other studies in which beneficial effects of furosemide have been evident only when nebulised rather than administered via tablet[157] iii) absence of haemodynamic changes with nebulised furosemide in a study assessing wedge pressure measurements in heart failure patients suggesting no systemic mechanism of action[183] iv) the desire to urinate has not been evident in some studies suggesting the systemic effects may be minimal.

Other support for use of this novel treatment option comes from the lack of any serious side effects. Most trials that have evaluated the adverse effects of nebulised

furosemide identified no significant toxicity. Kohara et al[175] found moderate nausea in 1 patient, mild sleepiness and nausea in 3 patients (n=15). Cough, sputum production, and nausea were the most common side effects, but these were tolerated and easily managed. These effects resolved in a few hours. Moosavi et al[29] found that intermittent cough accompanied the inhalation of furosemide for several naive subjects, but did not persist after inhalation. Naive subjects did not report ill effects when contacted the day after treatment. One study in advanced cancer patients suggested that dyspnoea worsened after nebulised furosemide although this was not significant and a clinical trial was recommended[186]. One study found no serious adverse events but noticed a small but significant fall in FEV1 and FVC with nebulised saline[177]. Many studies found no noticeable side effects or adverse events[176, 186-188].

Table 0.1 Summary of studies investigating dyspnoea relief by nebulised furosemide

Listed in chronological order. PEFR: Peak expiratory flow reading. FEV1: Forced expiratory volume in 1 second. VAS: Visual analogue scale. CDS: Cancer Dyspnoea Scale. QDS: four times a day

Trial	Study design	Subjects or Disease	N =	Age (mean)	Male	Dose	Component of dyspnoea studied and dyspnoea induction.	Parameter assessed	Results
Saba et al., 2020	Randomised, double-blind, crossover	COPD	69	65	55%	40mg	Physical activity at a level up to the level before the study	mMRC Borg	Adding nebulised furosemide to nebulised salbutamol significantly relieves dyspnoea (4 points on Borg and 1.5 on mMRC) more than single therapy without any side effect
Waskiw-Ford et al., 2018[189]	Randomised, double-blind, crossover	Healthy	24	25	100 %	40mg and 120mg	Intensity and unpleasantness of breathlessness using CPET	MBS	Compared with 0.9% saline, neither 40 nor 120mg of nebulised furosemide had an effect on ratings of perceived breathlessness during exercise or an effect on cardiometabolic, ventilatory, breathing pattern, or dynamic operating lung volume responses during exercise.
Morélot et al., 2018[185]	Randomised, double-blind, crossover	Healthy	11	32	73%	40mg	Breathing discomfort using a clinical ventilator	VAS MDP	Both saline and furosemide relieved breathing discomfort by 20% VAS (and by 16% VAS with IV furosemide). Effectiveness of nebulised furosemide was weakly correlated with larger tidal volumes. Response to nebulised furosemide was inversely correlated to furosemide blood level.

Trial	Study design	Subjects or Disease	N =	Age (mean)	Male	Dose	Component of dyspnoea studied and dyspnoea induction.	Parameter assessed	Results
Banzett et al., 2017[190]	Randomised, double-blind, crossover	Healthy	12	24	17%	80mg	Breathing discomfort using a clinical ventilator	VAS MDP	Both saline and furosemide relieved breathing discomfort with a mean treatment effect of 17% VAS for nebulised furosemide and 13% for nebulised saline.
Vahedi et al., 2013[174]	Double-blind, Randomised	COPD	100	73	63%	40mg	Dyspnoea during COPD exacerbation	VAS	Nebulised furosemide significantly relieved dyspnoea compared to nebulised saline. Dyspnoea improved with furosemide by 2.7 on VAS scale compared to 1.6 with saline
Newton 2012[191]	Randomised, double-blind, crossover	Stable Heart Failure	32	52	94%	40mg	Breathlessness, no dyspnoea induction	0 to 10 scale	No difference in dyspnoea with nebulised furosemide compared to saline. Nebulised furosemide did not exert an acute haemodynamic effect in patients with stable chronic heart failure.
Laveneziana et al 2008[192]	Double blind randomised crossover	Healthy	9	31	56%	40mg or 80mg	Respiratory effort Cycle exercise with external resistive load	10-point Borg	Furosemide did not affect the perception of respiratory effort and did not seem to have any clinical benefit in the treatment of severe dyspnoea.
Wilcock 2008[177]	Double-blind, Randomised	Lung Cancer/ Mesothelioma	15	66	47%	40mg	Breathlessness, Number reading test and arm exercise test	Dyspnoea exertion scale	No significant difference. 40% patients felt breathlessness improved with nebuliser compared to no treatment – 50% preferred saline, 17% furosemide and 33% reported equal benefit.

Trial	Study design	Subjects or Disease	N =	Age (mean)	Male	Dose	Component of dyspnoea studied and dyspnoea induction.	Parameter assessed	Results
Jensen 2008[28]	Randomised, double-blind, crossover	COPD	20	61	55%	40mg	Dyspnoea intensity CPET – constant load exercise	10-point Borg Exercise time	Furosemide led to a significant decrease in dyspnoea intensity (~1point reduction on Borg) at the highest equivalent exercise time and a significant increase in exercise endurance time.
Moosavi 2007[29]	Randomised, double-blind, crossover	Healthy	10	26	60%	40mg	Air hunger Hypercapnia with constrained ventilation	100mm VAS	Significant improvement in AH rating after furosemide. Mean treatment effect 13mm VAS.
Ong 2004[173]	Double blind randomised crossover	COPD	20	68	100 %	40mg	Respiratory discomfort Exercise (incremental & constant work)	100mm10 point VAS	Inhalation of furosemide alleviated the sensation of dyspnoea during constant-load exercise testing in patients with stable COPD. Mean reduction of 9mm VAS.
Kohara 2003[175]	Uncontrolled clinical series	Cancer	15	6		20mg	Sense of effort anxiety and respiratory discomfort No induction	CDS (12-point scale)	Inhalation of nebulised furosemide alleviated the sensation of dyspnoea for sense of effort, sense of anxiety, and total dyspnoea. In 80% of patients the total dyspnoea score improved significantly after inhalation of furosemide by 2points CDS.
Stone 2002[186]	Double blind randomised crossover	Cancer	7	72	57%	20mg	Breathing difficulty and distress No induction	100mm VAS	No significant difference between placebo and furosemide for difficultly or distress. Trend for worsening dyspnoea after nebulised furosemide.

Trial	Study design	Subjects or Disease	N =	Age (mean)	Male	Dose	Component of dyspnoea studied and dyspnoea induction.	Parameter assessed	Results
Shimoyama 2002[176]	Case report	Cancer	3	51	100 %	20mg QDS	Breathlessness No induction	VAS	Significant improvement in breathlessness in all 3 patients.
Minowa et al 2002[193]	Double blind randomised crossover	Healthy	10	25-37	80%	40mg	Respiratory discomfort, hypercapnia by steady state and rebreathe	200mm, 10-point VAS	Nebulised furosemide improves the dyspnoeic sensation produced during hypercapnic hyperpnoea. With mean treatment effect 10-15mm VAS.
Nishino et al, 2000[27]	Double blind randomised crossover	Healthy	12	25-40	6 (50%)	40mg	Urge to breathe i)Breath hold ii) Hypercapnia + resistive load	VAS (200mm 10 point)	Increased breath-hold (by 50%) with nebulised furosemide and slower development of respiratory discomfort with loaded breathing (median reduction of ~30mm VAS during loaded breathing).
Hinckley, 2000[167]	Double-blind placebo controlled	Acute asthma	35	NA	NA	40mg	Breathlessness No induction	10-point dyspnoea scale	No statistical improvement in dyspnoea by adding furosemide to standard care in acute exacerbations of asthma.
Stone 1994[178]	Case report	Cancer	1	NA	NA	20mg	Dyspnoea	None	Patient found nebulised furosemide had the most beneficial effect on his dyspnoea.

1.5. RATIONALE FOR THESIS

The mechanism of dyspnoea in heart failure is not yet fully understood. While ergoreceptors are likely to be involved in the aetiology of the sensation, it remains unlikely that this will explain the complexity of dyspnoea in CHF fully. As with respiratory disease, dyspnoea in CHF is probably a composite of several issues, both physiological and psychological and develops over time. Treatment of this disabling symptom should try to incorporate these different dimensions for the optimal alleviation for each individual patient.

Heart failure is a major health burden and continues to increase. Dyspnoea is subjective and its correlation to objective measures are weak but it is an important symptom and has been the primary outcome measure in recent acute heart failure studies[194-196]. One meta-analysis showed that loop diuretics in heart failure reduced mortality and improved exercise capacity but concluded that the trials used in this meta-analysis were small. The authors also stated that the evidence is insufficient to justify widespread use of diuretics to reduce mortality but that it will continue to be used for symptomatic benefit[197].

There is a wide range of treatments available for symptom relief for heart failure. The mainstay of treatments are beta blockers, angiotensin converting enzyme inhibitors, aldosterone antagonists and more recently, angiotensin receptor neprilysin inhibitor[3]. These all have prognostic benefit. Most studies in heart failure investigate new treatments to improve mortality rather than aiming for symptom relief. However, breathlessness is a key symptom of heart failure, even when it is optimally treated and

quality of life would improve by treating this symptom. Nebulised furosemide is an attractive treatment option as it may have a dual mode of action by both modulating the afferent signals reporting the prevailing level of ventilation, and acting on the Na^+, K^+, Cl^- channels within the lungs to ease any pulmonary congestion that may be present at the site[198]. As discussed earlier the exact mechanisms of dyspnoea in chronic heart failure are unknown with a variety of possible factors that may contribute. However, nebulised furosemide is not proposed to modulate the mechanoreceptors/ergoreceptors/metaboreceptors in the skeletal and respiratory muscles but to alter the signal reporting the ventilation, which is compared to the signal reporting the need to breathe. It is not known if altering this signal will ease dyspnoea in this patient cohort. Nebulised furosemide has also been shown to be beneficial in a randomised controlled trials of patients with COPD, and given that there is 30% overlap between the two conditions it may benefit these specific patients. The evidence for nebulised furosemide as a viable treatment option is uncertain due to the variability seen in the trials. This thesis aims to enhance the understanding of the mechanisms of dyspnoea in heart failure and to address the variability seen with nebulised furosemide in previous studies, Table 1.1. It aims to provide further evidence for the role of pulmonary vagal afferents in dyspnoea relief from nebulised furosemide, for example to assess whether it is due to a direct action on the lungs or via the systemic circulation. Recent advances within the field of dyspnoea research, such as the multi-dimensional questionnaires (e.g. Dyspnoea-12 and the

Multidimensional Dyspnea Profile), can now be used to guide trials assessing dyspnoea relief in heart failure.

1.5.1. Hypothesis and Aims

Hypothesis: Nebulised furosemide is a viable adjunct to optimal medical management of chronic heart failure by providing greater symptom relief via a direct action within the lungs.

General Aims

1) Address key outstanding questions with regard to dyspnoea relief by nebulised furosemide

One key outstanding question is how specific nebulised furosemide is with regard to the qualities of clinical dyspnoea relief. This could be accounting for much of the variability seen in previous studies of nebulised furosemide for dyspnoea relief. Recent advances in our understanding of dyspnoea include the recognition that there are different distinguishable qualities of clinical dyspnoea, that these arise from different neural pathways, and that the air hunger is the most unpleasant component of dyspnoea. Guided by this information Chapter 4 aims to verify that nebulised furosemide relieves dyspnoea and that this is primarily due to relief of the air hunger component via its effects on lung stretch receptor vagal afferents. Another recent advance includes the ability to experimentally induce the air hunger component of clinical dyspnoea in healthy volunteers using established reproducible and safe methods. Chapter 4 takes advantage of this and allows a hypothesis driven definitive study to be answered.

2) Determine the scale of the problem dyspnoea creates for heart failure patients in the community

It is now accepted that, like pain, dyspnoea is multi-dimensional. This has resulted in the development of validated multi-dimensional questionnaires, including the Dyspnoea-12 and MDP. Patients with heart failure were part of the original cohort used to develop the Dyspnoea-12 but this tool has not been used in clinical research in patients thus far. Previous studies have attempted to determine the extent of dyspnoea prevalence within various patient groups within the community but have not taken advantage of the new multi-dimensional questionnaires and none have been conducted in HF patients. Chapter 3 involves a postal survey of dyspnoea in the local heart failure community using the Dyspnoea-12.

3) Lay the groundwork for a future clinical trial of nebulised furosemide for dyspnoea relief as an adjunct to treatment of chronic heart failure

In order to conduct a meaningful clinical trial that will determine whether nebulised furosemide is a useful adjunct to optimal management of heart failure, there are a number of other potential factors that could be introducing variability into the efficacy of nebulised furosemide. These include: the breathing pattern used when inhaling the furosemide; the method used to induce dyspnoea in heart failure (exercise or hypercapnia); the type of exercise used to assess breathlessness (CPET or 6-minute walk test, 6MWT); the utility of various rating scales of dyspnoea (VAS, Borg etc.); and the variability in the systemic absorption of nebulised furosemide from the lungs. Chapter 5 aimed to conduct a pilot study in a representative sample of 12 patients with

advanced heart failure to specifically address these uncertainties. Cardiac biomarkers analysis was available during the course of the study and it was possible to incorporate these into this pilot study (Chapter 6).

2. METHODS

The methodology used in this thesis are as follows:

i) Laboratory-based experimental models of clinical dyspnoea

ii) Dynamic exercise testing

iii) Assessment of subjective ratings of respiratory discomfort

iv) Blood analysis

2.1. EXPERIMENTAL MODELS OF CLINICAL DYSPNOEA

As discussed previously, dyspnoea comprises of distinct components (Air Hunger-AH, Work/Effort-WE and chest tightness) that arise from different neural pathways. Studying clinical populations in order to understand the mechanisms of dyspnoea (or its relief with interventions) is particularly challenging, as clinical dyspnoea varies naturally throughout the disease trajectory, irrespective of the underlying pathology. At peak exercise, patients with cardiorespiratory disease are likely to experience both the AH and WE components of dyspnoea. In patient groups, it is therefore challenging to distinguish intervention related changes from clinical fluctuations and co-morbidities. Experimentally inducing dyspnoea in a laboratory setting offers a suitable environment to identify putative therapeutic targets that can inform future clinical trials, to maximise their effectiveness.

The methods used in this thesis to experimentally induce two of the individual components of dyspnoea are established, validated, reliable and safe, Fig. 2.1. These

tests induce specific components of dyspnoea in healthy volunteers. They do not aim to reproduce the exact sensations of dyspnoea that patients with heart failure experience. The amount of AH and WE that contribute to dyspnoea in patients with heart failure is unknown and is likely to vary for each individual patients. Experimentally induced breathlessness in healthy volunteers instead aims to offer an environment that is not affected by changes in clinical status. A study by O'Donnell et al., (2013) compared experimentally induced dyspnoea (increasing end-tidal CO_2 during restricted ventilation evoking AH) in healthy subjects and in patients with chronic lung disease[199]. Using the multi-dimensional dyspnea profile (MDP) to measure the sensory qualities as well as the immediate discomfort and secondary emotions they found that COPD patients and healthy volunteers reported similar levels of immediate discomfort relative to sensory intensity. There was no difference in the affective response to experimentally induced dyspnoea in those with COPD compared to healthy volunteers. They also found no difference in affective response between dyspnoea induced in the laboratory and that evoked by activities of daily living[199]. This study has not been performed in patients with heart failure. AH can be generated systematically by hypercapnia with constrained ventilation[29] and WE can be specifically induced by adding an inspiratory resistive load and targeting ventilation[200]. How these experimentally induced sensations are related to clinical dyspnoea is still open to debate. One study comparing chronic obstructive pulmonary disease (COPD) patients to healthy volunteers reported that the COPD patients experienced episodes of

dyspnoea during activities of daily living that were similar in intensity and unpleasantness to that of the experimentally induced dyspnoea test[199].

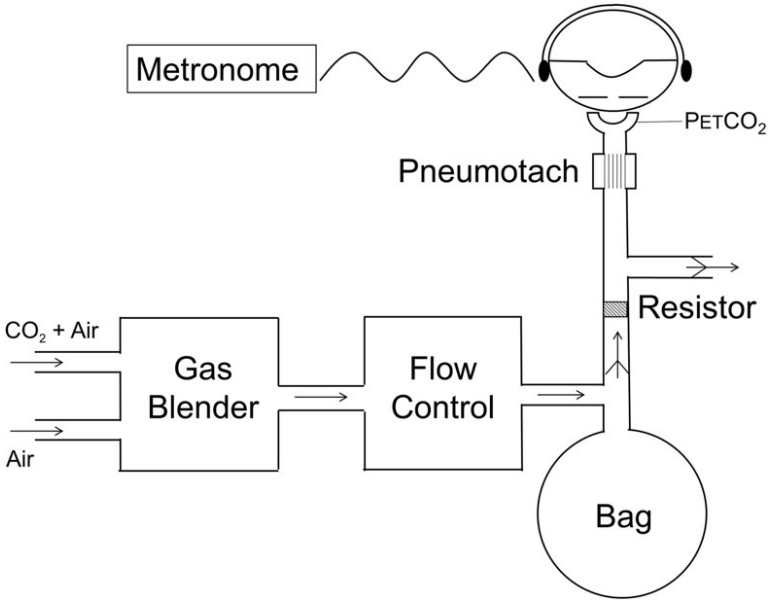


Figure 2.1 Experimentally induced dyspnoea circuit

The Breathing Circuit was identical for air hunger (AH) test and work/effort (WE) test, except that the external resistance was removed in the AH test. To elicit AH, CO_2 was added to the flow of fresh gas into the bag and this flow was fixed at baseline alveolar ventilation. To elicit WE, individuals were instructed to empty the bag with each breath while the flow of fresh bag into the bag was increased and CO_2 was added to maintain normocapnia. $P_{ET}CO_2$ = end tidal PCO_2

2.1.1. Air Hunger Test

Hypercapnia with constrained ventilation: This model of experimentally induced dyspnoea has been employed in a number of previous studies, in several laboratories around the world[22, 24, 29, 185, 190, 200-203]. Participants were semi-reclined in a padded chair, whilst wearing a nose clip, and breathing via a mouthpiece. Humidified warmed gas (Fisher and Paykel HCL150) was delivered into a 3-litre anaesthetic bag supplying

the inspiratory gas via a one-way valve (Hans Rudolph, model No.5710, USA). Expired gas was expelled via a second one-way valve. Minute ventilation was constrained as it could not exceed the flow into the bag. For each participant the level of ventilation was held constant at their normal resting level (i.e. the bag just collapsed with each breath). The participant's respiratory rate (fR) was fixed at 12 breaths per minute (healthy volunteer study) or to their baseline rate (heart failure study) by breathing in time with a metronome, resulting in a fixed tidal volume. Minor adjustments of each participant's tidal volume (VT) and fR (for the HF study) occurred at the start of each session to ensure that all participants were comfortable at baseline settings. Participants were informed that the amount of air at times would be limited. They were encouraged to relax as much as possible and were coached not to pull excessive pressures with ineffective efforts against the collapsed bag. During the test dyspnoea was induced by changing varying amounts of carbon dioxide (10% CO_2, 21% O_2, balanced N_2; BOC, England) to the inspired air (Sechrist air-oxygen mixer, USA), whilst fixing ventilation to resting levels. Only a small increase (<20mmHg) in the inspired fraction of CO_2 is required to generate a stimulus-response relationship between CO_2 and the perceived level of AH by the participant. This method has been shown to produce strong air hunger stimulation without any major noticeable work/effort sensation[21].

Two AH test protocols were performed:

i) Ramp: This involved a gradual increase in inspired CO_2 every minute until the maximal tolerated level of breathlessness (reached top of visual

analogue scale, VAS) or came off the mouthpiece. An example of a RAMP test in one individual is shown in Fig. 2.2.

ii) Steady state: The CO_2 was manipulated to give up to 5 minutes of a fixed level of end tidal CO_2 ($P_{ET}CO_2$), chosen to target a level of 50% ('test' level) of the visual analogue scale for AH (based on the ramp). Ideally 4 minutes are required to ensure the stimulus and perceptual ratings reach a steady state[203]. In the healthy volunteer study (Chapter 4), there was also up to 5 minutes of a 'masking' level that was chosen to target a level of 25% of the VAS for AH. The 'masking' level served to prevent the participants from 'expecting' a certain result. The order of the 5-minute test and masking steps were altered between runs. Brief periods of unrestrained breathing separated the two levels of hypercapnia during which participants performed an inspiratory capacity manoeuvre in order to facilitate rapid change in inspired CO_2 level and to reduce the chance of atelectasis. An example of a typical air hunger step produced from using this method is shown in Fig. 2.3.

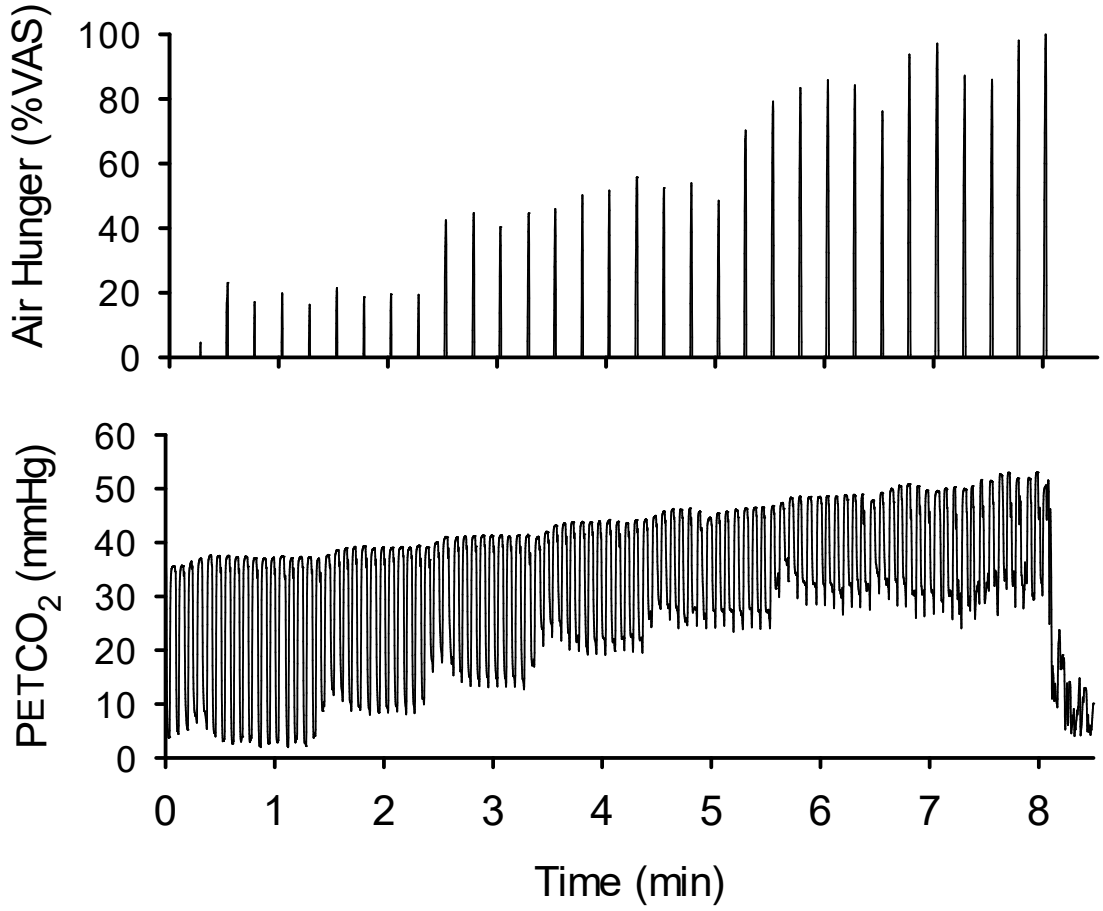


Figure 2.2 Typical AH 'ramp'

This is a typical example of a 'ramp' test for AH for one participant. The bottom panel shows the continuous PCO_2 measured from the mouthpiece and the top panel shows the AH ratings on the VAS scale every 15 seconds. Each minute the inspired CO_2 was increased to raise the end tidal PCO_2, which the participant notes to be an increase dyspnoea sensation and rates it accordingly on the 100mm VAS by providing a rating every 15 seconds.

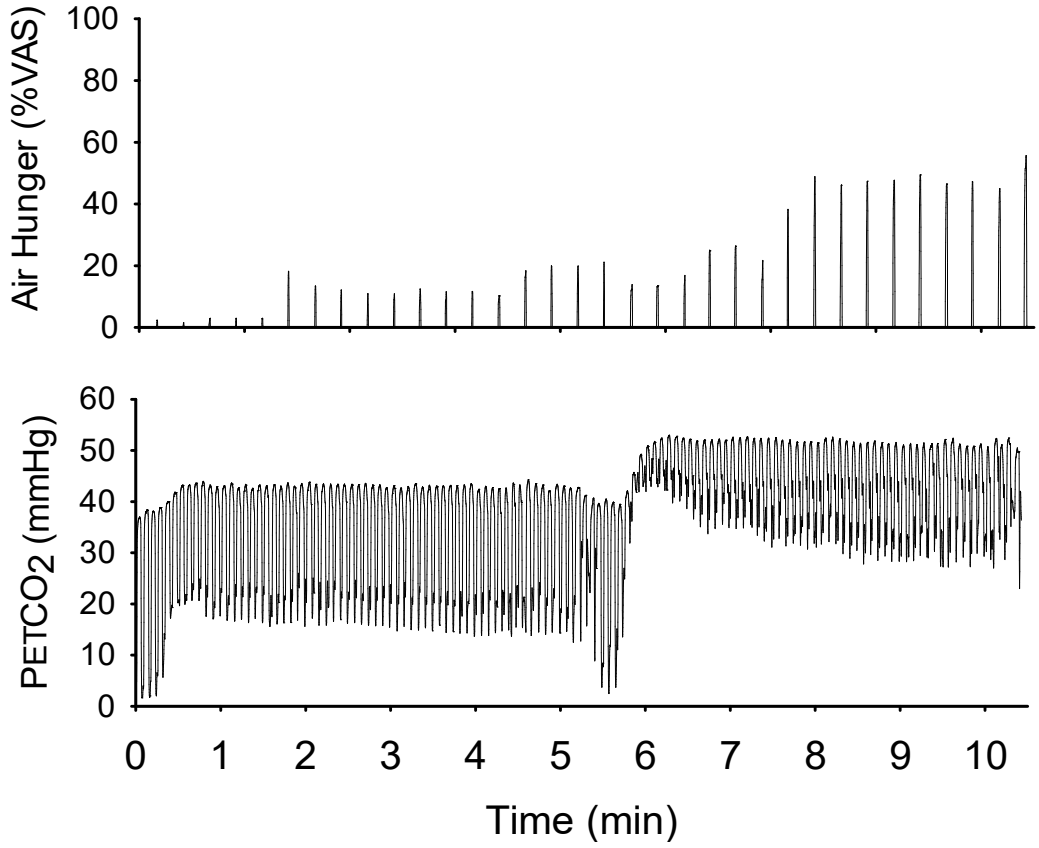


Figure 2.3 Typical AH 'steady state'

This is a typical example of a 'steady state' test for AH for one participant. There are two 5-minute steps with approximately 30 seconds of free breathing in between. The top panel shows AH ratings on the VAS scale every 15 seconds. The bottom panel shows continuous measurements of PCO_2 measured at the mouthpiece from which end-tidal PCO_2 was derived and the top panel shows AH ratings on the VAS scale every 15 seconds.

End-tidal carbon dioxide partial pressure ($P_{ET}CO_2$) is used as an indicator of arterial partial pressure of carbon dioxide ($PaCO_2$). For this assumption to be valid, gas exchange within the lungs should be normal. In the healthy volunteers' study presented in this thesis, this can be assumed. In the heart failure study, however, this may not be as accurate as these participants may have pulmonary oedema affecting

gas exchange. The heart failure patients selected for Chapter 5 are chronic stable heart failure patients, on optimal medical treatment so the pulmonary oedema should be minimal. The presence of a plateau on the continuous $P_{ET}CO_2$ waveform usually indicates a true reading.

It was noted during the AH test for the patient study (Chapter 5) that some of the patients were requiring high levels of flow. Therefore the accuracy of the flowmeter in the circuit was tested using a Douglas Bag and Harvard Dry Gas Meter (accurate to ±1.5%). This showed that at low flow rates there was good reliability and repeatability. However, at higher levels of flow there was reduced accuracy, with an increased coefficient of variation. This is likely to be due to a leak in the system at higher flow rates, Fig. 2.4

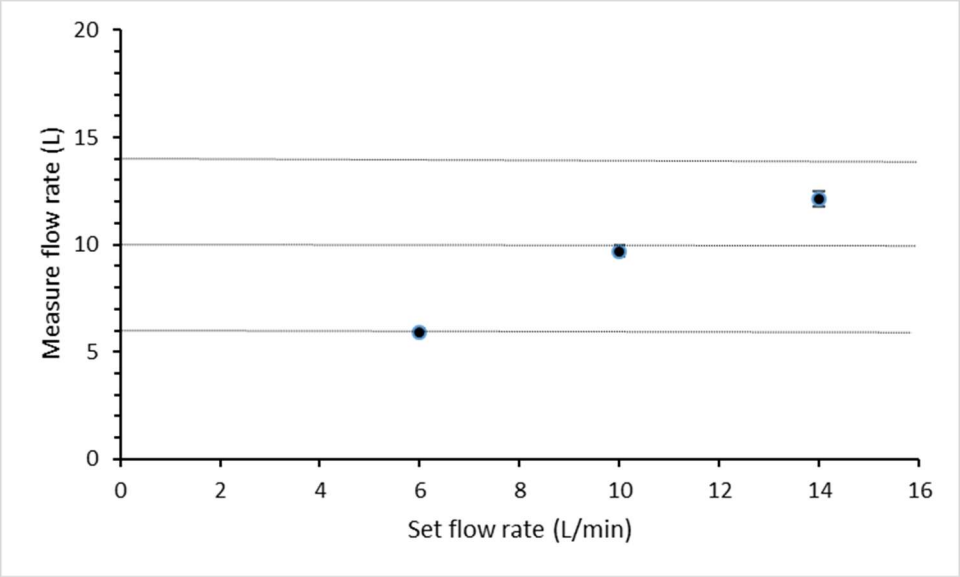


Figure 2.4 Accuracy of flow meter in the circuit

Flow rate measured at different set flow rates within the experimental dyspnoea circuit. Note: flow rate accurate at lower set flows but less accurate at high flow rate, with a lower flow measured.

Some criticisms of this AH test suggest that the negative airway pressure that is generated is the sensation that gives rise to the dyspnoea experienced during the test. However previous experiments within our laboratory have shown that even if this airway pressure difference is removed (by performing targeted breathing to a visual cue to fix tidal volume, rather than a bag) the sensation of air hunger still persists, Fig. 2.5. This confirms that the airway pressure during the AH test is a consequence rather than a cause of having AH.

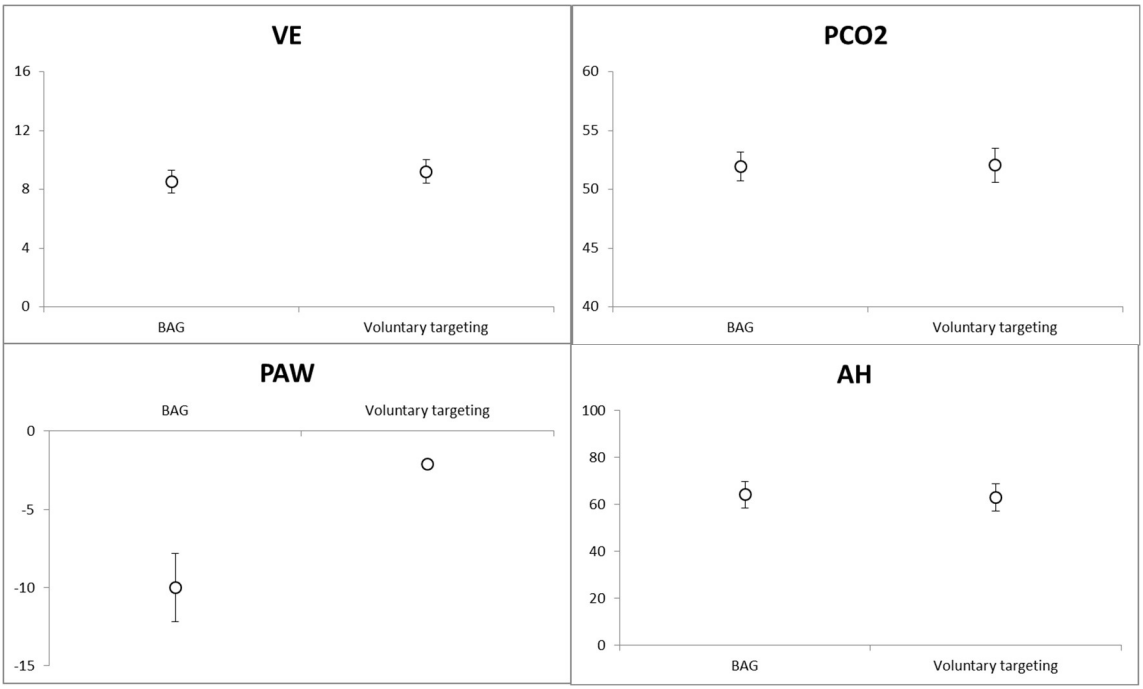


Figure 2.5 Negative airway pressure and air hunger

Air hunger test with tidal volume fixed by using a bag or a visual voluntary target. The use of the bag creates a negative airway pressure. If this airway pressure is removed the air hunger sensation persists for the same level of PCO_2 and ventilation (unpublished data.)

2.1.2. Work or Effort Test

Participants were semi-reclined in a padded chair breathing via a mouthpiece. Humidified warmed gas was delivered into a 3-litre anaesthetic bag supplying the inspiratory gas via a one-way valve. Two resistors (12cmH_2O and 8cmH_2O at 1L/s) were added, in series, to the inspiratory side of the circuit. Expired gas was expelled via a second one-way valve. The participant was instructed to just empty the anaesthetic bag with each breath, and the frequency of each breath was fixed by a metronome at 12 breaths per minute. Therefore, the amount of gas flowing into the bag determined the targeted minute ventilation.

Two WE test protocols were performed:

i) Ramp: This involved a gradual increase in flow. The flow began at a level that matched the individual's baseline alveolar ventilation and then was gradually increased until the maximal tolerated level of breathlessness, or until the participant could no longer empty the bag, or the limit of flow-meter (20l/min) was reached.

ii) Steady state: The flow was set at a fixed level for up to 5 minutes chosen to target a level of 50% ('test' level) of the visual analogue scale for WE, whilst keeping the $P_{ET}CO_2$ at normal resting levels. There was also up to 5 minutes of a 'masking' level that was chosen to target a level of 25% of the VAS for WE. The 'masking' level served to prevent the participants from 'expecting' a certain result. The order of the 5-minute test and masking steps were altered between runs. Brief periods of unrestrained breathing separated

the two levels of hypercapnia during which participants performed an inspiratory capacity manoeuvre to replicate what happens in the AH test.

As mentioned above, during the WE test two resistors were added in series (8 and 12cmH_2O/l/s) and the total resistance was estimated be 20cmH_2O, as it has been shown that resistances of series combination are approximately equivalent to the algebraic sum of the individual resistors[204]. To test if this was an accurate estimate each resistor was tested individually and in series, at different levels of flow and the resistance calculated, Fig. 2.6.

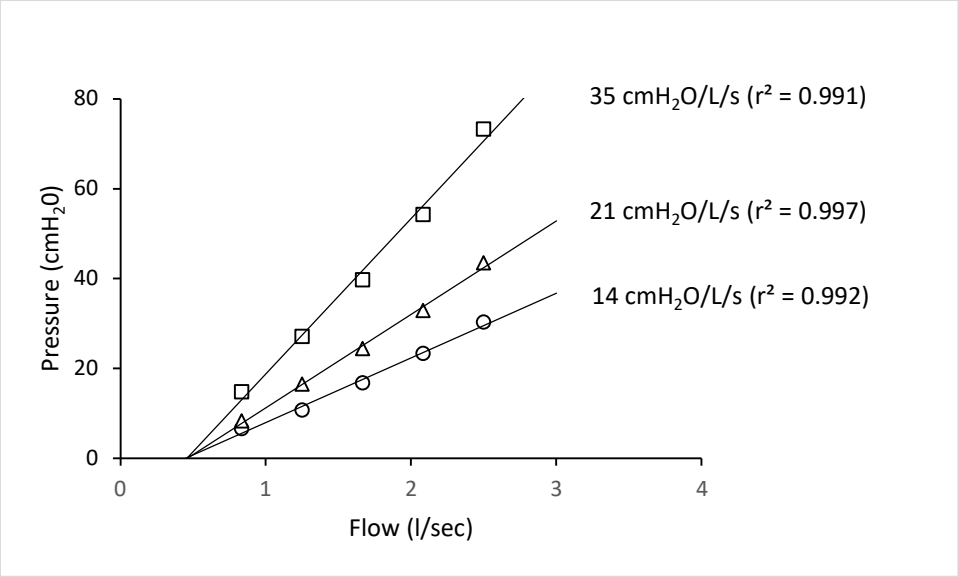


Figure 2.6 Pressure-flow characteristics of resistors

Pressure measured at several different flows whilst the resistor was in inspiratory line for the 8 cmH_2O/L/s resistor (O), 12 cmH_2O/L/s resistor (Δ), and both included in series (□). The actual measured resistance is shown as the slope of the linear regression through all data points for each dataset. Note 1: when in series the measured resistance is equal to the sum of the individual resistances measured for each resistor. Note 2: measured resistances are higher than the stated resistance of the resistors by a factor that increases with increased flow

The measured value of each resistance was higher than expected (i.e. the 8cmH_2O/l/s had a measured resistance of 14cmH_2O/l/s and the 12cmH_2O/l/s had a measure resistance of 21 cmH_2O/l/s.) However, the measured resistance of the two resistors in series was exactly the sum of the two (14 + 21 = 35cmH_2O/l/s). The higher readings from each individual resistor may be due to the circuit adding some resistance or the resistors need recalibrating. It is unlikely to be due to condensation or saturation as the resistors were tested when fully dry.

2.2. MEASUREMENTS

Airflow was measured at the mouth via a pneumotachometer (Respiratory Flow Head MLT300L, ADinstruments, Oxford, UK) and integrated (FV156 respiratory flow integrator, Validyne Engineering, CA, USA) to provide tidal volume (VT). Breathing pattern was recorded by DC-coupled respiratory inductance plethysmography (RespiTrace R250, Studley Data Systems, Oxford, UK). Pressure at the mouthpiece was measured via a fine-bore (1.5mm) sampling tube inserted into the mouthpiece connected to a pressure transducer (Differential pressure transducer, ±50 cmH20, Validyne Engineering, CA, USA). Tidal PCO_2 and PO_2 were measured with a calibrated, fast-responding, respiratory gas analyser (ML206, ADinstruments, Oxford, UK) via a separate fine bore sampling tube inserted into the mouthpiece. Blood pressure, oxygen saturations (SaO2) and electrocardiogram were also monitored (DatexOhmeda Cardiocap 5, Madison USA). Signals were sampled at 20Hz by an A-D converter and digitalised and recorded for offline analysis (Micro1401 with Spike 2 software, Cambridge Electronic Design, Cambridge, UK.)

Non-invasive blood pressure *(*BP) was automatically recorded every two minutes via an inflatable cuff on the upper arm (Datex-Ohmeda, F-CM1-04). 3-lead electrocardiogram (ECG; HME Lifetrak) and non-invasive arterial oxygen saturation (SpO2) using a finger-probe pulse oximeter (Nellcor) were monitored continuously throughout all experiments. Raising the level of inspired CO_2 can lead to increases in blood pressure and heart rate. Experiments were immediately stopped if any of the following occurred i) SpO2 <95%, ii) heart rate >150 beats per min, iii) frequent ectopic beats on ECG trace and iv) $P_{ET}CO_2$ was limited to a maximum of 65mmHg.

Prior to each visit the equipment was calibrated using a calibration gas and a 3litre syringe. Testing for leaks within the circuit was performed each visit by 'inspiring' using the 3-litre syringe attached to the mouthpiece whilst the gas supply was off. After each visit the breathing circuit was dismantled and soaked in an antiseptic solution for at least 15minutes. The circuit was then rinsed and dried.

Debrief: After each experimentally induced dyspnoea test, the participant was asked an open-ended question about the breathing sensations in the test they had just completed. This was followed by giving the participant a list of standard descriptors and asking them to select all of the descriptors that they experienced during that test. They were then asked to identify the top 3 descriptors that they experienced during the test, Table 2.1. During the first ramp test they were asked to rate any breathing discomfort on the VAS and then at the end of the test they were asked to identify the top 3 descriptors from the standard descriptor list. This is a non-validated questionnaire but established the quality of breathlessness experienced and allowed

instructions on which sensations the participant should focus on in the subsequent tests. For the AH test these were 'feel starved for air', 'feel a hunger for more air', 'feel an urge to breathe more', 'feel short of breath' and 'breaths feel too small'. For the WE test these were 'breathing requires more work', 'breathing requires more effort', 'size of breath feels too large' and 'feels like heavy exercise'. It also ensured that the dyspnoea tests were inducing the correct components of dyspnoea each time, allowing the stimulus to be comparable between test runs. The debrief also included a list of other symptoms/side effects that they may have experienced during the test (feeling flushed or warm, headache, dizziness etc.)

Table 2.1 Standard descriptors

After each experimentally induced dyspnoea test this list of sensations were shown and participants were asked to tick any that they felt during the test, and then to select the top 3 sensations that they experienced during the test.

	Tick	1, 2 or 3
A feeling of suffocation or smothering		
Size of breaths feels about right		
Breathing requires more work		
Breathing is comfortable		
Feel starved for air		
Feel short of breath		
Feels like heavy exercise		
Feel a hunger for more air		
Breathing requires more effort		
Size of breaths feels too large		
Feel an urge to breathe more		
Feel a tightness or constriction in chest		
Breaths feel too small		

Practice tests:

In the healthy volunteer study (Chapter 4) the participants underwent two practice sessions as they do not normally experience intense dyspnoea. This was deemed not to be required for the heart failure study (Chapter 5) as these patients are already familiar with experiencing dyspnoea. The practice sessions allowed the participants to become familiar with the breathing circuit and of how to rate their dyspnoea sensations. This included reliably using the full VAS scale. For this study it was necessary to ensure that the participants specifically rated air hunger for the AH test and sense of work/effort for the WE test. The practice sessions allowed this assessment. After the first test the instruction was to rate on the VAS 'any uncomfortable breathing sensations.' At the end of the test, the debrief (see above) determined if they had in fact rated the 'air hunger' cluster of descriptors. It was important that two different practice sessions are run in order to obtain consistency in data collection.

2.3. Exercise Induced Dyspnoea

2.3.1. 6 Minute Walk Test

The 6-minute walk test (6MWT) is used extensively in cardiology and it was first used in patients with heart failure in 1985[205]. It is a measure of the distance walked for 6 minutes at the patient's own pace, Fig. 2.7. In this thesis, the distance is calculated from laps achieved on a 20m course in a straight hospital corridor. The American Thoracic Society states the course should be 30m in length but this was not possible

within the department. This requires patients to take more time to reverse direction more frequently, thereby reducing the distance walked during 6 minutes. However, a recent multicenter study found no significant effect of the length on the distance walked, ranging from 15-50m corridors.[206] The test and equipment followed a standard operating procedure at each visit. Minimal equipment was needed and all patients were able to perform this test. It is not a maximal exercise test and does not give detailed information regarding the interaction between the pulmonary and cardiovascular circulation. It is known to be a reliable first-line test for quantification of exercise intolerance in patients with heart failure[207]. The correlation with prognosis from various studies has been inconsistent with some showing a strong correlation and others only showing a weak or no correlation[207-209]. One study suggested that the 6MWT was an inaccurate predictor of mortality[209]. A possible explanation for these varying results may be the different population groups studied (for example, ref 14, only studied New York Heart Association-NYHA II-III), different underlying aetiologies, different ages or due to the different protocols used (i.e. different levels of verbal encouragement). The use of the 6MWT was assessed in a systematic review and found poor correlation between the distance walked and the NYHA class[210]. However, a 6MWT distance of less than 300m did predict poorer prognosis in stable heart failure patients. Dyspnoea can be measured at the start and end (and during) using dyspnoea rating scales (see section below). In the patient study (Chapter 5) 6 patients performed the 6-minute walk test before and after mist inhalation.

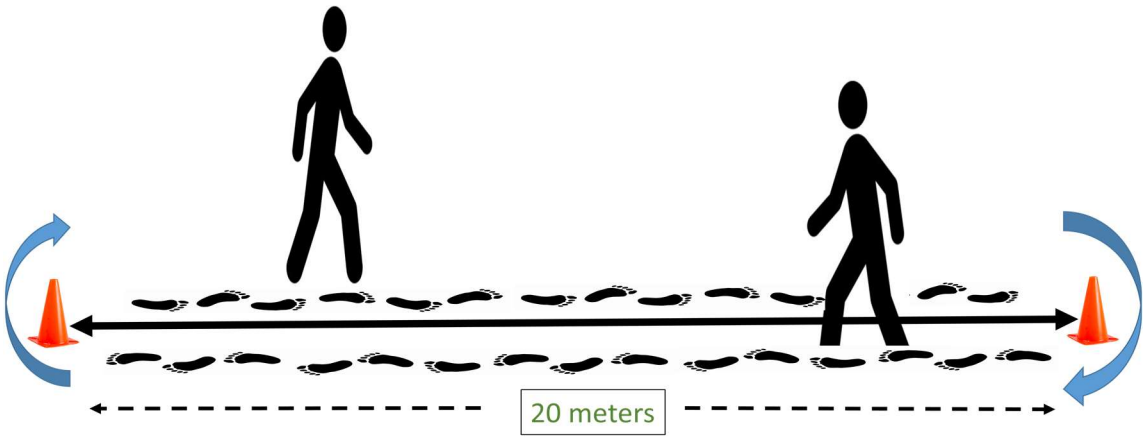


Figure 2.7 Six Minute Walk Test

Distance walked in 6 minutes at the patient's own pace.

2.3.2. Cardiopulmonary Exercise Testing

Cardiopulmonary Exercise Testing (CPET) is used less frequently than the 6MWT as it is more labour intensive and requires specialist equipment and training, Fig. 2.8. Clinically there is mounting evidence for using CPET, and within research its use continues to increase. In cardiovascular disease, CPET is used to assess the degree of exercise limitation, to determine the underlying mechanisms causing dyspnoea and to monitor response to treatment or deterioration in the condition[211].

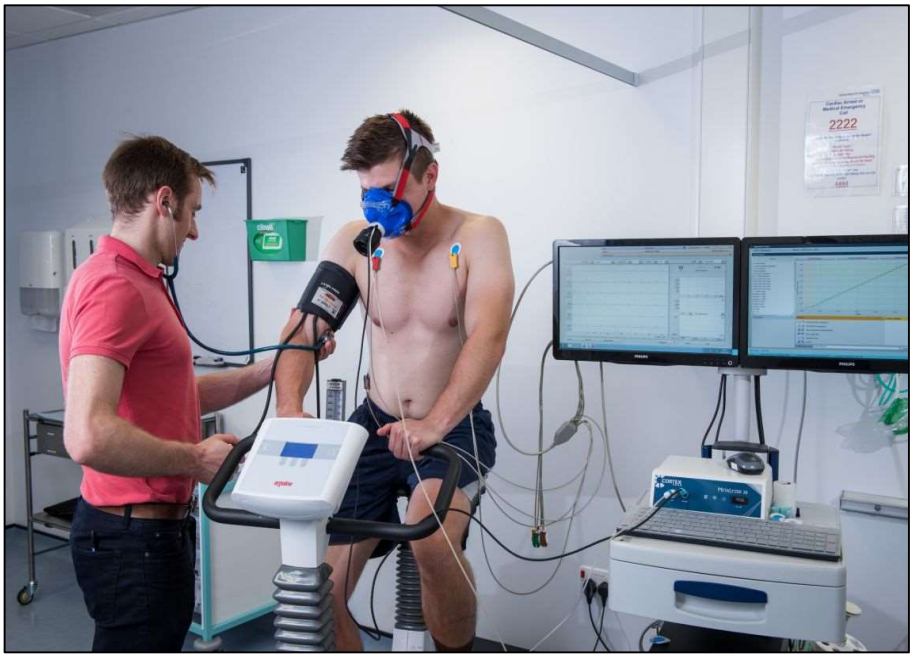


Figure 2.8 CPET using a cycle ergometer

CPET = Cardiopulmonary exercise testing. Image courtesy of the Cardiovascular Clinical Research Facility)

CPET enables information to be gathered about respiratory, cardiovascular and muscle function by providing measurements of oxygen consumption, carbon dioxide production and ventilation on a breath-by-breath basis. It also gives an indication of the effort the subject has given during the test. Table 2.2 shows some common parameters measured during CPET.

Table 2.2 Common CPET parameters

Parameter	Definition	Notes
VO_2	Amount of oxygen extracted from inspired gas per unit time (absolute value (ml/min)	Can be corrected for weight (ml/kg/min))
VCO_2	Amount of carbon dioxide exhaled from the body per unit time (ml/min)	
VO_2max	Maximum oxygen uptake achievable, despite further work rate increases	Ideally confirmed by multiple tests
Peak VO_2	Highest VO_2 achieve during presumed maximal effort	Aim RER >1.15
RER	Respiratory exchange ratio. Derived as the ratio of carbon dioxide production to oxygen uptake (VCO_2/VO_2)	This ratio can be used to estimate the respiratory quotient (RQ), an indirect measurement of calorimetry, at rest but not during exercise due to the accumulation of lactate.
VE	Volume of air inhaled or exhaled by the body in 1 minute	
MVV	The maximum potential ventilation achievable	Estimated as forced expiratory volume in 1 second (FEV1 x 40)
AT	Anaerobic Threshold. Exercise limit above which the subject's anaerobic high energy phosphate production supplements aerobic metabolism (V-slope method)	Denotes the break in the linear relationship between VCO_2 and VO_2 as subjects exercise past an intrinsic limit resulting in the disproportionate increase in ventilation and production of carbon-dioxide
BR	Breathing Reserve. The difference between MVV and the achieved maximum exercise minute ventilation.	This can be expressed as absolute values (L/min): $MVV\text{-}VE_{max}$ or as a percentage (%): VE_{max}/MVV
VE/VCO2 slope	The slope of the linear regression line of ventilation to VCO2 production	Measurement is taken using linear data points before the steeping associated with respiratory compensation.
Ramp exercise	Common style of protocol used for determining maximal work and gas exchange limits	Ideally with a duration of 6-12minutes

CPET was introduced to clinical practice in cardiology in 1985[211] and peak oxygen consumption (VO_2) has been shown to reliably predict prognosis[212]. Analysis of the results is useful in understanding the reason for exercise limitation. Ideally patients perform a maximal exercise test to provide a number of variables such as direct measure of oxygen consumption (VO_2 max) that have been shown to have prognostic value[213-215]. VO_2max is usually defined as the average of the final 30 seconds of VO_2 at peak effort. Before accepting a true VO_2max criteria for satisfying maximal effort must be met. This is usually defined as an RER >1.15, >80% of maximal predicted heart rate, or a plateau in the VO_2 versus time regression with increasing work resulting in no additional increase in VO_2. Patient with advanced heart failure are rarely able to perform a VO_2max test and peak VO_2 is used instead. Table 2.3 shows mean peak VO_2 for males and females in NYHA III and IV. There is a lack of data on the utility of these stress tests in patients with advanced heart failure, New York Heart Association Class III (NYHA-III) – indicating that patients are breathless on minimal exertion – or NYHA IV – indicating breathlessness at rest. Not all patients are able to perform CPET due to co-morbidities (e.g. severe osteoarthritis of the knees) or very advanced disease. Chronic heart failure is indicated by a normal breathing reserve, low VO_2 at anaerobic threshold (<40% of predicted), flattening oxygen pulse, and high VE/VCO2 slope[216].

Table 2.3 Gender differences in peak VO_2 according to NYHA class[217].

The difference in mean peak VO_2 (ml/kg/min) in men and women in NYHA class II and III[217].

	Mean Peak VO_2 (ml/kg/min)	
	NYHA II	NYHA III
Men	16.4	13.5
Women	14.8	11.7

NYHA Classification
Class I No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.
Class 2 Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
Class 3 Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20-100 m). Comfortable only at rest.
Class 4 Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients

CPET can either be performed on the treadmill or an electronically braked cycle ergometer. Cycle ergometers are useful for quantifying work-rate accurately and are better for those with balance problems, although leg fatigue can stop the test before a true VO_2max is achieved, with peak VO_2 on a cycle being 10-20% lower than on a treadmill[218].

Submaximal exercise testing

Criteria are used to define maximal exercise effort in healthy volunteers and those with cardiovascular disease[219]. These are a peak RER ≥1.10-1.15, post exercise lactate ≥8mmol/L, a plateau in the VO_2 to work rate relationship, a heart rate of ≥90% of

predicted, ≥8 on the Borg scale for perceived exertion, and the patients' appearance. Patients with advanced heart failure are not used to doing maximal levels of exertion during daily life. For those with a reduced ejection fraction, one guideline recommends an RER >1.05 and achievement of an anaerobic threshold on optimal medical treatment to be used to define a maximal CPET[220]. However, many patients are unable to reach this RER due to a combination of factors such as, muscle or ventilatory abnormalities, cardiac dysfunction, medication side effects or early fatigue[221]. Patients with heart failure are often unable to reach a heart rate >90% of predicted. This is due to chronotrophic incompetence which is common in heart failure[222, 223], as well as the result of beta-blockade, a standard therapy for heart failure. The worse prognosis arises from those with a low peak VO_2 (≤10ml/kg/min) who manage to attain a RER ≥1.15[224]. However, peak VO_2 and VE/VCO_2 slope are significant prognostic indicators irrespective of the RER[225].

Reproducibility of CPET

The reproducibility of CPET parameters has been investigated with a test-retest coefficient of variability of 5.9%[226]. This amount of 'noise' may prevent any useful data being gathered when trying to determine prognostic information and repeat testing.

In this thesis CPET was performed on a seated stationary electromagnetically braked cycle ergometer (Ergoline GmbH, Bitz, Germany) using an incremental value with respiratory gases collected via a facemask with an integrated system for collecting and measuring respiratory gases continuously (Metalyzer 3B, Cortex Biophysik, Leipzig, Germany). ECG and heart rate measurements were recorded continuously and blood

pressure was recorded every 2 minutes using a manual mercury sphygmomanometer (Accoson Freestyle, Essex, United Kingdom). Patients were instructed to maintain 55-65 rpm during the test. The test had an initial 2 minutes of unloaded cycling, with the workload increasing to 20 W at 2 minutes and then a gradual increase of 7.5 to 20 W depending on the patients reported level of activity, aiming to complete the test within 6 to 12 minutes. The patient then cycled continuously until exhaustion/maximum they were willing to do, or until they were unable to keep the rate at 55rpm. Patients then had a 2-minute cool down period with a revolution rate of their preference, with ECG monitoring continuing for 6 minutes. CPET was terminated prematurely if any of the following occurred; chest pain suggestive of ischaemia, ischaemic ECG changes, complex ectopy, second- or third-degree heart block, fall in systolic pressure >20 mm Hg from the highest value during the test, hypertension (>250 mm Hg systolic; >120 mm Hg diastolic), symptoms and signs of severe hypoxaemia, sudden pallor, dizziness or faintness, signs of respiratory failure or mental confusion.

2.3.3. Detecting changes in Exercise Tests with Interventions

Both the 6MWT and CPET have been used to measure the response to an intervention. In heart failure, the 6MWT has been used in a range of studies to assess the effect of new or established interventions, such as testing a new medication for cardiac amyloidosis (tafamidis), in the addition of intravenous iron therapy, beta-blockers, prostaglandins, optimal adjustment of medical therapy, use of cardiac resynchronization therapy or of exercise training and detraining[227-235]. Often a large variance is detected regarding the change in the 6MWT distance when testing an

intervention and this may be in part due to the test and re-test repeatability. However, 6MWT may not be a sensitive marker of change as proven therapies such as beta-blockers and angiotensin converting enzyme (ACE) inhibitors which significantly improve LV ejection fraction and NYHA class have not been shown to make a significant difference in the distance walked during a 6MWT.

In heart failure, CPET has been used less frequently to assess the effect of an intervention. Examples where it has been used include studies investigating the addition of oral or intravenous iron to standard medical therapy[236, 237]. Patients with NYHA class III-IV are rarely included in trials using CPET to assess outcomes of an intervention as this group may not be able to do enough exercise to generate meaningful data. However, there is an increasing view that CPET is underutilised in cardiology.

2.4. Assessment of Dyspnoea (patient reported outcome measure)

As discussed previously, dyspnoea comprised of different qualities and any measurement of dyspnoea should ideally cover the intensity, quality and emotional response of the respiratory discomfort. In patients, the severity of dyspnoea and the associated unpleasantness varies.

During incremental exercise testing, assessment of the intensity of dyspnoea was measured using the Borg Scale or Visual Analogue Scale (VAS). The Borg scale was initially used to measure perceived exertion and the scale ranged from 6 to 20. The Modified Borg Scale (MBS) was then created which was a 10-point scale with verbal

anchors to aid comparisons between tests. The MBS has good reliability and validity for the ratings of dyspnoea[238]. The VAS consists of a line 100mm long with word anchors at either end, 'no breathlessness at all' to 'worst possible breathlessness'. The reliability and validity is good[112].

Dyspnoea ratings in clinical trials

Over 30 ways of recording dyspnoea have been used in clinical trials[105, 106], each with a different focus, such as sensory perception versus emotional distress[107, 108], or addressing different time points (e.g. rest versus exercise). The most commonly used scales are the VAS, the numerical rating scale (NRS) and the MBS as they are convenient, quick and user friendly. Dyspnoea is present in many different conditions, such as heart failure, COPD and cancer and descriptors of dyspnoea overlap between these different conditions[51]. All of the scores above naturally will include both the severity and unpleasantness of dyspnoea unless specifically instructed otherwise.

In the AH and WE test an electronic 100mm vertical electronic visual analogue scale (VAS) is used to obtain subjective ratings of AH or WE. Ratings were cued by a 'light emitting diode (LED) light indicator every 15 seconds which instructed participants to rate how much AH or WE they were feeling at that point in time. In the AH test the participants were asked to focus on the sensations; 'feel starved for air', 'feel a hunger for more air', 'feel an urge to breathe more', 'feel short of breath' and 'breaths feel too small'. For the WE test the participants were asked to focus on the sensations; 'breathing requires more work', 'breathing requires more effort', 'size of breath feels too large' and 'feels like heavy exercise'. The range covered 0mm (no breathlessness)

to 100mm (tolerable limit) as previously described[21, 29, 239]. Additional word anchors ('slight', 'moderate', 'severe' and 'extreme') were placed at equal separation alongside the scale which enabled participants to remember how much of the scale represented how much sensation from one occasion to the next [239]. Extreme ratings activated an alarm, and the dyspnoea stimuli was immediately removed.

In the heart failure study patients, in addition to obtaining subjective ratings from the AH test, dyspnoea ratings were also taken pre-exercise (CPET and 6MWT) and immediately at the end of exercise, and for 1- and 2-minutes into recovery. Their dyspnoea ratings were recorded on both the VAS and the MBS, Figs. 2.9 and 2.10. A good correlation between these scales has been shown previously[240]. There were the word anchors mild, moderate, severe, extreme on the experimentally induced breathlessness tests electronic VAS but not on the VAS taken at the end of exercise. The word anchors were intended to make it easier for participants to remember how much of the scale represented how much sensation from one occasion to the next, as they were rating so frequently (4 every minute, approximately 40 ratings per test). This did not apply to the VAS taken at the end of exercise where only 3 ratings were taken.

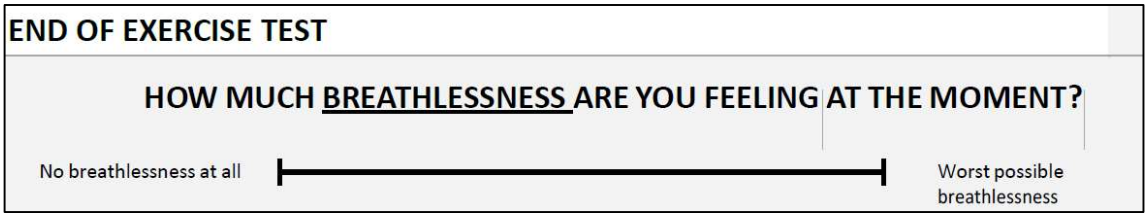


Figure 2.9 Visual Analogue Scale

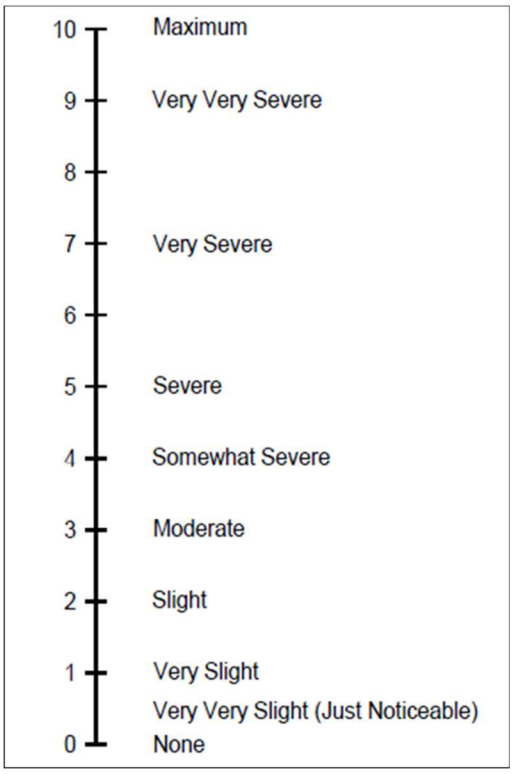


Figure 2.10 Modified Borg Scale

In addition to the dyspnoea rating scales, complementary questionnaires relating to breathlessness are also useful in the assessment of dyspnoea. In 1952, Fletcher first published a scale to rate the impact that dyspnoea is having on activities of daily living. A revised version focusing on the patients when walking or climbing stairs became known as the Medical Research Council (MRC) scale[241, 242]. This is the most commonly used dyspnoea scale in UK clinical practice.

Since the MRC Dyspnoea Scale two more recent multidimensional questionnaires have been developed, the Multidimensional Dyspnoea Profile (MDP) and the Dyspnoea-12 (D12)[7, 243]. These scales include both the physical and emotional aspects of dyspnoea. Utilising these questionnaires in conjunction with the dyspnoea rating scale allows a more focused approach to dyspnoea within research.

D12

In the heart failure studies (Chapters 3 and 5) the Dyspnoea-12 (D12) questionnaire was utilised, Table 2.4. This is a multi-dimensional questionnaire that comprised of both the physical and the emotional components of dyspnoea. Other questionnaires such as the NRS do not include the emotional components of dyspnoea and it is therefore difficult to ascertain how much this is contributing to their overall ratings of dyspnoea. The questionnaire was designed by collecting a pool of 88 common phrases used to describe breathlessness from published primary data studies. It has been validated in many different cohorts of patients, including heart failure. The total dyspnoea score is independent of underlying pathology and includes both physical and emotional components of breathlessness. It consists of 12 descriptors, divided into 7 physical and 5 emotional aspects and subjects can answer ‘none’, ‘mild’, ‘moderate’ or ‘severe’ with an associated score of 0, 1, 2 and 3, giving an overall maximal score of 36. It provides an overall total score of dyspnoea and while not designed to measure the physical and emotional individual components, it is possible to assess these separately. It is not designed to determine the different components of dyspnoea but some descriptors are terms more frequently used in patients with AH and some others in those with mainly the WE component of dyspnoea. The terms more aligned with AH are 'my breath does not go in all the way', I feel short of breath', and 'I cannot get enough air'. The terms more aligned with WE are 'my breathing requires more work' 'my breathing is exhausting.'

Table 2.4 Dyspnoea-12 Questionnaire

Item	None	Mild	Moderate	Severe
1. My breath does not go in all the way	0	1	2	3
2. My breathing requires more work	0	1	2	3
3. I feel short of breath	0	1	2	3
4. I have difficulty catching my breath	0	1	2	3
5. I cannot get enough air	0	1	2	3
6. My breathing is uncomfortable	0	1	2	3
7. My breathing is exhausting	0	1	2	3
8. My breathing makes me feel depressed	0	1	2	3
9. My breathing makes me feel miserable	0	1	2	3
10. My breathing is distressing	0	1	2	3
11. My breathing makes me agitated	0	1	2	3
12. My breathing is irritating	0	1	2	3

Minnesota Living with Heart Failure Questionnaire (MLHFQ)

The MLHFQ is a validated patient reported outcome questionnaire (United States Food and Drug Administration, FDA) that measures the adverse effects of heart failure on the patient's life. It has been approved to test the effectiveness of treatments for heart failure by assessing the reduction in the adverse impact of heart failure on the quality of life. It provides a total score (range 0–105, from best to worst HRQoL), as well as scores for two dimensions, physical (8 items, range 0–40) and emotional (5 items, range 0–25). The other eight items (of the total of 21) are only considered for the calculation of the total score.

2.5. DRUG DELIVERY

The nebulisers used throughout this thesis were MicroAir U22 Omron Nebulisers. This model has been used successfully in a previous study[29]. It is handheld and easy to use, with nebulisation possible at different angles. It is portable and will inform planned future clinical trials which involve patients taking the nebulisers home and using them independently. The aerosol is created by ultrasound (from a titanium vibrator, oscillating at high frequency) and can be battery operated or plugged into the mains power supply. It is easy to clean.

2.6. BLOOD TESTS

2.6.1. Biomarkers of Heart Failure

BNP and Troponin

The main cardiac biomarker used in heart failure is the brain naturetic peptide (BNP) or N-terminal pro-BNP) (NT-proBNP). BNP is strongly linked to dyspnoea and can be used to discriminate between acute dyspnoea caused by heart failure and that caused by primary lung disease[244]. Troponins (Troponin T (TnT) and Troponin I (TnI)) are most commonly used to diagnose myocardial infarction but are also often detectable in patients with heart failure. The use of high sensitivity troponin assays (hsTn) has significantly increased the number of patients with heart failure with detectable troponin to 92%[245, 246]. Elevation of these cardiac biomarkers are associated with increased mortality and hospital admissions[246]. Measuring hsTn over a few months strongly predicts all-cause mortality (HR 1.88)[247].

Cardiac biomarker response to exercise

Exercise improves morbidity and mortality for patients with heart failure with reduced ejection fraction[248, 249]. Myocardial infarction is diagnosed by a single cardiac troponin value above the 99th centile and a significant time-dependent change in the cardiac troponin concentration in the presence of clinical symptoms and signs[250]. The magnitude of the concentration change (i.e. the δ criterion) is not clear and varies from 20 to 250%. ESC recommends a change of 20-50% depending on whether the baseline troponin is below or above the 99th percentile. BNP is useful for both the diagnosis and monitoring of patients with heart failure. Exercise in heart failure is rarely mentioned and may be a confounding factor to diagnosing an MI using the δ criterion and may alter the BNP result. Biological variability in patients with chronic disease is critical for interpretation and analysis of both BNP and Troponin to understand their utility in clinical situations.

Collection of samples

Cardiac biomarkers were studied in patients with heart failure (Chapter 5). Blood samples were collected from 12 patients with stable heart failure with an ejection fraction <35%. Each patient attended on 4 days over a minimum of 21 days. Cardiac biomarkers were collected before and after exercise on two occasions each visit (4 samples per visit, Fig. 2.11) with a total of 16 samples per patient. Two series of blood samples were collected from the participants; one series included 4 samples collected within 3 hours, 2 pre-exercise and 2 post exercise; the second series consisted of, at most, weekly samples obtained over a period of at least 21 days (mean±SD

26.9±10.2days, range 22 to 53days). The samples were collected from an antecubital venous catheter.

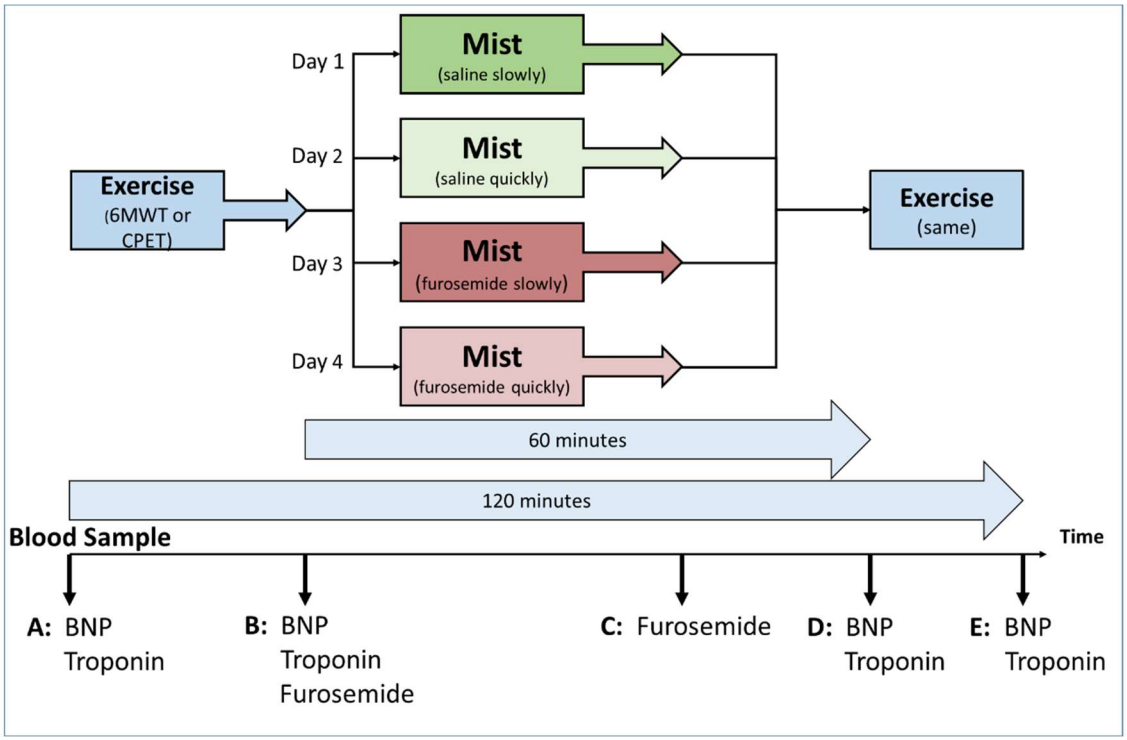


Figure 2.11 Protocol demonstrating timings of blood sample collection

Cardiac biomarkers are taken before exercise (A and D) and after exercise (B and E) at each visit. Each participant attends on 4 visits.

The interpretation of cardiac biomarkers over time and in relation to exercise allows us to assess the normal variability within each individual. The within-person variation (CVi), between person variation (CVg), RCV and II were calculated. The RCV is the maximum difference between two consecutive results that might be caused by analytical variation (CVa) and within patient variability (CVi). If the RCV is larger than the δ criterion used to diagnose disease then there is likely to be a high false positive

rate with low test specificity. The index of individuality (II) is the utility of conventional population-based reference values and diagnostic cut-offs (e.g. 99th percentile).

High sensitivity Troponin I assay (hsTnI) was determined on Abbott diagnostics. The limit of quantitation (LoQ) is 3.2 ng/L. The 99th percentile for the reference population has gender specific cut-offs with males at 34 ng/L and females at 17 ng/L. The functional sensitivity (CV=10%) is not specifically quoted by the lab. The manufacturer standard states that the assay performs with a CV of ≤10% across the concentration range 10 - 50,000 ng/L. BNP was analysed using Abbott Architect BNP. Lower limit of reporting = 2.9 pmol/L. The reference interval was up to 28.9 pmol/L. The manufacturer quotes CV of <12% across the reportable range.

2.6.2. Furosemide assay

The amount of furosemide absorbed from a nebulised dose of furosemide is unknown. The mean mass of the drug delivered to the lungs is thought to be less than 20% of the total mass nebulised and only 50% of the drug delivered is likely to be absorbed systemically[251-253]. To test this estimate blood samples were taken immediately before and immediately after nebulised furosemide. The furosemide assay was then processed, as below:

Chemicals and reagents

Organic solvents were of HPLC grade (Rathburns, UK). Other chemicals were of analytical grade (Fisher, UK). Furosemide 1mg/mL Certified Reference Material and

warfarin 1 mg/mL Certified Reference Material were purchased from Cerilliant (Sigma, UK).

Standards and quality control

Standards were prepared in house in 3% BSA in saline (Fisher, UK) at concentrations 0.05 – 1.0 mg/L and used to construct calibration curves. Quality control materials (two levels) were prepared in house in 3% BSA.

Extraction

Samples were stored frozen prior to analysis. Serum was acidified with 6M hydrochloric acid and internal standard (warfarin) was added to all samples. Furosemide was then extracted into hexane:ethyl acetate (70:30). The organic phase was evaporated and reconstituted into methanol for analysis by HPLC.

HPLC conditions

HPLC analysis was carried out on a Varian ProStar system with PDA/fluorescence detection. Separation used a Spherisorb 5 µm ODS 25cm x 4.6 mm ID column (Waters, Chromex Scientific, UK) at ambient temperature. Mobile phase was acetonitrile: phosphate buffer, pH3.0, using a gradient 35-55% over 10 min. Flow rate 2.0 mL/min. The detector used excitation and emission wavelengths 225 and 389 nm, respectively.

Method performance

Precision was assessed by running quality control material within (n=4) and between (n=24) batches. Intra-assay precision was <6% and inter-assay precision was <12%. Recovery of spikes at 0.025, 0.05, 0.1 and 0.5 mg/L gave mean 109% difference from

the spiked value (range 98-120%). All calibration curves were linear, r2>0.98. The lower limit of quantification, defined as CV<20% with accuracy 80-120% of expected value, was 0.03 mg/L.

2.7. STATISTICAL ANALYSIS

Descriptive statistics have been performed to present and summarise data. Test of significance have been performed to draw conclusions from the results. Statistical analysis was performed using SPSS (Version 25, IBM, Armonk, New York), Microsoft Excel 2016 and SAS 9.4. In this thesis, variability of data is indicated by either standard deviation (sd) or standard error (se). Tests of inference were primarily analyses of variance (ANOVA). This method allows estimates of independent and mixed effect of several factors on a dependent variable. The outcome variables were continuous. In this thesis most of the data was measured repeatedly in the same subject and so the analyses used most frequently was Repeated Measures ANOVA.

Repeated measures analysis of variance

Details are provided for individual tests within each chapter but all were analysed using SPSS Version 25. In general, the initial ANOVA included all the within and between factors. If the existence of a significance was indicated within the mixed effects then further ANOVA were performed to determine the individual contribution of the various factors. All ANOVA test were performed using SPSS Version 25. Statistic from the ANOVA was set at 5% level of probability of the null hypothesis being true.

Other statistical test employed in this thesis included Chi square, Student's t-test, Linear Mixed Model 'mixed' procedure, and tests of linear regression. Student's t-test were performed where ANOVA was unwarranted for unnecessary. This was usually a paired t-test.

Repeated measures ANOVA was most frequently used statistical analysis in SPSS. Chi squared was also used. The Linear Mixed Model 'mixed' procedure was used in Chapter 4. Chapter 5 was a pilot study and mainly descriptive statistics were used. Missing data was treated by using the pre or post mist value, or if not possible the average of that set of values.

Treatment of missing data

There were very few missing data points in this thesis. Any missing data resulted in the SPSS excluding the full set of data for that subject. To avoid this, missing data were replaced by representative values from the same subject. For example, for the missing blood test results (3 in total), the results on the same day at the same was taken as had occurred before (or after) the mist.

2.8. SUMMARY

Laboratory-based experimental models have been used in Chapters 4 and 5 to elicit dyspnoea in a controlled and systemic way to allow different components of dyspnoea to be investigated whilst minimizing confounding factors. Exercise tests have been used to elicit dyspnoea in Chapter 5 as closer representation of 'real life.' Subjective ratings of respiratory discomfort have been investigated throughout this thesis, from

simple visual analogue scales to more complex questionnaires, investigating both the physical and emotional components to dyspnoea. Finally, blood tests have been performed to gain a better understanding of how cardiac biomarkers change with exercise, as well as their within-subject and between-subject variability over time. Furosemide assay data allows direct measure of systemic absorption from a nebulised dose of furosemide.

3. DYSPNOEA PREVALENCE IN COMMUNITY HEART FAILURE

3.1. INTRODUCTION

The origins of dyspnoea are poorly understood in heart failure and there is a poor correlation with objective measures of heart or lung function[62, 63] yet dyspnoea is a key predictor of mortality, reduces quality of life and affects the will to live[254, 255]. Dyspnoea is one of the most frequent symptoms of heart failure, with over 50% experiencing daily dyspnoea and limiting the ability to perform activities of daily living[55]. However, this research by Barnes et al. (2006) used the Kansas City Cardiomyopathy Questionnaire which covered all symptoms related to heart failure, and was not specifically related to dyspnoea. Dyspnoea is not only a physical symptom and, like pain, is frequently associated with a strong emotional/psychological component. In 2010, a new questionnaire was developed focusing on dyspnoea called the 'Dyspnoea-12' (D12). It is a validated multidimensional tool comprising of 12 items and unlike some of the more commonly used measures of dyspnoea, such as the visual analogues scale, it incorporates both the physical and affective aspects of dyspnoea in a single global score of dyspnoea severity[7]. One of its strengths is that it has been designed using phrases that patients themselves used when describing their breathlessness. The variation of dyspnoea over the day or week is unknown in patients with heart failure. Dyspnoea is usually only assessed during GP or hospital appointment and anecdotal evidence from the community heart failure team suggest

that the nature and extent of dyspnoea may be different in the community setting. Readmission rates for heart failure are high (~17.5%) and are similar to that of chronic obstructive pulmonary disease-COPD (16.5%), compared to an average readmission rate of 6.6%[256] and have major financial implications in the NHS. Gaining a better understanding of the prevalence and unpleasantness of dyspnoea in the community may allow targeted treatment and reduce readmissions.

The relative contribution of the different components of dyspnoea (air hunger (AH), work effort (WE) and chest tightness) are unknown in patients with heart failure. How these components vary from rest to exercise has also not been studied. This study focuses on the prevalence, quantity and variation over the day and weeks rather than the quality of their dyspnoea.

Specific aims:

1) To assess the prevalence of dyspnoea at rest in patients with heart failure within the local community and understand to what degree the physical and emotional/unpleasant components contribute to the symptom of dyspnoea.

2) To assess the variations in dyspnoea in patients with heart failure within each day and over 1 week.

3) To correlate dyspnoea with other factors; ejection fraction (preserved or reduced), New York Heart Association class, gender and age.

Null hypotheses:

1) There is no variation in dyspnoea in patients with heart failure within each day or over a period of 1 week

2) There is no correlation with ejection fraction, NYHA class, gender or age

3.2. METHODS

Participants

One hundred and fifty patients were initially identified by reviewing consecutive heart failure clinic letters from the John Radcliffe Hospital, Oxford over a period of 1 month. The inclusion criteria consisted of a diagnosis of heart failure with reduced (<50%) or preserved (>50%) ejection fraction. Patients had to be ≥18years old, with no upper age limit. The exclusion criteria consisted of patients who had documented dementia or confusion which was likely to impair their ability to answer the questionnaire and/or an indication on the clinic letter that they required a translator.

Full ethical approval was obtained from the NHS Health Research Authority (REC reference number 16/YH/0360) and the Oxford Brookes Departmental Research Ethical Officer with Oxford Brookes University serving as the research sponsor.

Sample size

Re-examination of the raw data that was used in a previous publication[7] showed that the D12 score in 106 patients with heart failure had a standard deviation of 24% full scale. The minimally clinically important difference (MCID) for total D12 score is reported to be 9.7% full scale[257]. Using this information, it was determined that 50 subjects would be needed to be able to reject the null hypothesis for mean differences between time points (morning, noon, evening) with a power 0.8 and Type I error probability is 0.05. This calculation was performed using PS Power and Sample Calculations software V3.0 January 2009. When stratified according to age, sex, New

York Heart Association (NYHA) class and ejection fraction it is likely that any non-significant differences would need to be treated with caution as the research is underpowered for definitive conclusions with regard to these comparisons. Thus, for the secondary analyses this research is preliminary.

Barnes et al. (2006) showed dyspnoea present in 54% of patients with heart failure. For 50 patients to be the minimum number of patients with dyspnoea in the sample 108 patients are required, for a probability of 95%. Sample size affects the prevalence estimates. The more data available the more precise the estimate. As the sample size increases the confidence in the estimate increases, with a reduction in uncertainty and greater precision (standard error decreases). Protocol

Recruitment started in October 2016 and 150 consecutively identified patients with heart failure were each sent twenty-one D12 questionnaires to complete 3 times a day (morning, midday and evening) for 7 days. The D12 was used as it is a valid and reliable instrument to measure dyspnoea in different cohorts[125] and takes into account both the emotional and physical components of dyspnoea. It was designed using language that patients used themselves and can be filled in without the need of a trained healthcare professional. It is also quick and simple to perform. The MCID for the Dyspnoea-12 questionnaire is 3 points[124]. The questionnaire is included in the methods chapter. A reminder letter was sent after 1 month to non-responders. The instructions to the patient were to sit at rest for 5 minutes and then to rate their dyspnoea at that moment in time and to return the questionnaires by mail once complete. The original D12 validation paper tested the D12 using the reference frame

'these days' and it was pointed out in this paper that when using it in this way it is being used in a non-temporally specific way[7]. The paper does not however explicitly state that it should not be used in a temporally specific way. A recent paper by Ekstrom et al., 2020 has shown that the MCID does not change if you use various specific timeframes, for example, average, best, worst and current intensity[123]. In this chapter the patients were asked to rest for 5minutes to fill this questionnaire in and to rate their breathlessness at that moment in time. Any returned without completion was taken to mean that they withdrew from the study. On each questionnaire the 12 items were presented in a random order. Chapter 2 provides details of the validity and features of this questionnaire.

Statistical analysis

A repeated measures analysis of variance was performed with 2 within factor; time of day (3 levels; morning, noon and evening) and day of the week (7 levels; Monday to Sunday). This analysis was performed for D12 total score and for the component D12 physical and emotional scores, all expressed as %full scale. A further analysis was performed with the addition of between factors of age, sex, NYHA class and ejection fraction (two levels: reduced, <50% and preserved, >50%).

3.3. RESULTS

A total of 109 males (73%) and 41 females (27%), with a mean age of 77 years were sent an invitation to participate. Fifty three percent (male: female 75:25%) had a reduced ejection fraction and 47% (male: female 69:31%) had a preserved ejection

fraction. Twenty eight returned the questionnaires without completing them, indicating that they did not wish to participate, and 30 returned the fully completed set of questionnaires giving an overall response rate of 20%. This was a lower than anticipated response rate and the study is underpowered. Analysis of the results has been undertaken to generate preliminary data but when 'significance or lack of' is stated this must be interpreted with caution. Of those that participated 14 (47%) experienced some dyspnoea and the remaining 16 (53%) did not experience any dyspnoea.

For those that experienced dyspnoea (n=14) the overall mean% full scale±sd total, physical and emotional D12 scores were 23±22, 18±17 and 5±6 respectively. The within subject standard deviation was 6.2% full scale. The 95% range was 0 to 67% full scale. When averaged over 7 days for morning, noon and evening times the scores were 23±22, 24±23, 23±23 for the D12 total, 18±18, 18±18, 18±20 for D12 physical and 5±7, 6±8, 5±7 for D12 emotional respectively. Repeated measures ANOVA did not detect any main effect of time of day or day of week or any significant interaction between these within factors for D12 total, D12 physical or D12 emotional components, all $p>0.05$, Fig. 3.1. However, the study is underpowered for this analysis.

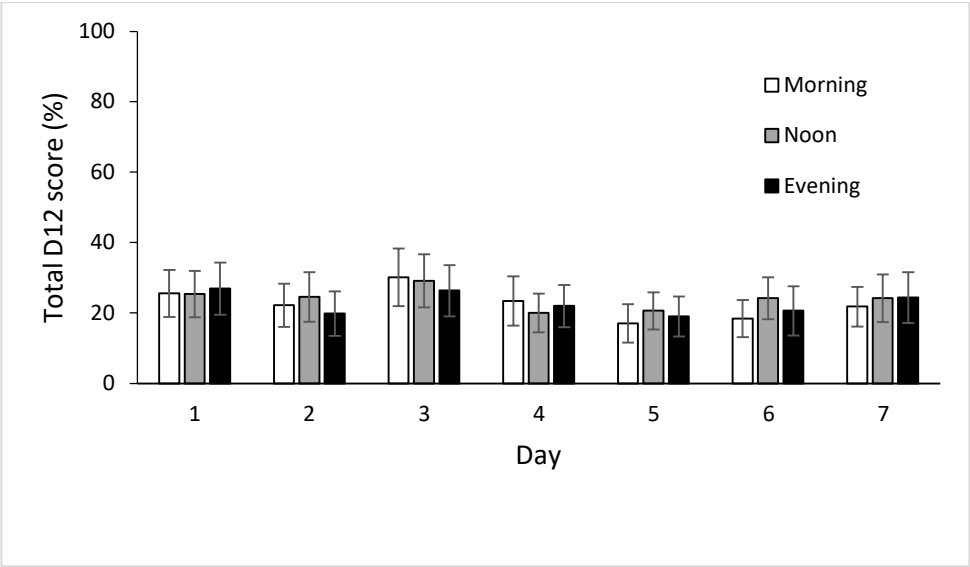


Figure 3.1 D12 scores at different timepoints

Variation in total D12 scores at morning, noon and evening time over 7 days. The mean±sem total, physical and emotional scores for dyspnoea expressed as %full scale recorded at morning, noon and evening over 7 consecutive days.

i) <u>Type of heart failure (reduced or preserved ejection fraction)</u>

Accepting that the study is underpowered, preliminary analysis showed that in those with a preserved ejection fraction 67% experienced dyspnoea, compared to 38% in those with a reduced ejection fraction but this did not reach significance, chi squared p=0.15, Table 3.1. This calculation includes all patients who reported any degree of dyspnoea on the D12. Repeating this analysis with no dyspnoea defined as never rating above a threshold of 9.7% (the MCID) did also not result in a significant difference (chi squared p=0.16), although the proportion of reduced ejection fraction patients with dyspnoea decreased further to 29%.

Table 3.1 Dyspnoea prevalence in preserves and reduced ejection fraction subsets.
Chi-squared analysis of the ratio of dyspnoea presence and absence among preserved and reduced ejection fraction subgroups of patients with heart failure. No dyspnoea indicates a score of zero on the total D12 score. The chi-square statistic is 2.07. The p-value was not significant at 0.15, however the study is underpowered for this analysis.

	Dyspnoea	No dyspnoea	*Marginal Row Totals*
Reduced	8 (9.8)	13 (11.2)	21
Preserved	6 (4.2)	3 (4.8)	9
Marginal Row Totals	14	16	30 (Grand Total)

In those patients where dyspnoea was present, the D12 scores were similar between those with a reduced and preserved ejection fraction (25±24 vs. 20±22 respectively, mean%±sd). There was a slight trend for an increase in the physical component for those patients with a reduced compared to the preserved ejection fraction (21±19 versus 14±16).. There were no significant differences in the emotional components in reduced compared to preserved ejection fraction (5±7 versus 6±7), Fig. 3.2. However, the study is underpowered for this analysis.

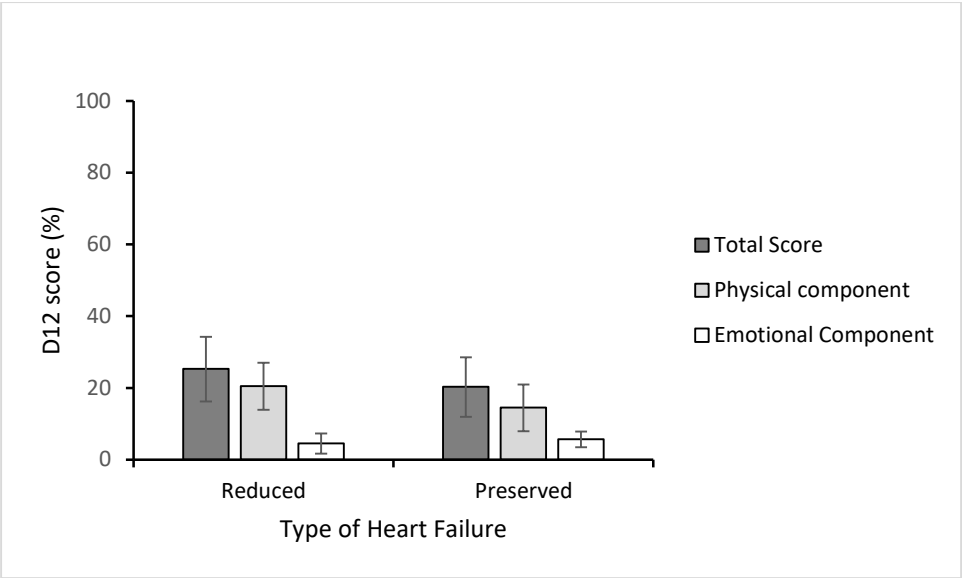
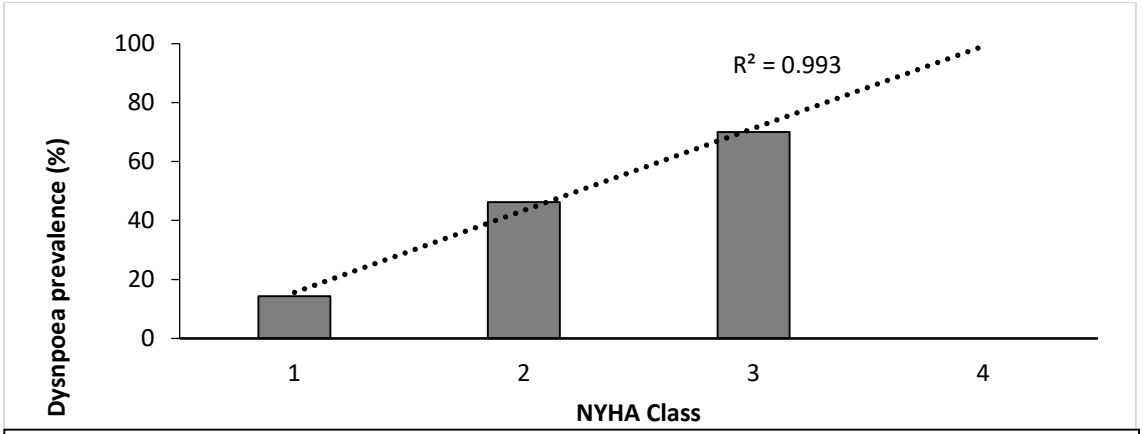


Figure 3.2 D12 scores in heart failure with a reduced or preserved ejection fraction.

The mean±sem total, physical and emotional scores for dyspnoea expressed as %full scale averaged for morning, noon and evening over 7 days in 14 patients with reduced and preserved heart failure in the community.

ii) <u>New York Heart Association (NYHA) class</u>

Preliminary data of this study showed the presence of dyspnoea on the D12 questionnaire correlated with the NYHA class. For the 14 patients who reported dyspnoea 7 were in NYHA class III, 6 were in class II and 1 was in class I. For the 16 patients without dyspnoea 3 were in NYHA class III, 7 were in class II and the remaining 6 were in NYHA class I, Fig. 3.3. The study was underpowered, however preliminary analysis showed there was a significant difference in D12 scores between Class I and III, p=0.02. There was a trend for a difference in D12 scores between Class II and III, p=0.05, Fig. 3.4. There was no significant difference between Class I and II, p=0.39.



Class I No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.
Class 2 Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
Class 3 Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20-100 m). Comfortable only at rest.
Class 4 Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients

Figure 3.3 Proportion of patients with dyspnoea within each NYHA class

The proportion of patients experiencing dyspnoea increases with each NYHA class.

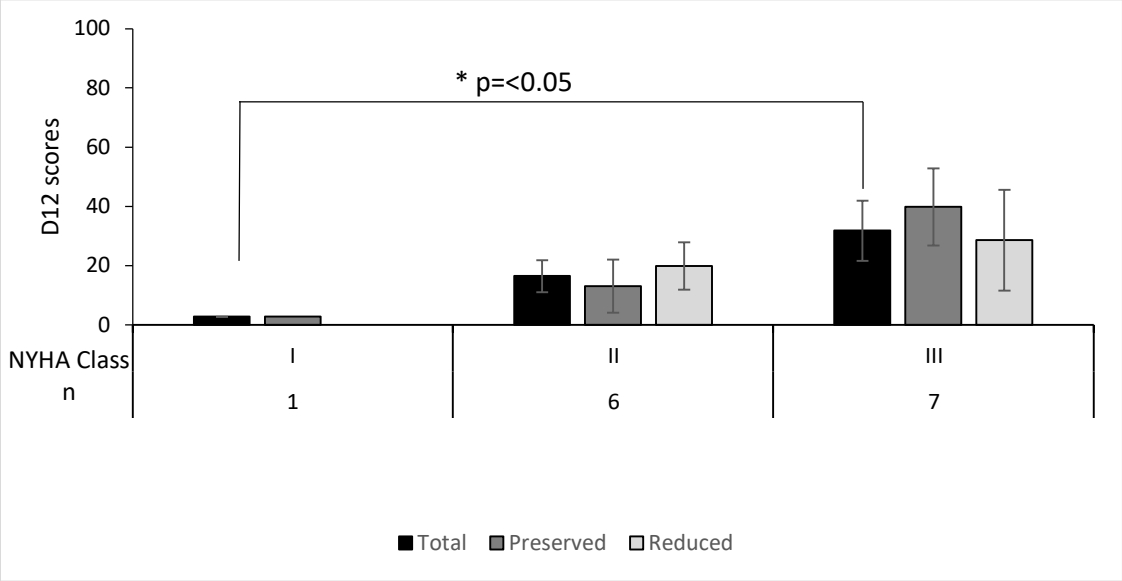


Figure 3.4 D12 score in each NYHA class

The mean±sem total D12 expressed as %full scale averaged for morning, noon and evening over 7 days in 14 patients with reduced and preserved heart failure in the community according to NYHA class.

iii) Sex and Age

For the 30 patients who responded 10 (33%) were female and 20 (67%) were male. In the 14 who reported some level of dyspnoea 5 (36%) were female and 9 (64%) were male. In the 14 who reported dyspnoea the total D12 was 22±30 for females and 24±19 for males. Gender nor age had any significant effect on the total D12 score or for the individual physical and emotional components Table 3.2. However, the study is underpowered for this analysis.

Table 3.2 D12 scores according to gender and age

The mean±sd total D12 expressed as %full scale averaged for morning, noon and evening over 7 days in 30 patients with heart failure in the community.

		N	D12 total (% ±sd)	D12physical (% ±sd)	D12emotional (% ±sd)
Sex	Female	10	11±23	13±30	7±13
	-reduced	8	12±26	16±33	7±15
	-preserved	2	5±3	4±3	7±9
	Male	20	11±17	15±23	5±11
	-reduced	14	7±14	11±20	2±7
	-preserved	6	19±23	24±29	11±17
Age	50-60	1	0	0	0
	60-70	3	21±31	25±37	43±19
	70-80	13	8±12	13±20	1±4
	80-90	11	14±24	18±31	9±16
	90-100	2	3±4	1±2	7±8
	TOTAL	**30**	**11±20**	**15±26**	**6±14**

iv) Mortality

Mortality increased with age. Approximately 15% of those experiencing dyspnoea in the age group 70-80years old died compared to none of the patients in that age group who reported no dyspnoea, Fig. 3.5. In the older age group of 80-90years the reverse was true, with more patients dying in those that reported no dyspnoea.

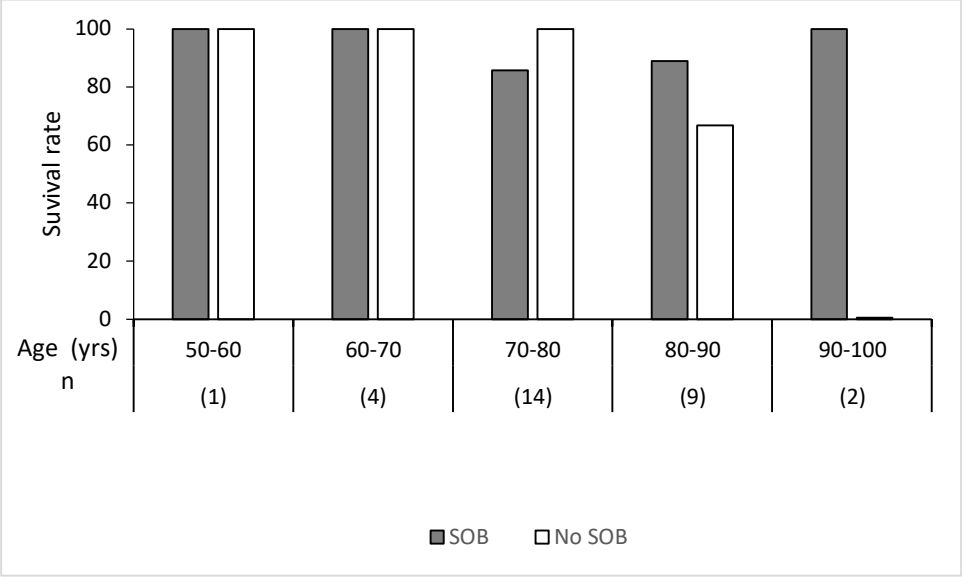


Figure 3.5 Dyspnoea and survival rate

Survival in each age group, stratified according to presence or absence of dyspnoea. SOB = shortness of breath/dyspnoea

3.4. DISCUSSION

The study has found that patients with heart failure in the local community report a relatively low level of dyspnoea at rest (23±22, mean%±sd) that did not vary with time of day or day of the week over a 1-week period. This information has not previously been reported and provides insight into the experience of dyspnoea for those patients

living with heart failure in the community. However, the correct sample size was not achieved and therefore the data including the prevalence estimates should be treated with caution.

Comparison with the literature

In the paper that originally presented the D12 (Yorke et al., 2010), re-examination of the raw data for the levels of dyspnoea reported by 106 heart failure patients for D12 total, physical and emotional scores were higher than the corresponding data in the current study; 30±24, 33±25, 26±26 versus 23±22, 18±17 and 5±6% respectively, mean %full scale±sd[7]. This difference is likely due to our methodology which asked patients to sit for 5 minutes before completing the questionnaire, whereas the original study asked about breathlessness 'these days' in terms of how patients currently experience breathlessness in their daily lives, and not at a specific time-point or after a specific activity. Asking them to sit for 5 minutes was done to standardise the results but likely had the effect of minimizing any dyspnoea experienced and, in retrospect, added value may have been achieved if patients were asked to complete the D12 after a short walk or after climbing a flight of stairs. Another reason for these differences may be due the definition of 'chronic heart failure' which is not reported in the Yorke et al. (2010). The distribution of reduced and preserved ejection fraction is unknown. However, this may not affect the results as a study by Bhatia et al. (2006) found dyspnoea present in 96.2% of those with a reduced ejection fraction and 94.9% in those with preserved ejection fraction ($p=0.11$)[258] and this study showed no significant difference in the

severity of dyspnoea experienced in each group. However, the study is underpowered for this analysis.

The proportion of patients who indicated any level of dyspnoea on the D12 amounted to 47% which is similar to the 54% reported previously by Barnes et al. (2006) using the Kansas City Cardiomyopathy Questionnaire which is not specific to dyspnoea but covers a range of symptoms related to heart failure[55].

In acute exacerbation of COPD, Nishmura et al. (2018) recorded D12 throughout admission [259]. Our data fits most closely with day 1 of an acute exacerbation of COPD, with a much higher score (29%full scale, total D12) than at 84 days after an acute admission (11%full scale, total D12)[259]. This suggests that patients with heart failure are experiencing a similar level of dyspnoea to those experience by patients on day 1 of an admission with acute exacerbation of COPD. It is not clear in the Nishimura et al. (2018) study as to whether the scores were taken before or after initial treatment for COPD had been administered on the day of admission. The patients previous admissions to hospital are not recorded and for both heart and lung disease the disease trajectory tends to be the same with a gradual decline in the disease, which is populated by exacerbations (more severe symptomatic brief episodes) which then resolve but never return to the baseline set before the exacerbation[260, 261]. It is unknown as to where on the trajectory these patients are in either study.

<u>Heart failure with reduced or preserved ejection fraction</u>

There was a trend for more dyspnoea in those with preserved compared to reduced ejection fraction (25±24 vs. 20±22 respectively, mean%±sd), with a greater proportion

attributable to the physical components of the D12 for those with a reduced ejection fraction (21±19 versus 14±16.) Heart failure with a preserved ejection fraction is more common in females (66%) and heart failure with a reduced ejection fraction is more common in males (63%)[258]. Eighty percent of the females in this study had a reduced ejection fraction and this is not representative of the normal distribution of heart failure. It is possible that this bias existed in this study, as those with preserved ejection fraction are often more frail with multiple co-morbidities and therefore may not have been able, or are less willing to complete the multiple questionnaires that were required. Although both preserved and reduced ejection fraction appear to experience similar levels of dyspnoea, the study by Bhatia et al. (2006) did not separate this into males and females so it is unknown if this is likely to alter the results.

Correlation with NYHA class

Preliminary analysis of data obtained by stratifying the data according to age, sex, NYHA class and ejection fraction, taking into account the study is underpowered, found there were no significant differences apart from between the overall D12 score for NYHA class I and NYHA III ($p=0.02$). This is expected as breathlessness is a component used for classifying patients into their NYHA class. There was a trend seen for between Class II and III, however this lack of significance should be treated with caution as the research is underpowered with regard to this comparison. The definition of NYHA III is "marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20-100 m). Comfortable only at rest." However, this

study shows that 66% of patients in NYHA III do experience a degree of dyspnoea at rest within the community.

Correlation of dyspnoea severity with time of day

In COPD there are several studies describing the fluctuation of symptoms over the day, with the most severe symptoms in the morning[262, 263]. Unlike in COPD there were no such fluctuations in dyspnoea seen during this study for the morning, noon or evening, 23±22, 24±23, 23±23 respectively. The highest dyspnoea scores were recorded on Day 3 (29±27) compared to Days 1, 2, 4, 5, 6 and 7 (26±24, 22±24, 22±22, 19±20, 21±22, 23±24 respectively). The MCID for D12 is at least 9.7% full scale[257] so this change in symptom intensity is unlikely to be recognised by the patient. This increase on day 3 was mainly down to two participants having significantly higher scores on this day compared to the other days..

Situational dyspnoea

The preliminary analysis of this study does not support the notion that the nature and extent of dyspnoea varies between a healthcare environment (GP/hospital) compared to the community setting. When comparing the D12 scores recorded in our laboratory (Chapter 5) to those in this study, where they completed it within their own home, the total D12 scores (21±15, 23±22 mean %full scale±sd respectively) were not significantly different (SPSS independent t-test; p=0.70), and this was also true for the individual emotional and physical components (27±18, 31±29 and 11±16, 12±1, mean %full scale±sd, respectively.) However, it is noted that the mean D12 scores were all higher in the community than in the laboratory. This may be due to the fact that the

instructions for completing the D12 questionnaire were not identical, such that the instruction for the laboratory test was for them to rate how their dyspnoea is 'these days' compared to 'at this moment' for this study, which was performed after 5minutes of rest. A previous study reported as an abstract by Russell et al. (2016) showed that D12 scores reported "these days" are greater than those reported "today" which is also consistent with the pain literature which shows recalled sensation is reported as more intense than symptoms in the moment[264]. This data does not fit with the pattern seen in this thesis (higher when asked to rate 'now' than 'these days'). The Russell et al. (2016) study was performed in those with lung disease rather than heart failure and this may account for the differences seen in this study. It is possible that psychological factors at home relating to a diagnosis of heart failure may raise the scores of self-reported dyspnoea, although the emotional component of the D12 was similar (11±16 vs. 12±15 % full scale emotional±sd).

Mortality

As expected the mortality rate increased with age in this study, however, only those that reported experiencing dyspnoea in the younger age groups died, compared to those reporting 'no dyspnoea' on the D12 questionnaire. This agrees with previously published literature showing that dyspnoea is a predictor of mortality[254]. For the older age group of 80-90years the absence of dyspnoea was associated with an increased mortality compared to those with dyspnoea. This may be related to the increased risk of mortality from all conditions in these age groups, or due to the small number of patients in these groups.

Critique of study and future directions of study

The main limitation is that the study is underpowered due to a lower than anticipated response rate. Nevertheless, most of the trends reported are consistent with the published literature. The NYHA class is a widely accepted classification, although this limited study suggests that some patients classified as class III are not comfortable at rest. This raises the possibility that some patients should be re-classified as class IV or the text amended to take this into account.

This study utilises the D12 score to assess the dyspnoea in a multidimensional way that previous tools for assessing dyspnoea had ignored, by including the emotional component of dyspnoea. Another questionnaire has been developed that also separates the physical and emotions components of dyspnoea. This is known as the Multidimensional Dyspnea Profile (MDP)[243]. This questionnaire required more input for the patient and healthcare professionals than the D12. The D12 was used in this study as it is straight-forward and easy for the patient to complete without the need for any healthcare professional input. It therefore works well in this study.

This study excluded patients who required a translator as only the English version of the D12 questionnaire was used in this study. The D12 has been translated into a number of different languages (e.g. Italian, Portuguese, Arabic) and future studies could include a wider population where English is not the first language[265-267].

In this study, participants were asked to complete the questionnaire after sitting for 5 minutes to standardise the results, however, this had the effect of minimizing the participants' level of dyspnoea; it would have been interesting to assess the results of

completing the D12 immediately after climbing a flight of stairs and how this would alter the results as one of the common features of heart failure is dyspnoea on exertion, rather than at rest, which is thought be related to a more advanced stage of heart failure.

3.5. CONCLUSIONS

This study has advanced our understanding of the prevalence of dyspnoea at rest in heart failure patients in the community setting using a questionnaire survey that considers the multidimensionality of dyspnoea. Almost half the patients experience some dyspnoea at rest. Unlike in COPD, this preliminary data provides no evidence for any significant variability within each day or between days. As expected the dyspnoea scored by the D12 correlated with NYHA class. Experiencing symptoms at rest applies to NYHA class IV however this study shows a significant proportion of patients in class III are experiencing dyspnoea at rest. Clarification is needed regarding the term 'comfortable at rest' in the NYHA class III classification to address this.

4. SPECIFICITY OF DYSPNOEA RELIEF WITH NEBULISED FURSOSEMIDE

4.1. INTRODUCTION

Dyspnoea accounts for over 15% of symptom burden among hospitalised patients and contributes to poor quality of life by limiting activity, increasing anxiety levels and undermining the will to live[56, 268]. It is present in a wide range of conditions such as chronic obstructive pulmonary disease (COPD), chronic heart failure, advanced cancer and neuromuscular disease[269]. Given its prevalence and impact, there is an urgent clinical need for more effective treatments. Nebulised furosemide offers a potential complementary treatment for dyspnoea relief[270].

Furosemide is a loop diuretic. It is usually taken orally or intravenously and acts through inhibition of the sodium-potassium-chloride co-transporter in the thick ascending limb of the loop of Henle in the kidneys[271]. In rats, nebulised furosemide has been shown to sensitize slowly adapting pulmonary stretch receptors (saPSR) in the lung parenchyma[30]. Stimulation of these receptors has been shown to relieve air hunger (AH; an uncomfortable urge to breathe) in high level quadriplegic humans in whom afferent information from the chest wall is blocked but vagal afferents from lungs remain intact[24]. Along with AH, clinical dyspnoea is comprised of other distinguishable components including the sense of breathing work/effort (WE) and chest 'tightness'[43]. These components can vary based on interactions between

physiological, psychological, social and environmental factors[4]. The mechanisms underlying dyspnoea are complex with multiple voluntary and involuntary triggers as well as feed-forward and feed-back mechanisms[4]. Measuring breathlessness is difficult as the sensation of breathlessness is subjective and does not correlate well with objective measures of lung or heart function[272, 273]. The distinct components of clinical dyspnoea are thought to arise from separate neural pathways[43]. This theory comes from studies showing that despite complete paralysis of the respiratory muscles, subjects show the same AH stimulus-response to CO_2 and that increasing the tidal volume using a ventilator can relieve AH in C1-C2 quadriplegics, suggesting a vagal pathway rather than feedback from chest wall afferents[13, 22, 24]. For AH a corollary discharge of the drive to breathe from the brain stem has been proposed[274] whereas for WE a corollary discharge from the motor cortex driving voluntary breathing has been proposed as the source of the sensation[13].

The optimal solution for relief of dyspnoea is to treat the underlying pathology but this is not always possible and does not always lead to symptom relief. In chronic conditions, such as heart failure or COPD, symptom control becomes a priority in order to improve quality of life. A newer focus is to alter the perception of dyspnoea via altering the activity of neural signals sent to the brain reporting the prevailing level of breathing. The mechanism of action of nebulised furosemide has not been fully elucidated but current theory suggests that it acts by modulating pulmonary stretch receptor activity. There is evidence to support this theory, both in animal and human studies[30, 275].

The current study hypothesized that nebulised furosemide would relieve AH but not the sense of breathing effort. The result of this study have been published in a peer-reviewed journal[276]. t.

Specific Aims:

1. Determine the specific type of dyspnoea relief by nebulised furosemide
2. Assess the effect of intravenous furosemide on dyspnoea relief

4.2. METHODS

Sixteen healthy volunteers (9 male) attended the Oxford Brookes Cardiorespiratory Research Laboratory on 4 occasions. Eligibility criteria included; age above 18 years, no regular prescription medication in the previous 2 weeks and if female, not pregnant or planning pregnancy. Oxford Brookes University Research Ethics committee approved the protocol and all participants provided written informed consent.

All participants and all healthcare professionals apart from those who administered the interventions were blinded to the medications. Each participant visited the laboratory on 4 occasions; two practice sessions to familiarise themselves with the equipment and to become accustomed to rating the sensation of dyspnoea and; two 'test' sessions where participants nebulised the mists, with different dyspnoea stimuli (AH or WE) on different days in random order. On these days the participants were randomised to either inhale aerosolized mist (nebuliser; MicroAir U22 Omron, Milton Keynes, UK) in the order of furosemide (40 mg, 10 mg/ml; hameln pharmaceuticals gmbh, Langes Feld, Hameln, Germany), saline (4 ml; B.Braun, Melsungen, Germany), furosemide (FSF)

or saline, furosemide, saline (SFS) for both study days. Prior to each mist inhalation they gargled with a menthol mouthwash. The nebulisation duration of the furosemide mist was approximately 10-15 min and the saline mist 5-10 min. Each mist inhalation started after 6–11 min of the steady state test level of each pre-mist AH or WE test. The post mist steady state test level was between 9 and 14 min after the end of the mist inhalations. Each AH or WE test lasted 10 min, with a total visit duration of around 3 h (7 AH or WE tests, and 3 mist inhalations.)

4.2.1. Dyspnoea Stimuli

Two different dyspnoea stimuli were tested in each volunteer on different days. The air hunger (AH) and work/effort (WE) tests are described in detail in the methods section. In both situations the initial test was 'ramp' and this was followed by the 'steady state' protocol. Two 5 min steady state levels of end tidal CO_2 ($ETCO_2$) were chosen to target a level of 50% ('test' level) and 25% ('masking' level) of the visual analogue scale (VAS) for AH, Fig 4.1. This method has been shown to produce strong AH stimulation without any major WE sensation[21].

For the WE test, there were two 5min steady state levels of targeted ventilation, a 'test' level that generated 50% WE on the visual analogue scale and a 'masking' level generating 25%, Fig. 4.1. This stimulus was always limited by participants failing to meet a higher ventilation target and not because they reached the top of the VAS for WE. Normocapnia (mean ± SD: 41.9 ± 1.2 mmHg) was maintained throughout.

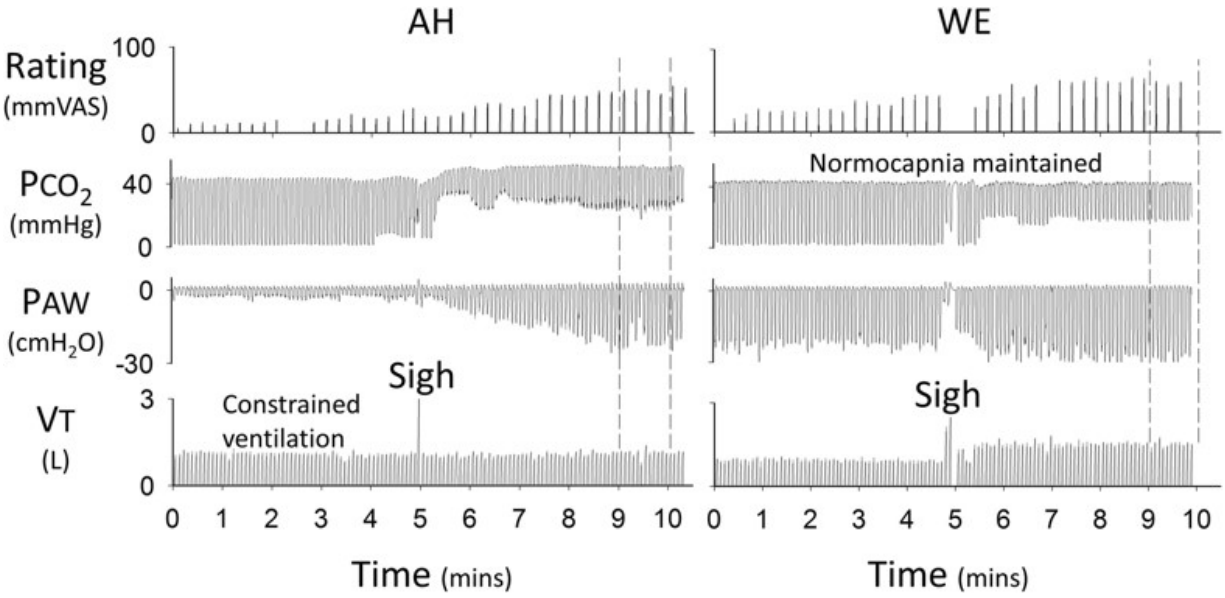


Figure 4.1 Standard tests of air hunger and work/effort

Left: Typical raw data set for the air hunger (AH) test during which two levels of end-tidal PCO_2 were imposed and ventilation was constrained. The vertical dashed lines indicate the steady state level of AH associated with the test level of CO_2 chosen to elicit 50%VAS ratings in pre-mist trials. Right: Typical raw data set for the work effort (WE) test in which two levels of targeted VT were imposed and normocapnia was maintained. The vertical dashed lines indicate the steady state level of WE associated with the test level of VT chosen to elicit 50% VAS ratings in pre-mist trials. During both tests ventilatory constraint or targeting was suspended briefly and participants were instructed to take a sigh. VAS ratings were provided every 15 s in response to a LED cue. VT Tidal volume, PAW continuous airway pressure measured at the mouth.

4.2.2. Intravenous infusions

During each inhalation period, participants also received a 15min (1 ml/min) intravenous infusion of 0.1 mg/ml solution furosemide if nebulised substance was 0.9% sodium chloride, or 0.9% sodium chloride if nebulised substance was furosemide (i.e. SFS infusions for FSF inhalations versus FSF infusions for SFS inhalations.) Infusions

were administered via a catheter inserted in the antecubital vein using a syringe driver (Graseby In-line Pressure Syringe Pump 3200, Graseby Medical Ltd., Ashford, UK). Furosemide (1.5mg) was infused over a period of time set to match the duration of inhalation. This dose was chosen as this was the amount expected to be systemically absorbed from a 40 mg nebuliser dose[277]. This ensured that both participants and the researchers did not know which mist was furosemide or 0.9% sodium chloride since the systemic effects (diuresis) were similar in each case. It also allowed an assessment of the systemic effect on furosemide on dyspnoea relief.

An overview of the protocol is shown in Fig. 4.2.

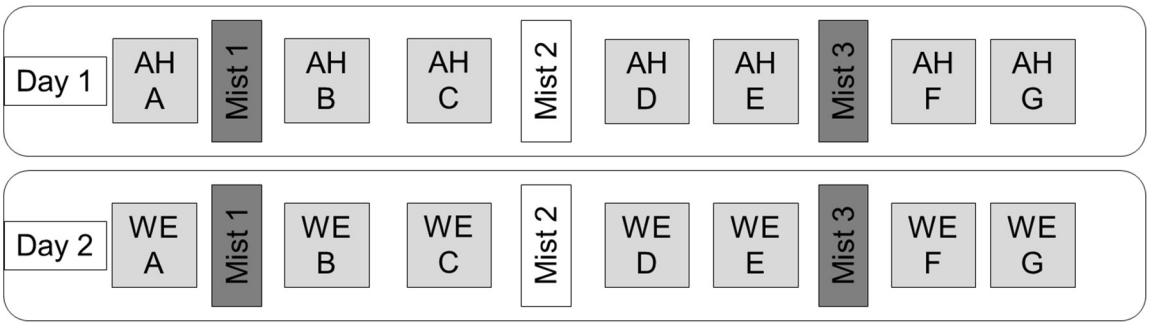


Figure 4.2 Schematic of protocol

Each participant attended on two 'test' days where they nebulised either the furosemide or the saline in the order of furosemide/saline/furosemide or in the order saline/furosemide/saline, with corresponding IV infusions on both 'test' days. On one day they performed the AH test and other day the WE test on 7 occasions, before and after mist inhalations.

4.2.3. Measurements

A 100 mm electronic VAS was used to obtain subjective ratings of both AH (during AH tests) and WE (during WE tests). Ratings were cued by a 'rate now' light every 15 s, which instructed participants to rate how much AH or WE they were feeling at that point in time, throughout the 10 mins of each AH and WE test, Fig. 4.3. In the practice sessions, participants were immediately asked to select descriptors from a set list for any of the sensations they felt during that test, and to rank the top 3. In future test sessions they were asked to focus on those descriptors that matched the AH sensation for the AH test and WE sensation for the WE test. The range covered 0 mm (no breathlessness) to 100 mm (tolerable limit) as previously described[21, 29, 239]. Additional word anchors ('slight', 'moderate' and 'severe') were placed at equal separation alongside the scale, which enabled participants to remember how much of the scale represented how much sensation from one occasion to the next. The order of test sessions (AH or WE) were randomly allocated and counterbalanced.

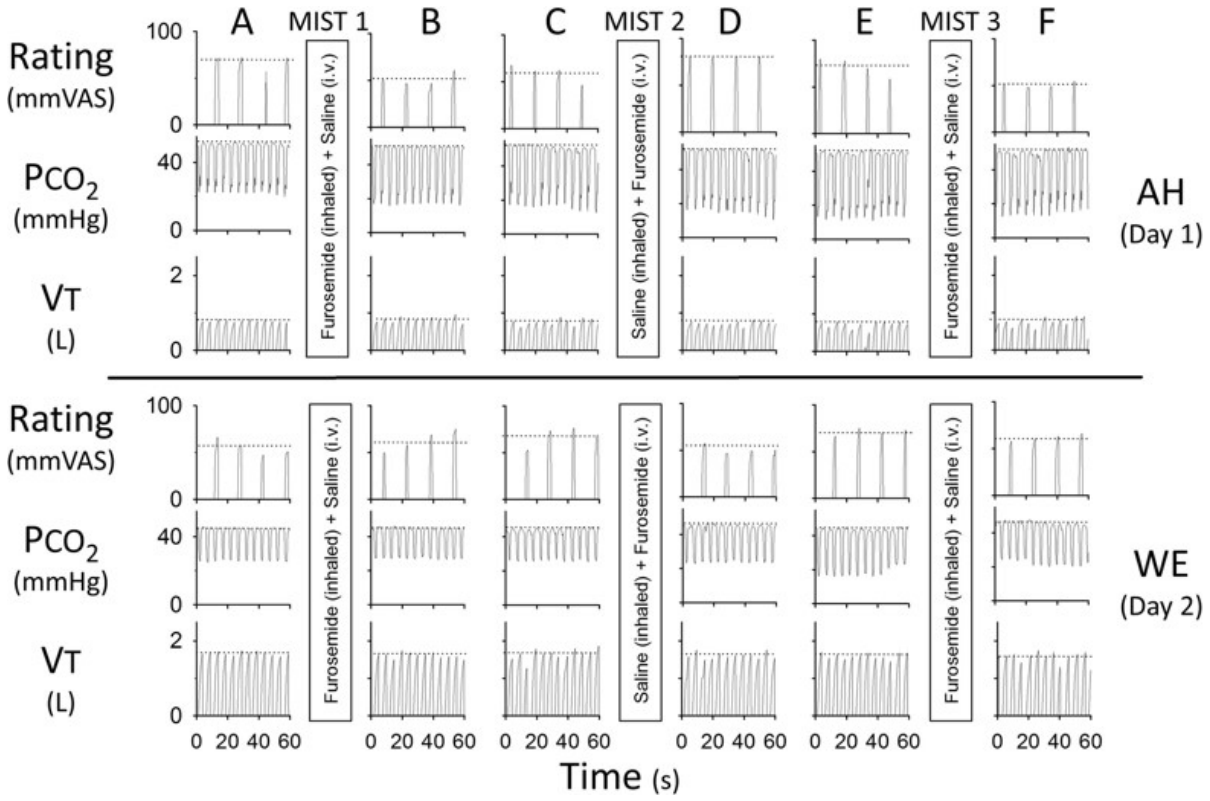


Figure 4.3 Effect of mist inhalations on steady state air hunger and work/effort

This dataset is from an individual who received the mists in the order of furosemide-saline-furosemide (FSF) with the corresponding saline-furosemide-saline (SFS) intravenous infusions on both days. Panels A to F show the last minute of each test level of end-tidal CO_2 (AH) or of VT (WE) –these regions of interest are shown by the vertical dashed lines in Figure 4.1. AH test day: Air hunger ratings were reduced after furosemide inhalation (A to B and E to F) but not after saline inhalation (C to D). WE test day: No obvious differences in ratings were evident before and after any mist inhalations

Participants voided prior to the start of each test session and the output was measured at approximately 25 min after each mist inhalation by urinating into a measuring flask.

AH = air hunger, WE = work effort

4.2.4. Data analysis

The visual analogue scale (VAS) in the last minute of each test step for the two furosemide mists presented for half the participants were averaged (those in the FSF group) and for the 2 saline mists presented for the other half (for those in the SFS group). The Linear Mixed Model "mixed" procedure of SAS 9.4 was used to analyse the data. Initially a full mean model with three factors; two levels of 'condition' (AH or WE), two levels of 'mist' (Furosemide or Saline), and 7 levels of 'time' (tests A-G; Fig. 4.2). All the 2-way and 3-way interactions were examined. Reducing the mean model by removing non-significant terms individually, resulted in the final model with 3 main effects and one interaction between condition-mist.

4.2.5. Sample size

In a preliminary study 10 healthy volunteers rated 13% lower AH on VAS with nebulised furosemide relative to nebulised saline[29], with a standard deviation of 16% resulting in an effect size of 0.81. Based on this observation, it was determined that 16 participants were required to detect an effect size of 0.81 using matched pairs t-test at 5% significance level and 86% power.

4.2.6. Randomisation

Participants were randomised to mist allocations after completing the two practice sessions. One of 17 recruited participants did not progress to randomization, Fig. 4.4; this was because despite increasing levels of hypercapnia (up to end tidal carbon dioxide partial pressure-$PETCO_2$ of 54 mmHg), they rated near zero dyspnoea and self-

terminated the test due to light-headedness during practice sessions. They denied any experience of dyspnoea during the test.

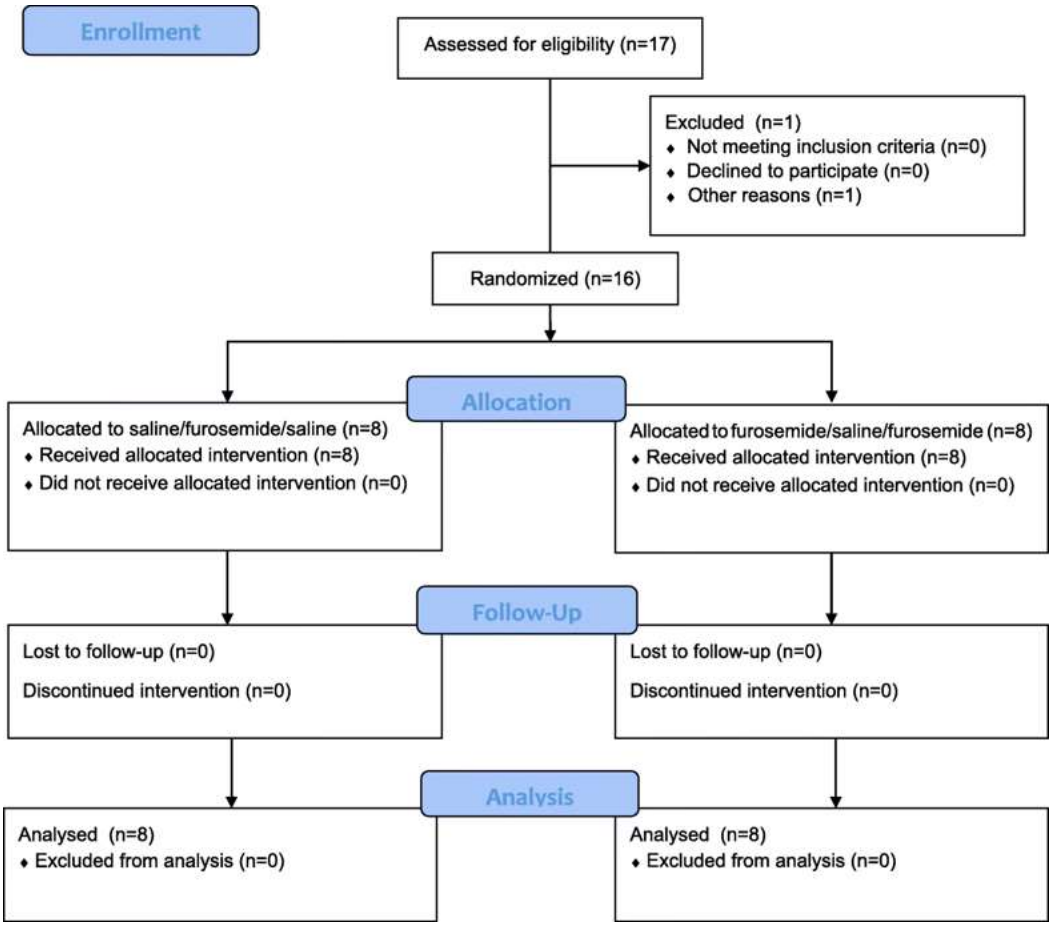


Figure 4.4 Participant flow diagram

The mist order allocation (FSF or SFS) was randomly assigned by the unblinded researcher to 16 sequential numbers, ensuring that 8 participants were allocated to the SFS group and 8 to the FSF group. A blinded researcher assigned each consecutive participant to the next available allocation number. Apart from the unblinded researcher, all other investigators and participants did not know whether the allocated number corresponded to FSF or SFS group. Once full analysis had been completed the principal investigator was provided with the allocation code.

Participants were provided with detailed written information about the interventions and protocol. They were aware they were going to receive furosemide or a placebo (control substance, saline) to inhale as a mist and to have as a solution via a vein in the arm (that was not the same as the mist) 3 times per visit. The diagram in the participant information leaflet showed the mist order as A, B, A.

4.3. RESULTS

Participants were recruited between 1st October 2015, with the first participant enrolled on 6th October 2015 and the last participant enrolled on 26th February 2016. The last visit for the last participant was on 11th March 2016. The median duration for all visits was 19 days.

The baseline characteristics of the participants who completed the study are shown in Table 4.1. The FSF mist order group and the SFS mist order group were well matched apart from by chance a higher proportion of participants who were Caucasian in the FSF compared to the SFS groups ($p = 0.031$). It is notable that 2 of the 3 (S9, S12) individuals who had an increase in AH (rather than a relief) following nebulised furosemide had a history of asthma. These two and S15 who also had a history of asthma were in the SFS group. No other notable differences were observed for individuals with a history of asthma. Almost 70% had some previous experience of breathing apparatus and this was driven by most of the participants having snorkelled before. One participant had used a mouthpiece before during a cardiopulmonary exercise test.

Table 4.1 Participant characteristics

	Total	**FSF**	**SFS**	***p* value**
Number	16	8	8	NS
Males: Female	9: 7	4: 4	5: 3	NS
Mean age, yr. (mean ± SD)	24.3 ± 3.7	23.6 ± 3.1	25 ± 4.3	NS
Caucasian: Non-Caucasian	11: 5	8: 0	3: 5	*0.031
Mean height, m (mean ± SD)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	NS
Mean weight, kg (mean ± SD)	79.5 ± 24	88.4 ± 28	70.6 ± 17	NS
History of Asthma	3	0	3	NS
Smoker/Ex-smoker: Never smoked	5: 11	3: 5	2: 6	NS
Previous experience with breathing apparatus	11	6	5	NS
Regular Sport: Sedentary	14: 2	7: 1	7: 1	NS

Abbreviations: FSF Mist order inhalation furosemide-saline-furosemide, *SFS* Mist order inhalation saline-furosemide-saline, *NS* not significant, *$p < 0.05$

Note: Experience of breathing apparatus included snorkelling, scuba diving or previous testing

4.3.1. Effect of mist inhalations on AH and WE.

A treatment effect (relief with nebulised furosemide relative to relief by nebulised saline) was seen with furosemide for the AH test, Fig. 4.5. Mean VAS for dyspnoea was significantly lowered by furosemide relative to saline inhalation (Difference of Least Squares Mean ± SE of − 9.7 ± 2.1%VAS) for the AH test ($p = 0.0015$, Tukey-Kramer adjusted), but was not significantly changed by furosemide relative to saline inhalation (+ 1.6%VAS ± 2.4SE) for the WE test ($p = 0.903$).

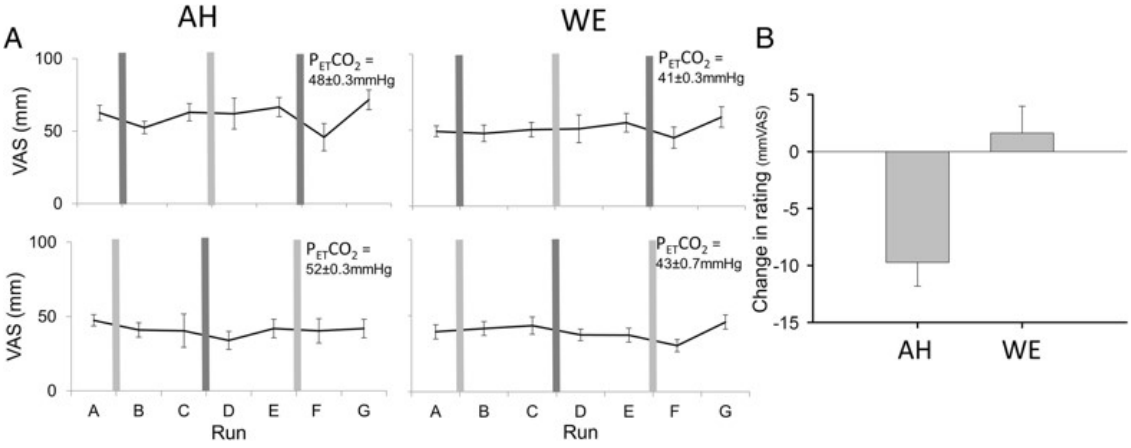


Figure 4.5 Change in AH and WE associated with mist inhalation

Panel A. Mean ± SEM AH (left panels) and WE (right panels) before and after furosemide inhalations (black bars) and before and after saline inhalations (grey bars) in the 8 individuals who were allocated to the saline-furosemide-saline order of mist inhalations (top panels) and in 8 individuals who were allocated to the furosemide-saline-furosemide order of mist inhalations (bottom panels). VAS ratings improved to a greater extent after furosemide compared to saline mist inhalations for AH, but this pattern was not evident for WE. Panel B. Least Squares Mean change in VAS ratings before and after nebulised furosemide relative to the change before and after nebulised saline for AH and WE. AH = air hunger, WE = work/effortETCO2 = end tidal CO2 (mean ± SD mmHg).

Four of the 16 participants showed a relief of over 20%VAS with nebulised furosemide for AH but no relief of this magnitude was seen in any participants for WE, Fig. 4.6.

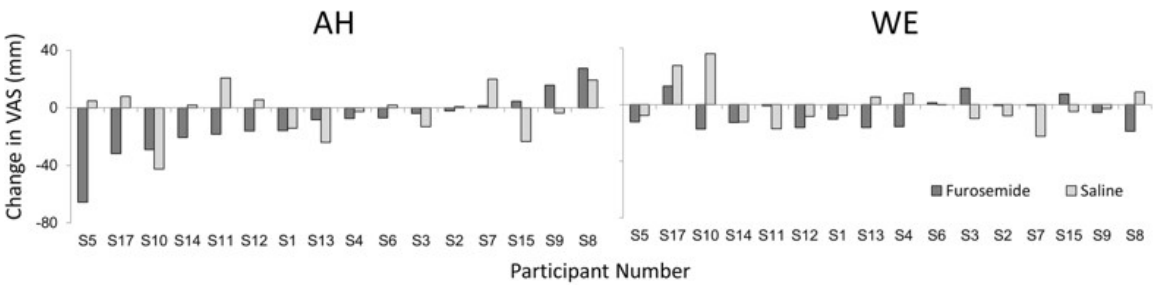


Figure 4.6 Individual data for change in VAS for AH and WE

Individual data for change in visual analogue scale for AH and WE. Individual change in VAS, % full scale, of AH at fixed test levels of $PETCO_2$ (left panel), and of the sense of breathing WE at fixed test levels of tidal volume (right panel) following nebulised furosemide (dark bars) and nebulised saline (grey bars). Closed bars indicate the average change in VAS for two furosemide inhalations in half the participants (S1, 2, 4, 7, 10, 11, 14, 17) or the change in VAS for one furosemide inhalation in the other half. Open bars indicate the average change in VAS for two saline inhalations in half the participants (S3, 5, 6, 8, 9, 12, 13, 15) or the change in VAS for one saline inhalation in the other half. Inhalation of furosemide tends to produce a reduction in VAS after furosemide more often than after saline for the AH test. For WE test reductions were evident for both nebulised furosemide and nebulised saline. Participants are arranged in order of response to furosemide for the AH test. AH = air hunger, WE = work/effort, VAS = visual analogue scale

4.3.2. Single versus two doses of furosemide

The average relief of AH from furosemide inhalations (averaged response for mists 1 and 3; 2x40mg) in the FSF group was greater than the relief seen with the single furosemide inhalation (mist 2; 1x40mg) in the SFS group; this group-wise comparison

did not achieve statistical significance (mean ± SD − 15.5 ± 12 versus − 6.6 ± 27%VAS, unpaired t-test with unequal variance; $p = 0.42$). However, within the FSF group, 7 of the 8 participants had substantially greater relief of AH after the second inhalation (mist 3) of furosemide compared to the first (mist 1) - a doubling of relief (mean ± SD − 10 ± 12 versus − 21 ± 13%VAS) which was highly significant (paired t-test, $p = 0.002$). In contrast, comparing the mean change for WE between the first and second doses of furosemide in the FSF group revealed no significant difference (paired t-test, $p = 0.41$). There were no significant differences between the first and second dose of saline within the SFS group for either AH or WE tests (paired t-test $p = 0.6$ and 0.3 respectively), Fig. 4.7. In addition, there was a strong correlation ($R^2 = 0.8$) between the amount of relief of AH with the first dose of furosemide and the subsequent amount of relief with the second dose of furosemide. This was not seen for saline, Fig. 4.8.

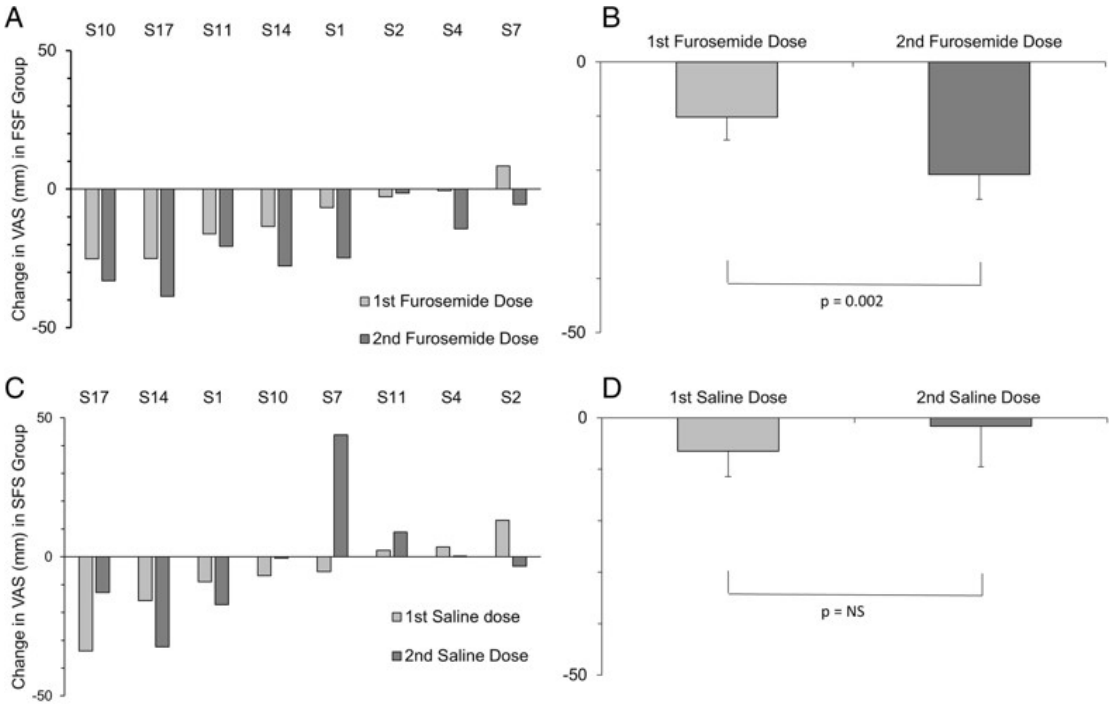


Figure 4.7 Second dose effect

Left panels (A and C): Individual (n = 8) changes in VAS ratings of AH in response to first and second doses of nebulised furosemide in the furosemide-saline-furosemide (FSF) group (A). Corresponding changes for the first and second doses of nebulised saline in the saline-furosemide-saline (SFS) group (C). For the FSF group the 2nd dose of furosemide had a greater reduction in AH relief than the first dose in all but one participant. This was not true for the 2nd dose of saline in the SFS group. Participants are arranged in order of response to first dose of furosemide for the FSF group or first dose of saline for SFS group. Right panels (B and D): The mean reduction in AH for the first and second dose of furosemide (B) and saline (D)

A B

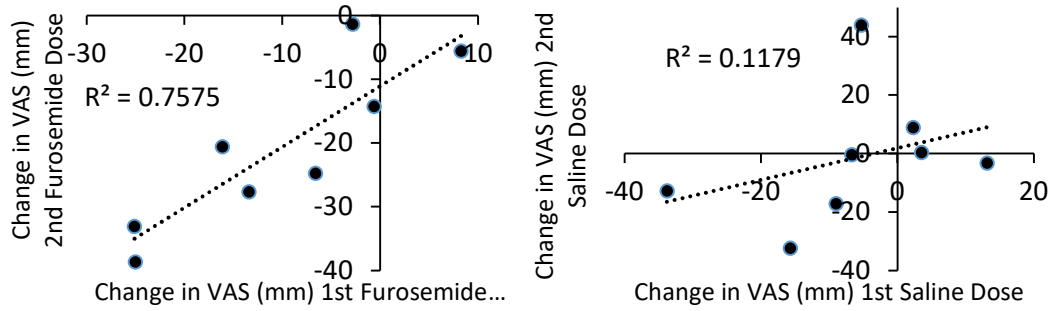


Figure 4.8 Correlation between response to 1st and 2nd doses

A) Strong correlation between dyspnoea relief with 1st and 2nd dose of nebulised furosemide. B) Weak correlation between 1st and 2nd dose saline.

4.3.3. Distinguishability of stimuli and blinding of participants

AH and WE stimuli were clearly distinguishable; subjective selection of descriptive phrases from a set list immediately following breathing tests verified that the AH test predominantly elicited phrases consistent with 'air hunger' whereas the WE test predominantly elicited phrases consistent with 'work/effort', Fig. 4.9. Choice of descriptors following AH and WE test showed a low level of conflation in sensation ratings, with 6% choosing WE descriptors for the AH test and 10% choosing AH descriptors for the WE test.

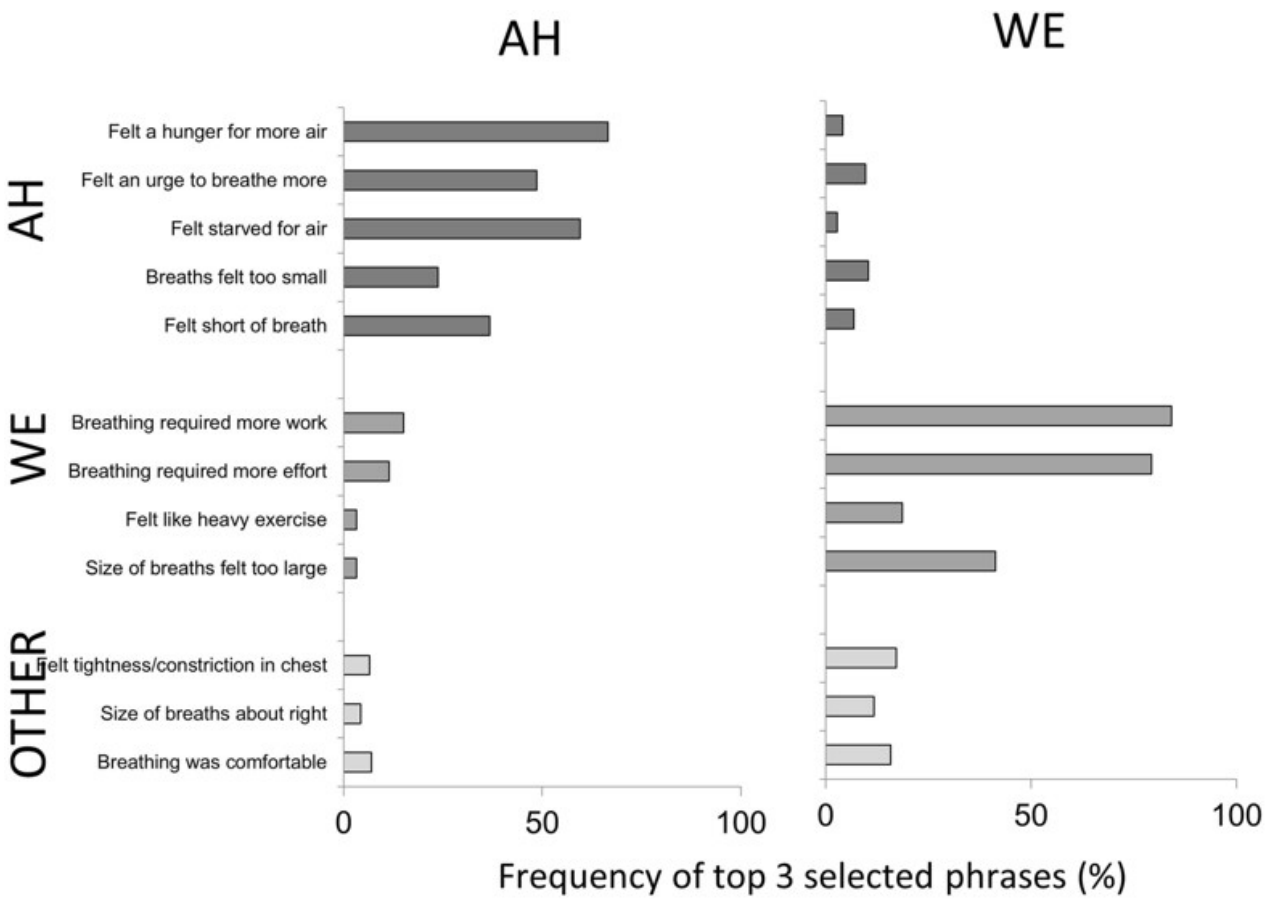


Figure 4.9 Dyspnoea Descriptors

Frequency with which each descriptive phrase was selected by participants to describe their experience during air hunger tests (AH; left panel) and WE tests (WE; right panel). AH cluster of descriptors dominated the participants' choice of the respiratory sensations felt during the AH tests while the WE cluster of descriptors dominated the participants' choice of the respiratory sensations felt during the WE tests.

Cumulative urine output was matched for FSF and SFS groups. There was no significant difference in the cumulative urine volume between participants in the FSF versus the SFS group with their concomitant intravenous infusions (mean ± SD, 1.6 ± 0.4 l versus 1.5 ± 0.5 l; $p = 0.4$). No other side effects related to the furosemide or saline inhalation were reported.

4.4. DISCUSSION

This study verifies that experimentally induced AH in healthy individuals is substantially relieved by nebulised furosemide compared to nebulised saline control. Furthermore, this study shows for the first time that this effect was specific for the AH component of dyspnoea rather than the sense of breathing effort.

4.4.1. Confirmation of AH relief by nebulised furosemide

The same stimulus to generate AH (hypercapnia with constrained ventilation) and the same dose of nebulised furosemide, delivered by the same method was used in the Moosavi et al. (2007) study, which had demonstrated a borderline treatment effect with nebulised furosemide relative to saline inhalation[29]. This study was powered to support a definitive outcome and had 16 participants compared to 10 in the original study. It showed a significant treatment effect with nebulised furosemide compared to nebulised saline for dyspnoea relief, specific to AH.

In contrast to the current findings, Banzett et al. (2017) have recently published a study using similar methods in 11 healthy volunteers indicating no significant difference between relief of breathing discomfort by nebulised furosemide and by nebulised saline[190]. The effect of nebulised furosemide reported by Banzett et al.[190] was greater than in this study (mean ± SE: − 17 ± 3 versus − 11 ± 5 %VAS); this is likely to reflect the fact that they used a higher dose (80 mg versus 40 mg) with controlled delivery on a mechanical ventilator. However, the effect of saline was far greater in the study by Banzett et al.[190] compared to this study (mean ± SE: − 13 ± 4 versus − 2.5 ± 4 %VAS); this

is likely to be due to an enhanced placebo effect in their study as participants were informed they were going to receive 2 active treatments and one saline, whereas in reality they had one active substance and two saline controls. Likewise, a second study published by this group[185] also reported a significant effect of nebulised saline (− 20 %VAS); this study also used a similar deception to that alluded above which is again likely to have enhanced the placebo effect. While this second study used the same dose (40 mg) to this current study they also reported a larger relief of 'breathing discomfort' with furosemide (− 20%VAS). This could be explained by the different delivery method used, which reduced loss of aerosol to the atmosphere during expiration[185]. The different delivery method they used reduces loss of aerosol to the atmosphere during expiration and also assuming a similar absorption efficiency (both studies in healthy volunteers) could have led to a greater and more prolonged interaction of furosemide with lung stretch receptors thereby accounting for a greater relief.

4.4.2. Specificity of relief

The relief of experimentally induced dyspnoea in healthy individuals by nebulised furosemide was first shown by Nishino et al. (2000) who induced dyspnoea by i) a combination of inspiratory resistive load and hypercapnia, and ii) breath-holding[27]. The first of these stimuli was likely to have induced both WE and AH components of dyspnoea. Since participants were instructed to rate respiratory discomfort, both of these sensations could have contributed to their ratings. The breath hold task may have generated AH specifically but breath-holding is a non-steady state. A subsequent

study in healthy individuals which specifically focused on experimentally induced steady state AH generated similar levels of relief[29]. This suggests that the nebulised furosemide may well have specifically relieved the AH component in the stimuli used by Nishino et al.[27].

In contrast, there is direct evidence that nebulised furosemide does not affect the sensations associated with respiratory effort during expiratory flow limited exercise[192] or during exercise in the presence of external thoracic restriction in healthy individuals[189]. External thoracic restriction during exercise will elicit both AH and WE component of dyspnoea[189, 278]. In the study by Waskiw-Ford et al. (2018) individuals were asked to rate the intensity and unpleasantness of their perceived dyspnoea without specifying which component of dyspnoea to focus on[189]; it could be that the reported lack of relief was because of the participants focusing on WE due to the increased metabolic demand in this situation. Breathing effort is assumed to arise from non-vagal afferents from the chest wall, though a role for vagal afferents from the lungs in the sense of breathing effort cannot currently be discounted. Nebulised furosemide does appear to confer some benefit to exercising COPD patients but clinical dyspnoea in this scenario is likely to be multifactorial and may not be specifically related to respiratory effort[28]. It is generally accepted that AH and WE components of clinical dyspnoea likely arise from different neural pathways[43]. Previous reports have provided evidence that the AH component of dyspnoea is relieved by increased vagal afferent input from the lungs[24, 33, 36, 37] or accentuated by absence of vagal afferents from the lungs[279]. It is not known whether the vagal afferent information has any role

in the sense of breathing effort - our data would suggest that vagal afferents (and indirectly, stretch receptors) have no role in generation of WE, but are involved in AH. This also fits with the theory that work/effort sensation of dyspnoea arises from non-vagal afferents from the chest wall.

Nebulised furosemide demonstrated a statistically significant treatment effect that reached the accepted level for the minimally important clinical difference (MCID) for AH but not for WE[280]. There was some evidence of a placebo effect with, on average, a slight reduction in AH with nebulised saline. Some studies have shown a substantial relief of laboratory-induced dyspnoea with saline in over 30% of participants[185, 190]. However, O'Donnell and colleagues found that overall the administration of aerosol saline had little effect on experimentally induced AH, provided the expectation of a treatment effect is minimized[281]. In the current study, the careful blinding procedures and instructions participants received ensured that they were unable to guess correctly when they had received the active or placebo substance.

This is the first study to compare the effect of nebulised furosemide and nebulised saline on AH and WE induced separately in the same individuals allowing a direct comparison of treatment effects. Our data confirms that the mechanism of dyspnoea relief by nebulised furosemide, presumed to be via modulation of vagal afferents from the lungs, specifically relates to AH and not WE.

4.4.3. Action of nebulised furosemide in the lungs

Nebulised furosemide is known to have many beneficial effects all of which appear to be mediated by actions on the airway epithelium such as; improvements in exercise induced asthma [282], inhibition of cough in asthmatics and healthy volunteers[156, 158, 170], and induced bronchodilation in constant-load exercise testing in COPD[173].

The most likely explanation for relief of dyspnoea with nebulised furosemide is modulation of lung mechanoreceptor feedback which replicates the sensation of larger tidal volumes; thus experimentally induced AH is relieved when tidal volume is increased[24, 283]. This is further supported by recent studies that have reported a weak to moderate correlation between the extent of dyspnoea relief with increased tidal volumes (whilst free breathing) on the one hand, and relief by nebulised furosemide on the other hand[185, 190]. Thus, nebulised furosemide may be acting at least in part via the same pathway.

There are many different mechanoreceptors in the lung including; slowly adapting pulmonary stretch receptors (saPSR), rapidly adapting pulmonary stretch receptors (raPSR), pulmonary and bronchial C-fibre receptors (irritant receptors). These receptors collectively transmit information to the central nervous system reporting the tidal volume or the presence of airway irritants[284]. Exposure of anaesthetised rats to nebulised furosemide has demonstrated sensitization of saPSRs and desensitization of raPSRs[30]. Evidence points to the saPSRs being the most likely mechanoreceptor involved since the raPSRs could not signal maintained volume changes as they provide feedback relating more to transition between inspiration and expiration rather than

the magnitude of lung stretch[284, 285]. Studies in humans have suggested that it is feedback concerning the overall ventilation rather than intra-breath variables that account for the level of breathlessness perception[15, 286].

The diuretic effect of furosemide occurs via its chloride channel blocking property affecting the sodium-potassium-chloride co-transporter in the loop of Henle[271]. Because the same membrane co-transporters are expressed on vagal sensory neurons present in the airways[287], it is possible that the modulation of pulmonary stretch receptor sensitivity by nebulised furosemide may occur by the same mechanism. In vitro studies of isolated human lung tissue are needed to verify the precise mechanism of action of nebulised furosemide on pulmonary stretch receptors.

4.4.4. No evidence for systemic action for dyspnoea relief with nebulised furosemide.

A potential alternative mechanism of action of furosemide in relief of dyspnoea is via systemic effects from absorption of the nebulised furosemide into the circulation. Morélot-Panzini et al. (2018) estimated an absorption efficiency of up to 30% of the nebulised dose[185]. From this information and assuming the maximal level of efficiency, gives an estimate that a 40 mg nebulised dose would result in 5 mg entering the systemic circulation assuming a respiratory frequency of 12 breaths per minute and a duty cycle of 0.4. This appears to be higher than our estimate of systemic load of 1.5 mg from inhalation of a nebulised dose of 40 mg in this study. They also found that when given 15 mg intravenously participants had an average 16%VAS improvement in dyspnoea. It is suggested that in heart failure systemic furosemide relieves dyspnoea

by easing pulmonary congestion and thereby reducing activation of pulmonary C-fibre receptors[288, 289]. This cannot explain relief of dyspnoea by intravenous furosemide in healthy volunteers with no pulmonary congestion. The authors suggest that this result could be explained by the placebo effect as the participants were informed that they would only receive active substances. In the current study a dose of intravenous furosemide (1.5 mg) that more closely matched the amount absorbed from the nebulised dose was infused concurrently with nebulised saline. To maintain blinding, intravenous saline was infused during furosemide inhalation. In both cases the rate of infusion was set to match the period of inhalation. We therefore consider the findings of the current study showing no significant relief from intravenous furosemide (mean ± SEM, − 2.5% ± 4) to demonstrate more clearly that the AH relief by nebulised furosemide is via direct actions within the lungs.

Further support for a mechanism of relief of AH via direct actions in the lungs is provided by: i) direct exposure of the lung tissue to furosemide in rat preparations resulted in modulation of PSR afferent activity but not when administered intravenously[30] ii) other studies in which beneficial effects of furosemide have been evident only when inhaled rather than administered via tablet[158] iii) absence of haemodynamic changes with nebulised furosemide in a study assessing wedge pressure measurements in heart failure patients suggesting no systemic mechanism of action[290] iv) absence of any detectable difference in cumulative urine output between the two groups in this study (SFS and FSF) which discounts a mechanism of action related to diuresis.

4.4.5. Suggestion of 'second dose' effect

This study shows a beneficial cumulative effect of repeated furosemide inhalations (2x40mg). This is the only study that has to our knowledge investigated the effect of a second dose of nebulised furosemide on experimentally induced AH in healthy individuals. We noted a significant reduction in AH ratings with the second dose of furosemide in those who had the mists in the order FSF (mean ± SEM -10.2 ± 4.2 versus -20.8 ± 4.6%VAS). This was not seen for saline in those who had SFS so it is unlikely to be an order effect (mean ± SEM -6.5 ± 5.0 versus -1.6 ± 7.9). It is possible that the first dose of furosemide sensitizes the receptors so that the second dose has an additive/cumulative effect. Another possibility is from a carry-over effect where the nebulised furosemide is still active in the lungs for up to at least an hour after the first inhalation. Supporting this theory is Morélot-Panzini et al. (2018) study reporting that the rate of systemic absorption of nebulised furosemide is inversely related to the extent of dyspnoea relief[185]. This suggests that when the furosemide remains in the lungs, in contact with the pulmonary stretch receptors for a longer duration, the action of furosemide on dyspnoea relief is increased. An enhanced 'second dose' effect due to the pharmacokinetics is a recognised phenomenon in psychopharmocology[291].

The question remains whether the 'second dose' effect is related to sensitisation of stretch receptor-furosemide interaction or a carry-over effect from the first dose of furosemide due to incomplete removal of furosemide from the lungs before the second dose. In the current study the time between first furosemide mist and the second was approximately 90 min. If we accept that on average the furosemide stays

within the lungs for up to 1 h based on the effect of a single dose on dyspnoea relief[29] the length of time between the first and second dose would go against a carryover effect to explain the greater relief with the second dose. This is further supported by the fact that in the SFS group the time between the middle furosemide mist and the second saline mist was less than 1 h and the pre-second saline mist AH remained below the pre-first saline mist level (consistent with a carryover effect of the middle furosemide mist). We would therefore favour a sensitisation explanation to account for the bigger relief from the second dose of furosemide in the FSF group. This is also supported by the strong correlation between the air hunger relief with the first dose and second dose of furosemide which suggests it is related to an individual's receptor sensitivity to furosemide, and not due to a carryover effect. The clinical ramifications of the second dose effect, if due to sensitisation, may affect the dosing regimen. For example, prescribing nebulised furosemide twice a day and taking the medication regularly, rather than 'as required.'

4.4.6. Technical considerations

It was difficult to achieve a target level of 50% VAS with the WE test compared to the AH test. This resulted in the average VAS recordings for WE being lower (40-50mmVAS) compared to the AH average VAS recordings (50-60 mm VAS). A higher resistance in the circuit may have enabled both sensations to be studied at more comparable levels of the VAS. Though unlikely, we cannot discount the possibility that nebulised furosemide would not have been effective in relieving WE if the WE test had also been performed with a target level above 50% VAS.

It was noted that the time taken to nebulise 4 ml saline to 4 ml of furosemide differed (duration of saline mist was approximately 5-10 min and the furosemide approximately 10-15 min), and this was also reported independently in another recent study[185]. In the current study, the unblinded researcher added saline or pretended to add a solution to the nebuliser to ensure the time taken to nebulise either solution was equal thereby maintaining blinding.

The participant selection of descriptive phrases after each breathing test confirmed that the different stimuli elicited the required sensations and that the participants were able to distinguish the different forms of dyspnoea (AH vs WE). For the AH tests participants were instructed to focus on and rate the form of dyspnoea indicated by the phrases they had previously selected following the initial exposure to the AH stimulus (during practice sessions). If the participant reported other sensations such as 'breathing required more work' during the AH tests, they were coached not to include this sensation in their ratings and to report them after each trial if present. For the WE tests the participants were instructed to focus on and rate the form of breathlessness indicated by the phrases they had previously selected following the initial exposure to the WE stimulus (during practice sessions). If the participant reported other sensations such as the AH descriptors, they were coached not to include this sensation in their ratings but to report them after each trial if present. After completing each trial, the participant described their sensations and picked phrases from a given list of descriptive phrases and identified the top 3 most relevant. Subject selections following

the AH and WE tests were consistent with the type of stimulus. Participants were also queried about any non-respiratory sensations or external clues.

The participants and investigators were successfully blinded to the study drugs and neither were able to correctly identify the correct order of mist inhalation. There was no detectable taste difference detected by the participants.

4.4.7. Applicability of conclusions

This study was performed in a narrow age range (20-28 years). It is therefore not known whether the same results will apply to older population. It is possible that the sensitivity of PSRs alters with increasing age or is affected by lung/heart disease. Most patients with chronic dyspnoea will be much older than these study participants.

4.4.8. Validity of conclusions

The test level of end tidal CO_2 ($ETCO_2$), the level chosen to generate 50% VAS full scale for AH at baseline, was different in the FSF group compared to the SFS group (48 ± 0.4 mmHg vs 52 ± 0.3 mmHg). We do not believe that this affects our data but it is interesting to consider why. A post prandial rise in $ETCO_2$ has been demonstrated[292] but in our study there was no difference in consumption between the groups. They were also tested over both morning and afternoon sessions (FSF group: 3 in the morning, 5 in the afternoon. SFS group: 4 in the morning, 4 in the afternoon). There were no significant sex differences between the groups or differences in smoking habit. By chance there was an uneven distribution of ethnicity among the SFS and FSF groups ($p = 0.031$). All participants in the FSF group were Caucasian whereas the SFS group

were not (Caucasian =3, Others = 5). There is some suggestion in the literature that the level of dyspnoea is associated with ethnicity[293]. This may explain some of the differences seen in this study. There was also a trend for increased weight (88 kg vs 70 kg) and for playing a wind instrument (3 vs 1) in the FSF group. In the SFS group more participants had a history of asthma (3 vs 0.) Although these were not statistically significant some of these differences in characteristics may explain the different $ETCO_2$ levels in each group.

4.4.9. Limitations

Ventilation, tidal volume and inspiratory reserve volumes were targeted at substantially different levels to generate AH and WE rated at approximately 50% on the VAS (9 vs 17 L/min; 0.75 vs 1.6 L, 1.8 vs 1.0 L respectively). For both AH and WE the levels of these variables were well matched before and after mist inhalations. However, the frequency of vagal feedback from PSRs will have been at a higher level for WE compared to AH test. We cannot therefore discount the possibility that had the WE test been done at the same level of afferent feedback from PSRs that the nebulised furosemide would have relieved WE as well. As discussed above (specificity of relief section) there is a lack of evidence for the role of vagal afferent feedback from PSRs in WE modulation. Furthermore, from a practical viewpoint it would have been very difficult to strictly control the ventilatory parameters between AH and WE tests while maintaining a clear distinction in the quality of the dyspnoea generated; and a far greater resistive load would have been required to generate 50% full scale on the VAS for WE.

In the VAS ratings of AH and WE, participants were not specifically asked to rate intensity or unpleasantness and it is likely that they rated a combination of both of these. From this study we cannot say which component was more predominant, however from previous studies we know that AH is more unpleasant than WE[200].

Since instructions prior to intervention could influence the outcome (e.g. amplify the placebo effects) we asked participants at the end of the study which order they thought they received the active and placebo substances. They were either unsure or chose an order that was not feasible (e.g. thought they received FFS or SSF or FSS etc.) We do not have any evidence that the small placebo effect we observed in this study arose from biasing the participant expectations through the instructions given prior to the start of the protocol.

4.5. CONCLUSIONS

Nebulised furosemide was effective at relieving the AH component of dyspnoea but not the WE component. This is consistent with a mechanism involving sensitization of slowly adapting pulmonary stretch receptors leading to dyspnoea relief that specifically applies to the AH component, the most unpleasant form of dyspnoea. We suggest that multi-dimensional dyspnoea assessment tools should be used to identify patients where AH predominates the symptom burden and future clinical studies with nebulised furosemide should target these patients, irrespective of their underlying pathology, to optimise dyspnoea relief.

5. PILOT STUDY OF NEBULISED FUROSEMIDE FOR DYSPNOEA RELIEF IN ADVANCED HEART FAILURE

5.1. INTRODUCTION

Nebulised furosemide has been used for over 20 years in research (see Introduction chapter for details) but its use has not been transferred to clinical practice. More recently the option of using nebulised furosemide for dyspnoea relief has emerged due to a lack of alternative safe and effective treatments[294]. However, the variability of response to nebulised furosemide for dyspnoea relief has prohibited its use as a viable treatment option; some participants experience a large relief in dyspnoea and others a small or no response[185, 275, 295]. This pilot study seeks to explore this variability in order to inform future fully powered clinical trials. The study also aims to see if it is feasible in heart failure patients to perform the air hunger sensitivity test (see chapter 2-methods) that has never been trialled in this patient cohort.

There have been only two studies using nebulised furosemide in patients with heart failure and these were focused either on the haemodynamic response[183, 295] or the diuretic effects of systemic absorption of furosemide from the lungs[182]. In the current study, the focus is on optimising dyspnoea relief with nebulised furosemide through manipulation of breathing patterns of inhalation, determining the best method for eliciting dyspnoea to test the intervention and for quantifying dyspnoea in patients with heart failure.

Specific aims included:

a) To assess the optimal dyspnoea measurement tool in heart failure (VAS, MBS, D12)

Quantifying dyspnoea can be challenging with a variety of tools available, as described in Chapter 2-Methods[105]. Simple scales include the Visual Analogue Scale (VAS) and Modified Borg Scale (MBS), whereas more detailed multi-dimensional information regarding dyspnoea can be gathered from questionnaires such as the Multidimensional Dyspnoea Profile (MDP) and Dyspnoea12 (D12), which include assessment of emotional content[6]. Patients with heart failure were included in the validation and development of the D12, but the D12 has not previously been used in heart failure clinical trials, whereas the MBS and VAS have both been used[126]. Minnesota Living with Heart Failure Questionnaire (MLHFQ) is a measure of quality of life in heart failure and includes a statement on shortness of breath with some reference to activity levels. This sub-study therefore; i) addressed concurrent validity of D12, VAS and MBS in assessment of hypercapnia induced dyspnoea and exertional dyspnoea, and ii) explored the relative sensitivity of these dyspnoea measurements for detecting change with nebulised furosemide.

b) Determine the optimal breathing frequency during nebulised furosemide

Breathing pattern of inhalation (fast and slow) may affect region of deposition and extent of systemic absorption potentially accounting for some of the variability previously noted[28, 29, 177, 192]. A couple of recent studies have attempted controlled delivery of furosemide via volume controlled non-invasive mechanical ventilation; however, the focus there was to maximise the amount of drug delivered, rather than

to manipulate the site of deposition[185, 295]. The aim here was to manipulate breathing frequency to determine the optimal pattern for drug administration. It was hypothesised that inhaling the mist with different breathing patterns would alter the deposition within the lungs[296]; slow (deeper) breaths would result in the particles depositing deeper within the airways compared to faster (shallow) breaths where they would deposit more in the upper airways. Given that the pulmonary stretch receptors are located deep within the airways it was hypothesised that the slow deep delivery method would result in greater relief with nebulised furosemide compared to the fast shallow breaths. An alternative method to achieve this would have been to alter the particle size[297].

c) Feasibility of Cardiopulmonary Exercise Testing and air hunger tests in assessing nebulised furosemide for dyspnoea relief

In clinical cardiology, Cardiopulmonary Exercise Testing (CPET) was first used in 1985 to evaluate the degree of heart failure[211] but it is not commonly used to assess the effect of an intervention. For this purpose, 6-Minute Walk Test (6MWT) is used more frequently, due to ease of use and lack of requirement for specialist training and equipment. Thus, there is a lack of data on the utility of CPET in patients with advanced heart failure. New York Heart Association (NYHA) class III and IV in particular are often excluded from clinical trials on the assumption that they are unable to exercise enough to generate meaningful data. There is mounting evidence supporting greater use of CPET clinically and its use in research is increasing.[298-300] CPET is included in this study to review if the parameters assessed during CPET are able to determine if the intervention is having a significant effect, and to compare it to the

6MWT. CPET requires more training and more complex equipment but multiple clear-cut endpoints are possible, compared to the 6MWT. In comparison to the 6MWT, it is also possible to use parameters gained during CPET to provide useful information despite a suboptimal effort during the test. Cardiac pathology is indicated by a normal breathing reserve, low oxygen consumption ($\dot{V}O_2$) at anaerobic threshold (<40% of predicted), flattening oxygen pulse, high minute ventilation/carbon dioxide production ratio ($\dot{V}E/\dot{V}CO_2$) slope and low oxygen uptake efficiency slope (OUES)[216, 301]. This data can be obtained from a low workload incremental test lasting 6-12 minutes. Testing hypercapnic air hunger sensitivity has never been trialled in heart failure patients previously. It was unknown as to whether patients with heart failure who are already experiencing dyspnoea would be able to perform this test. The aim here was to perform CPET and air hunger sensitivity tests to elicit dyspnoea and assess the response to nebulised furosemide.

5.2. METHODS

5.2.1. Patient characteristics

Twelve patients diagnosed with NYHA class III or IV attending the heart failure clinic at John Radcliffe Hospital or those under the care of the community heart failure nurses in Oxfordshire participated in the study (Table 5.1). The cause for their heart failure was either ischaemic or dilated cardiomyopathy.

Table 5.1 Patient Characteristics.

*HR = heart rate, BP = blood pressure, JVP =jugular venous pressure, NYHA = New York Heart Association. * Prior breathing experience includes scuba diving/snorkelling, playing a wind instrument, singing in a choir regularly, previous similar breathing tests or spirometry.*

Age (mean, median, range), years	77, 82, 48-90
Gender (Male: Female)	8:4
Ethnicity	All Caucasian
Height (cm, mean±sd)	169±12
Weight (kg, mean±sd)	80±25
HR (beats/min, mean±sd)	71±13
BP (systolic/diastolic, mmHg, mean±sd)	127±23/69±14
JVP (normal: raised)	12:0
Peripheral oedema: no/yes	10:2
Never smoked/Ex-smoker	7:5
Prior breathing experience * no/yes	8:4
NYHA class (III:IV)	12:0

The inclusion criteria were patients aged 18 years or above with no upper age limit, treated with oral furosemide and clinically stable in the previous 3 months (no hospital admissions for heart failure). The main exclusion criteria were a history of allergic reaction to furosemide (rare reports of hypersensitivity including anaphylaxis due to the sulfonamide group have been reported), symptomatic postural hypotension, renal (eGFR <15) or hepatic impairment, hypo- or hyperkalaemia (<3.0 or >5.9mmol/L) or

hypo- or hypernatraemia (<130 or >150mmol/L) over the preceding month, or co-existent history of lung disease or nasal polyps.

This research study was reviewed and approved by South Central - Oxford C Ethics Committee (NHS REC reference 15/SC/0480) and complied with the Declaration of Helsinki. All participants provided written informed consent.

5.2.2. Dyspnoea measurements

The dyspnoea measurement tools VAS, MBS and D12 are described in more detail in Chapter 2-Methods. The D12 was used in this study to gain insight into both the physical and emotional aspects of dyspnoea in patients with heart failure. The only other dyspnoea questionnaire that also offers this is the multi-dimensional dyspnoea questionnaire (MDP). The MDP is more labour intensive to fill in and requires a trained personnel to administer it with the patient. The D12 can be completed by the patient alone and takes a couple of minutes to complete. The D12 has not been used in this patient population before and its comparison to the VAS and MBS is unknown in this group. Patients provided discrete ratings of air hunger on a word-labelled electronic VAS (0-100mm) every 15 secs throughout the hypercapnic air hunger breathing tests, cued by an LED. The output of the VAS was recorded by an analogue to digital converter (Micro1401, Cambridge Electronic Design, UK) and stored for off-line analysis using Spike 2 software (Version 6.18, Cambridge Electronic Design, UK). The D12 was administered immediately after each exercise and air hunger (AH) test, with the instruction to score their dyspnoea for how they were feeling at the point of termination of the test. A horizontal paper VAS and vertical MBS were administered

immediately before, immediately after, 1 minute post, and 2 minutes post exercise; patients were instructed to focus on how they were feeling at that instant in time. The D12 ('these days') was also administered at the start of each visit, along with the MLHFQ for how they were feeling over the previous week. Scoring of the D12 and MLHFQ are described in Chapter 2 – Methods.

5.2.3. Assessment of optimal breathing pattern

During nebulisation patients targeted a fast or a slow breathing pattern, by breathing in time to a metronome, set at 20-30% below or above their baseline rate. The achieved breathing patterns were recorded using respiratory induction plethysmography (Respitrace, Ambulatory Monitoring Inc, New York, USA). This incorporated elasticated bands comfortably strapped around the chest and abdomen. Insulated wires stitched into the bands were stretched by displacement of the chest and abdomen during breathing, thereby changing the inductance of the bands. The sum of these two displacements were calibrated by the patients breathing in and out of a bag with a known volume. The gain of the signal from the chest band was set at twice the gain of the abdominal band in order to optimally reflect tidal volume changes[302]. From the Respitrace sum signal the inspired $T\dot{V}$, Ti, Te and Ttot and respiratory frequencies were measured on a breath-by-breath basis throughout nebulisation and these were averaged for all breaths in the first minute, middle minute, and final minute of the nebulisation period. The nebulisation period ranged between 10-20 minutes for all of the solution to be aerosolised.

5.2.4. Using hypercapnic air hunger and exercise to assess dyspnoea

Air hunger

The air hunger (AH) test involved hypercapnia with constrained ventilation. Tidal volume was fixed by limiting flow into a 3 litre anaesthetic bag (held constant at a level that matched their normal resting ventilation) and fixed respiratory frequency to their baseline rate, by breathing in time to a metronome. The test is explained in detail in Chapter 2 - Methods. In this study a 'ramp' protocol was initially performed which involved a step increase in inspired CO_2 every minute until patients reached the top of the VAS (maximum they are willing to tolerate). This was followed by presenting a list of standard descriptors from which patients selected the best three descriptors of how they were feeling. This ensured the patients were identifying AH as the predominant sensation elicited. The ramp was then repeated with the patients instructed to focus on the AH descriptors only in their VAS ratings. Additional word anchors ('slight', 'moderate' and 'severe') were placed at equal separation alongside the scale, which enabled patients to remember how much of the scale represented how much sensation from one occasion to the next. A steady state protocol was then performed with CO_2 manipulated to give up to 5 minutes of a fixed level of end tidal CO_2 ($P_{ET}CO_2$), chosen to target a level of 50% ('test' level) of the VAS for AH based on the ramp data. The steady state protocol was repeated after the nebulisation.

Exercise

Half the patients were randomly allocated to perform the 6MWT while the other half performed CPET. Details of the 6MWT is described in detail in Chapter 2. CPET was performed on a seated stationary electromagnetically braked cycle ergometer, with patients wearing a facemask to enable breath-by-breath analysis of respiratory gases. Patients were instructed to keep pedal rate at 55-65rpm and underwent a 'ramp' protocol, depending on their self-reported baseline fitness level. They cycled continuously until they were at the maximum level they were willing to do or were unable to keep up with the required pedal rate. Electrocardiogram (ECG) and blood pressure (BP) were recorded for safety monitoring. See Chapter 2 for more details.

5.2.5. Study protocol and equipment

This was a randomised double-blind placebo-controlled crossover pilot study. Twelve patients with advanced heart failure attended the Cardiovascular Clinical Research Facility at the John Radcliffe Hospital on four visits over a minimum of 21 days and a maximum of 53 days. Patients followed the same protocol at each visit. On the first visit, they were randomised to either CPET or 6MWT and they continued with this allocation for all visits. This randomisation was to assess if patients with heart failure in Class III and IV were able to generate useful data during CPET and to assess which test may be more sensitive in detecting a change with an intervention (nebulised furosemide). Patients were also randomised to the order in which they received the mist and the breathing pattern for that mist. This resulted in the four following options for randomisation; 'saline nebulised slowly', 'saline nebulised quickly', 'furosemide

nebulised slowly' and 'furosemide nebulised quickly' which were allocated randomly to visit number. The protocol is shown schematically in Fig. 5.1. The figure includes details about blood sampling however, this data is reported in Chapter 6. All participants and all healthcare professionals apart from those who administered the interventions were blinded to the medications. On each visit the participants were randomised to either inhale nebuliser (MicroAir U22, Omron Healthcare, Milton Keynes, UK) of furosemide (40 mg, 10 mg/ml; hameln pharmaceuticals gmbh, Langes Feld, Hameln, Germany) or saline (4 ml; B.Braun, Melsungen, Germany). The nebulisation duration of the furosemide mist was approximately 10-15 min and the saline mist 5-10 min. Total visit duration of around 3 h.

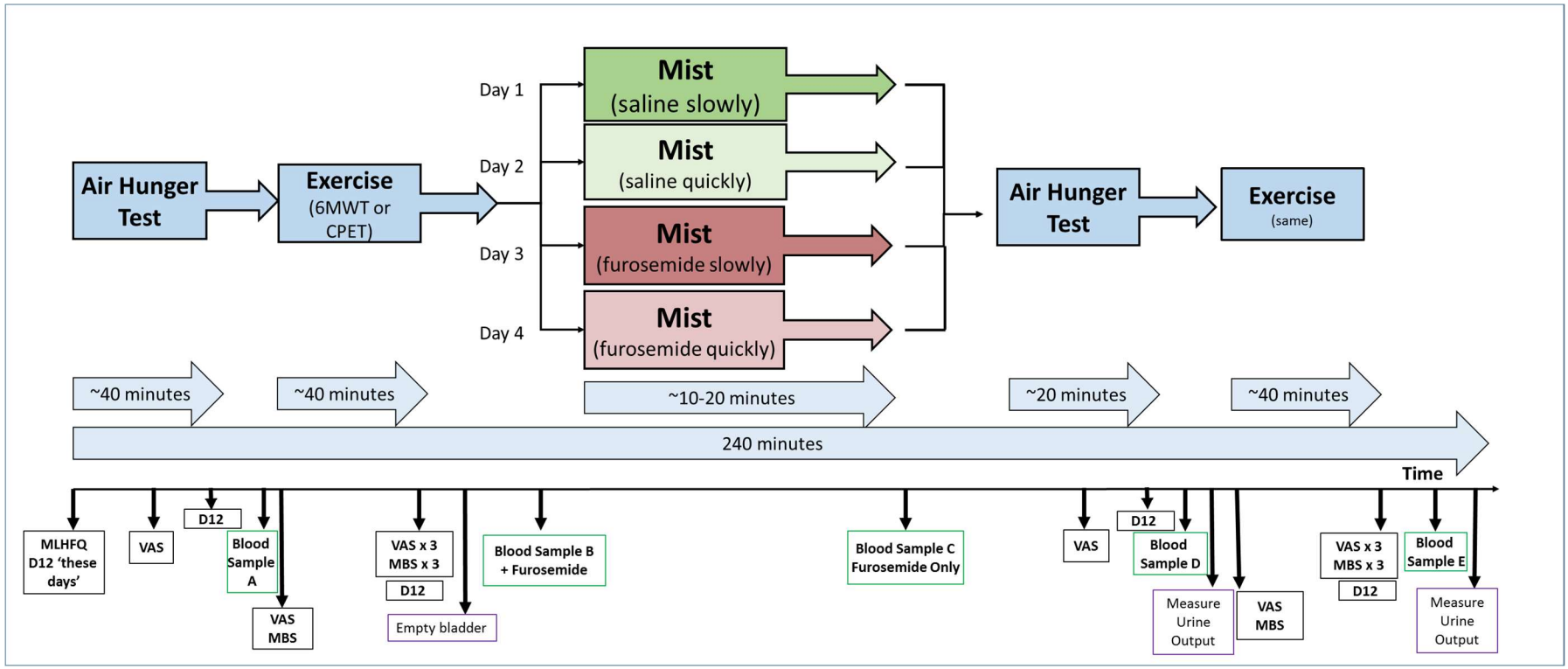


Figure 5.1 Flow diagram of study protocol

VAS ratings were recorded electronically every 15 sec during the air hunger. The D12 was administered after each AH and Exercise test. The VAS and MBS were administered immediately before and after exercise, and at 1- and 2- minutes after exercise. VAS = Visual Analogues Scale. MBS = Modified Borg Scale. D12 = Dyspnoea-12. MLHFQ = Minnesota Living with Heart Failure Questionnaire

5.2.6. Data analysis

A sample size was not determined prior to this study because the primary measures of breathlessness have not previously been used in this patient group. A sample size of 12 patients reflected a conservative estimate of the likely number of suitable advanced heart failure patients (of the required NYHA classes) that can be expected from the sources of prospective recruitment over a one-year period at a single centre. All patients were included in the analysis up to the point of completion of the study or withdrawal.

This was a pilot study of novel assessment and outcome measures; thus, the recently developed multidimensional Dyspnoea 12 questionnaire has not previously been used in a clinical trial and the air hunger has not been used to induce dyspnoea in patients with heart failure. Descriptive statistics are presented for the air hunger test and exercise test. To address the aims of the study both will be considered in relation to the following between factors: pre and post treatment (furosemide, saline) and breathing frequency during inhalations (slow, fast).

Some post-hoc significance testing has been performed in addition to the descriptive statistics, however this is not definitive due to the small number of patients studied in this pilot trial, and is intended to be hypothesis generating. This preliminary data is descriptive; however some statistical analyses were performed;

1) The D12 was compared to the; i) MLHFQ, using Pearson Correlation ii) original heart failure cohort for the validation of D12, using repeated measures ANOVA and iii) VAS, MBS-dyspnoea and MBS-perceived exertion, using Pearson Correlation. The effect of nebulised furosemide on exertional

dyspnoea using the VAS, Borg and D12 and the change in D12 across visits 1 to 4 was analysed using repeated measures ANOVA.

2) The optimal breathing pattern (slow or fast breathing frequency) whilst nebulising furosemide to achieve the greatest level of exertional dyspnoea relief was analysed using paired samples t-Test. The difference between fast and slow breathing, and the effect of nebulised furosemide and saline on MBS level of exertion was also analysed using the paired samples t-Test.
3) The optimal exercise test (CPET or 6MWT) for eliciting dyspnoea in patients with chronic stable heart failure was analysed using the independent-samples t-test.
4) The change in spirometry after nebulised furosemide and saline on was assessed using paired samples t-Test.
5) The treatment effect of nebulised furosemide on air hunger sensitivity was analysed using the independent-samples t-test.
6) The effect of nebulised furosemide on urine output was analysed using a paired sample t-Test.

Distance walked from 6MWT were converted to estimates of peak $\dot{V}O_2$ based on the following equation in order to make this comparable with peak $\dot{V}O_2$ during CPET.

Mean Peak $\dot{V}O_2$ (ml/kg/min) = 4.948 + 0.023 * mean 6MWD (m) (Standard Error of the Estimate 1.1ml/kg/min)[303].

5.3. RESULTS

5.3.1. Optimal method for measuring dyspnoea

The main methods used for measuring dyspnoea in this study were the VAS, MBS and D12. The MLHFQ also has some statements relating to dyspnoea. The VAS is a continuous scale; the Borg scale is a category scale with ratio properties; the D12 is a multi-dimensional questionnaire quantified using a Likert scale. The VAS in the AH test had verbal anchors which makes it similar to category scale with ratio properties[239]. To enable comparison between the scales they have been converted to % full scale although this has limitations. It may also limit the provision of useful data for future clinical trials. The minimum clinically relevant difference for each these scale is similar at around 10% of full scale. The MCID for VAS assessing dyspnoea is 8-10 points out of 100, for the MBS is 1 point out of 10 and for the D12 is 3 points out of 36[114, 123, 280]. VAS and MBS have been shown to be reproducible subjective measures of symptoms change before and after medication during exercise[110]. The VAS at maximal exercise has been shown to be reproducible, with a within-subject coefficient of variation of 6% (range 2-10%). However, submaximal VAS ratings were highly variable (21%, range 11 to 28%)[304]. MBS had a higher within-subject coefficient of variation at peak exercise (14%, range 6 to 31%). At submaximal exercise this increased to 25% (range 12 to 50%)[305]. The sample size reflects of the repeatability of the test. However, in this pilot study, which was designed to be more explorative rather than definitive, the low sample size means repeatability cannot be assumed. However, D12 has been shown to have good test-retest reliability in different diseases but larger studies are needed to confirm the repeatability of this measurement[7].

Comparing D12 'these days' with MLHFQ

The overall average score for D12 and MLHFQ taken at the start of each visit was 20.5±15.4% and 24.4±12.7%, mean±sd, respectively. They were moderately correlated (R^2 = 0.41, p = 0.02, n = 12, Fig. 5.2). This was similar when analysing the physical and emotional components of both the MLHFQ and D12 separately (R^2 = 0.32, p = 0.04 and R^2 = 0.32, p = 0.06, respectively, n=12). When analysing visit 1 only (prior to any interventions or mist) the R^2 = 0.43, 0.52 and 0.17 for the total, physical and emotional correlation, respectively.

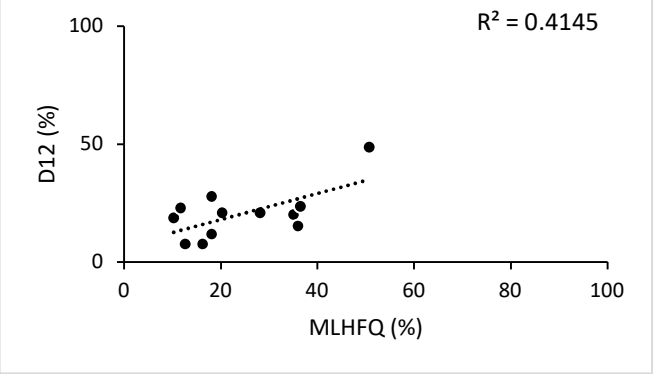


Figure 5.2 D12 'these days' correlated to the MLHFQ

The D12 'these days' total score moderately correlated with the MLHFQ. The four D12 and MLHFQ questionnaires for each patient were averaged. D12 = Dyspnoea-12 'these days', MLHFQ = Minnesota Living with Heart Failure Questionnaire.

There was no significant difference between D12 'these days' scores taken at the start of successive visits. Both the D12 'these days' and MLHFQ had higher mean scores for visit 1 compared to the other visits, but there was no similar trend for the subsequent visits, Table 5.2.

Table 5.2 Baseline dyspnoea ratings

Dyspnoea ratings taken at the start of each visit. D12 = Dyspnoea-12, MLHFQ = Minnesota Living with Heart Failure Questionnaire

	Visit 1 Mean±SD	Visit 2 Mean±SD	Visit 3 Mean±SD	Visit 4 Mean±SD
D12 'these days' (%)	27.5±17.5	19.7±14.6	18.1±15.7	16.7±13.0
MLHFQ (%)	33.2±14.9	21.0±12.1	23.8±16.6	19.8±15.5
D12 at peak exercise (%)	21.5±19.7	23.3±14.3	24.3±19.8	23.7±20.9

The D12 score taken at peak exercise averaged for all 4 visits was slightly, but non-significantly, increased compared to the D12 score for 'these days' averaged for all 4 visits (23±19 vs 20±15, %total±sd, p=0.38).

<u>Comparison of D12 compared to the original heart failure validation cohort</u>

The original cohort of patients with heart failure used in the validation of the D12 had a total D12 score of 30±24% (unpublished data from the original validation paper[7]). In this study the D12 (total, physical and emotional) taken at the start of each visit ('these days'), at peak exercise and at steady-state air hunger is shown in Table 5.3. The D12 'these days' and D12 at peak exercise were significantly less than the original data ($p<0.05$). The physical components of the D12 were similar for 'these days' and at peak exercise but were significantly higher for the AH steady state D12. The emotional components were significantly reduced for all of the D12 questionnaires ('these days', peak exercise and AH steady state) compared to the original cohort, Table 5-3.

Table 5.3 D12 comparisons with original D12 heart failure cohort

*D12 questionnaire was taken at different timepoints during the study. 'D12 'these days' = at the start of each study visit. $D12_{Ex\text{-}peak}$ = at peak exercise, $D12_{AH\text{-}steady\ state}$ = at the end of each steady state of the air hunger test, *=p<0.05.*

	Total D12 (% total)	Physical (% physical)	Emotional (% emotional)
$D12_{'original\ cohort'}$	**30±24**	**33±25**	**26±26**
$D12_{'these\ days'}$	20±15*	27±18	11±15*
$D12_{Ex\text{-}peak}$	24±18*	37±24	7±15*
$D12_{AH\text{-}steady\ state}$	29±18	44±23*	8±16*

Comparison of exercise dyspnoea ratings by the different tools

Peak exercise ratings were highest using the VAS, followed by MBS and D12, when expressed as %full-scale, Table 5.4. When averaged over 4 visits the dyspnoea ratings by the different tools differed significantly (repeated measures ANOVA, p<0.005, Table 5.4). D12, VAS and Borg scores for dyspnoea at peak exercise were reproducible over repeated visits. VAS shows the greatest magnitude of change at peak exercise, from baseline, with a mean±sd increase of 49±19% compared to MBS which was 34±20%.

Table 5.4 Exertional dyspnoea ratings over 4 visits

*Mean±sd of 24 scores at peak exercise (2 per visit for 12 patients). Scores are normalized to %full-scale and averaged over 4 visits and compared using repeated measures ANOVA VAS = Visual Analogue Scale, MBS = Modified Borg Scale, D12 = Dyspnoea-12. *= p<0.05 compared to VAS ł = p<0.05 compared to MBS ǂ = p<0.05 compared to VAS*

		Visit 1 Mean±SD	Visit 2 Mean±SD	Visit 3 Mean±SD	Visit 4 Mean±SD	Average of all 4 visits
Pre-mist	VAS (%)	65.3±25.3	60.2±21.2	61.8±25.6	66.0±19.8	63.3±2.8
	MBS (%)	45.9±20.3	47.3±21.7	55.5±25.2	53.2±17.5	50.5±4.6*
	D12 (%)	24.8±21.1	22.7±14.9	26.6±20.6	26.4±21.1	25.1±1.8łǂ
Post-mist	VAS (%)	66.3±28.9	61.3±20.1	65.8±25.7	66.0±23.0	64.9±2.4
	MBS (%)	46.8±20.9	48.0±17.5	56.3±21.8	57.1±20.4	52.1±5.4*
	D12 (%)	19.9±18.6	23.8±14.3	22.0±19.5	23.0±21.1	22.2±1.7łǂ

There was a strong correlation between the VAS and MBS measurements (R^2=0.81, p = 0.0003, n = 12), when all data was pooled (pre-exercise, peak exercise, 1- and 2-min recovery). This strong correlation persisted when data only from the CPET was analysed (R^2 = 0.84, p = 0.01, n = 6) but was only mildly correlated for the 6WMT (R^2 = 0.30, p = 0.26, n = 6) data. The D12 showed a weak correlation with the VAS (R^2 = 0.22, p = 0.13, n = 12) and MBS (R^2 = 0.28, p = 0.07, n = 12). When only the D12 physical component was included in the comparisons, the correlation improved to 0.63 (p = 0.002, n = 12) for the VAS and 0.71 (p = 0.0006, n = 12) for the MBS, Fig. 5.3.

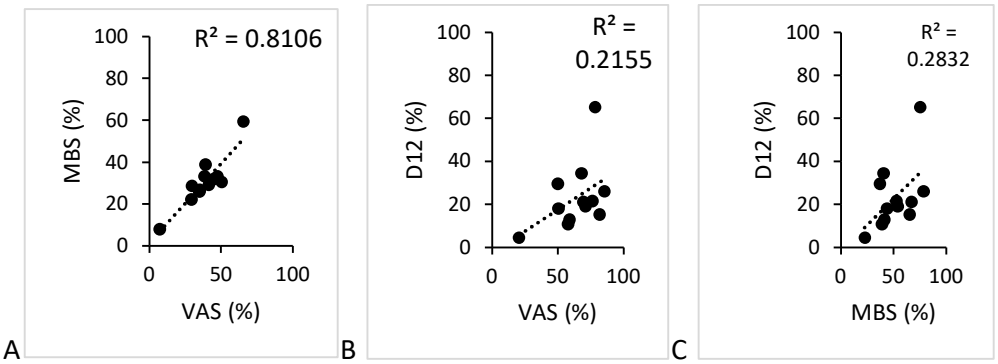


Figure 5.3 Correlations between dyspnoea ratings

The MBS and VAS were strongly correlated (A). The D12 and VAS had a weaker correlation (B) as did the D12 and MBS (C). Rating for dyspnoea using the VAS and MBS were averaged for all ratings (pre-exercise, immediately at the end of exercise, 1-min and 2-min post exercise, pre and post mist) for the 12 patients. For D12 comparisons the VAS and MBS scores immediately post exercise were used, pre and post mist for all 12 patients. D12 = Dyspnoea-12, MBS = Modified Borg Scale - Dyspnoea, D12 = Dyspnoea-12.

<u>Comparison of peak perceived level of exertion ratings and the different dyspnoea tools</u>

MBS perceived level of exertion correlated closest with MBS dyspnoea (R^2 = 0.80, p = <0.001, n = 12) but also correlated moderately with the VAS dyspnoea (R^2 = 0.63, p= 0.002, n=12). It correlated poorly with the D12 (R^2 = 0.14, p = 0.23, n =12, Fig. 5.4). There was no correlation with the work rate achieved on CPET and the patients reported level of exertion (R^2 = 0.04, p=0.18, n=12).

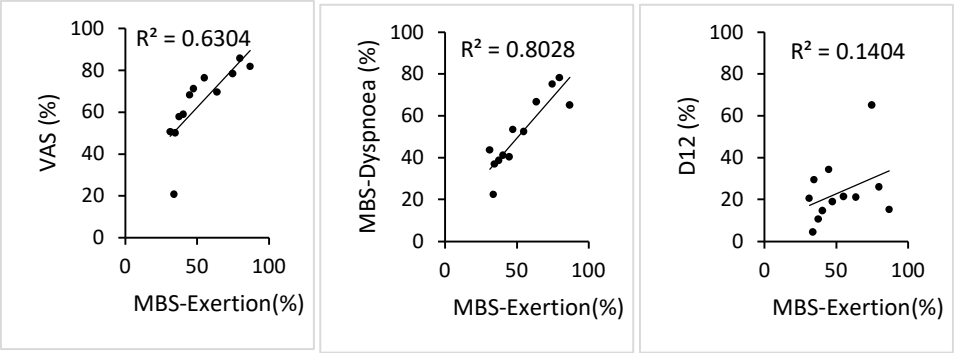


Figure 5.4 Correlation between level of exertion and dyspnoea

Correlation between perceived level of exertion at peak exercise measured using MBS with dyspnoea rated at peak exercise. VAS, MBS and D12, all expressed as % full scale.

Measuring the effect of nebulised furosemide on exertional dyspnoea using the different dyspnoea tools

Nebulised furosemide did not significantly alter the exertional dyspnoea ratings (VAS, MBS or D12) taken immediately at the end of exercise, irrespective of exercise type (CPET or 6MWT). The magnitude of relief of exertional dyspnoea recorded on the VAS, MBS and D12 is shown in Fig. 5.5 separately for saline and furosemide nebulisation. The greatest difference detected was on the D12 with the furosemide mist (nebulised furosemide and saline, -6.1±10.9 vs -1.5±10.7 %full scale, respectively, p = 0.40). The results were not significantly altered if the baseline ratings were deducted from the immediate peak exercise rating, for the VAS and MBS. None of these analyses reached statistical significance.

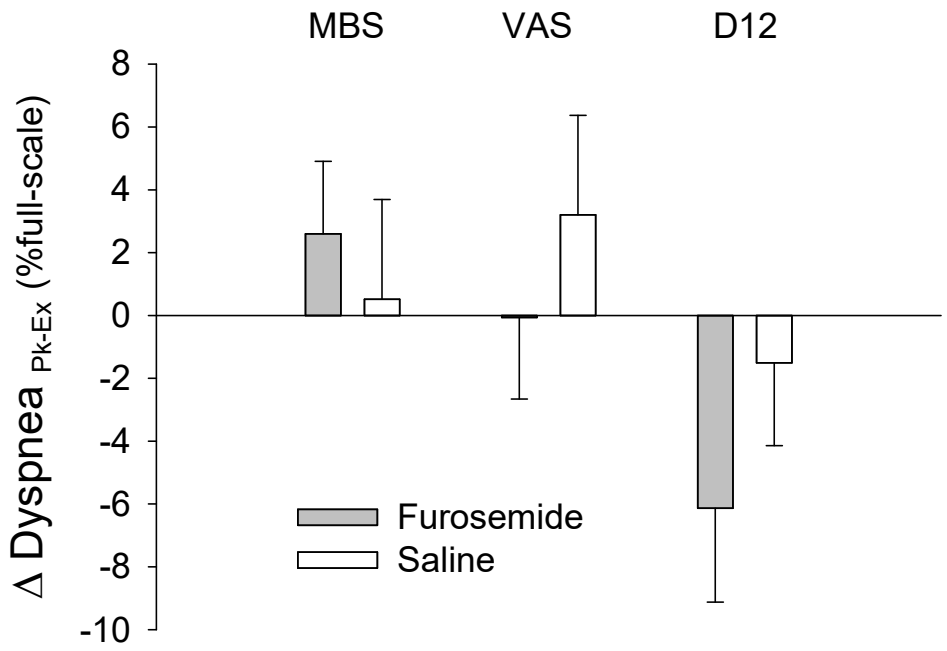


Figure 5.5 Change in dyspnoea ratings before and after mist inhalation

Error bars show SEM. Greatest difference detected on the D12 with the furosemide mist. VAS = Visual Analogue Scale, MBS = Modified Borg Scale, D12 = Dyspnoea-12

Nebulised furosemide did not affect the rate of recovery at 2 mins for the VAS, Fig. 5.6, or MBS-dyspnoea (figure not shown, shows same pattern as VAS).

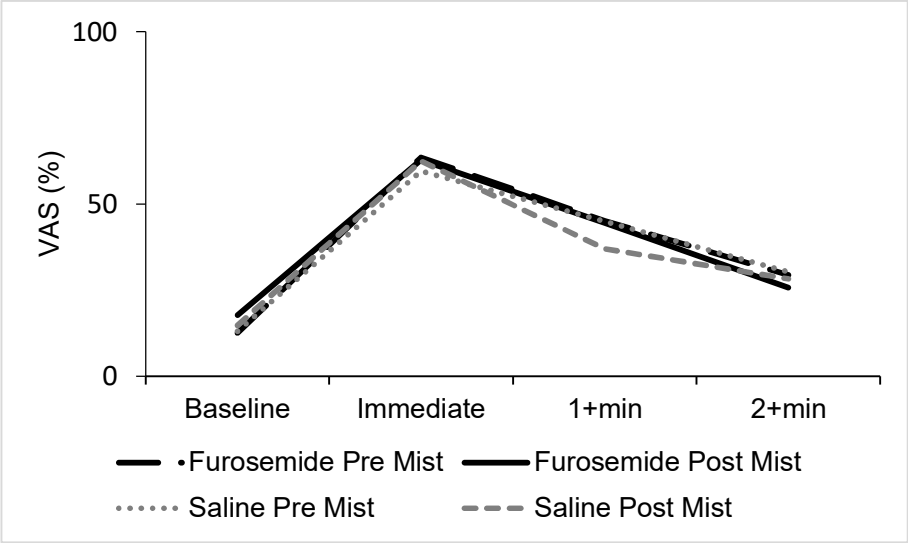


Figure 5.6 VAS at baseline, immediately, 1 and 2 mins after exercise

The data is averaged for the 12 participants. VAS = visual analogue scale.

5.3.2. Optimal breathing pattern

The mists nebulised with a faster respiratory rate, as dictated by the metronome, resulted in an average breathing frequency of 15±3/min compared to the slower respiratory rate of 9±2/min, p=<0.001 (student t-Test), from a spontaneous level of 12.2±3.6 breaths per minute. The tidal volume was 701±253ml for the faster rate compared to 841±343ml for the slower rate, p=0.52. The overall ventilation was 10±4l/min for the faster rate and 8±4l/min for the slower rate, p=0.09. The difference in the achieved ventilation for fast and slow targeting became greater over the period of nebulisation. The achieved tidal volume for both the fast and slow targeting fell over the period of inhalation, Fig. 5.7.

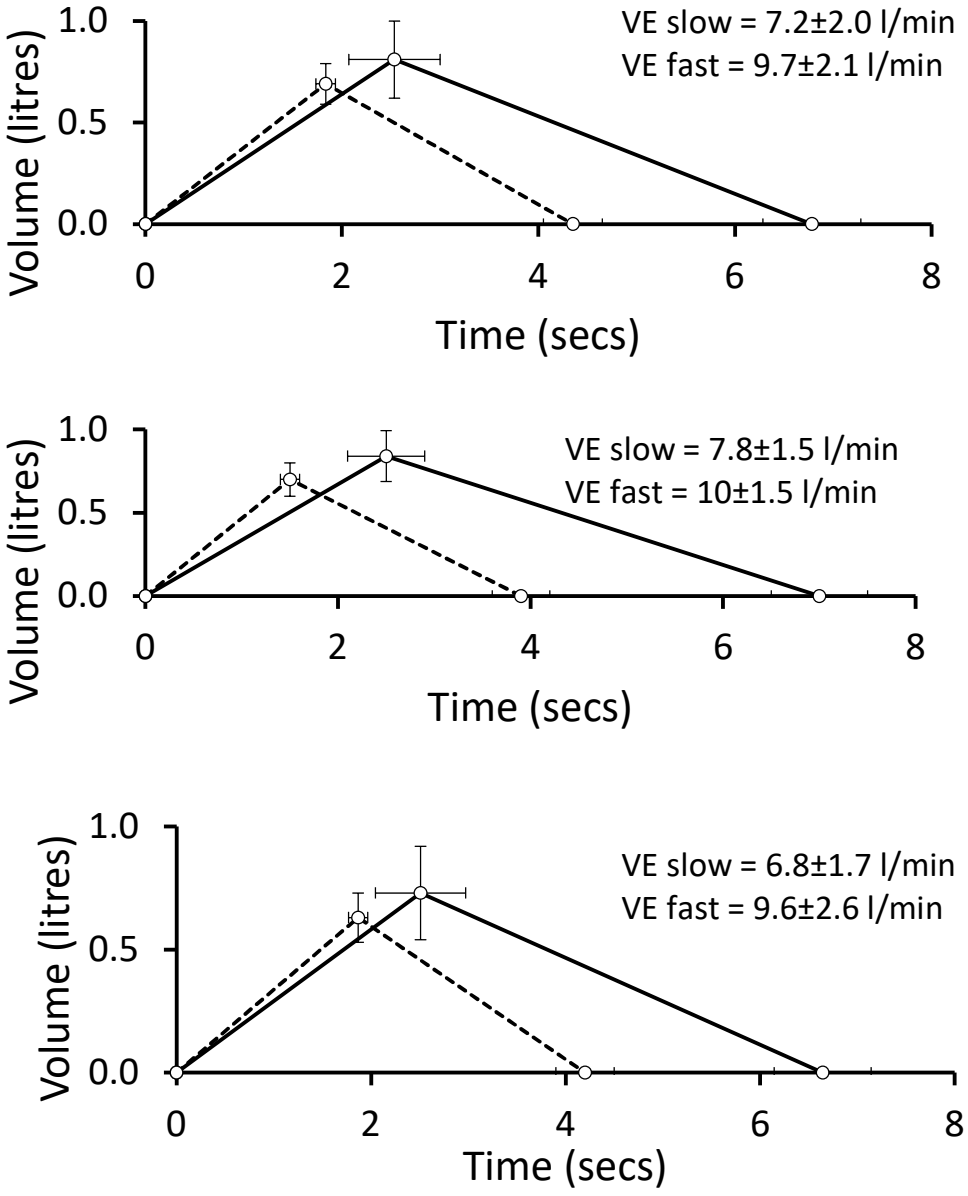


Figure 5.7 Changes in breathing pattern during furosemide inhalation

Tidal volume recorded at the start (A), middle (B) and end (C) of mist inhalation (duration of mist inhalation ~10minutes). Dashed line = faster rate, solid line = slower rate.

Effect of breathing pattern during mist inhalation on exertional dyspnoea relief

The VAS data shows that nebulising furosemide with the fast breathing pattern resulted in greater exertional dyspnoea relief relative to saline for both the CPET and 6MWT. In contrast, the D12 data showed that nebulising furosemide with the slow breathing pattern resulted in a greater treatment effect (dyspnoea relief with nebulised furosemide relative to nebulised saline) for the 6MWT but not the CPET (Table 5.5, please note, a negative number indicates greater exertional dyspnoea relief). None of these trends showed statistical significance. Nebulising the furosemide with a slow breathing pattern resulted in less distance walked during the post-mist 6MWT compared to the pre-mist 6MWT but no reduction was seen for peak work in CPET. However, nebulising the furosemide with a fast breathing pattern resulted a negligible reduction in the distance walked during the post-mist 6MWT and in the peak work achieved during the post-mist CPET. Slow or fast breathing during nebulised furosemide did not affect the maximum work rate achieved with slow inhalation resulting in a change of 0.5±5.7, compared to saline at -0.7±3.4watts.

Table 5.5 Breathing pattern during mist inhalation on dyspnoea relief

Effect of breathing pattern during mist inhalation on exertional dyspnoea relief

VAS = Visual analogue scale, D12 = Dyspnoea-12, ΔEx test peak = change in exertional dyspnoea relief at peak exercise, CPET = cardiopulmonary exercise test, 6MWT = 6 minute walk test, WR = work rate. NB a negative result indicates a greater relief in dyspnoea

Mist inhalation			Faster respiratory rate	Slower respiratory rate
VAS (% full scale)	ΔEx test peak	Furosemide	-3.0±9.2	2.9±15.3
		Saline	5.3±13.4	1.1±17.1
		Treatment Effect	-8.4±15.4	1.8±20.7
	- ΔCPET peak	Furosemide	-4.5±11.7	1.1±21.0
		Saline	3.0±18.9	-6.7±18.5
		Treatment Effect	-7.5±20.4	7.8±28.3
	- Δ6MWT	Furosemide	-1.6±6.6	4.8±8.1
		Saline	7.7±5.0	8.8±14.3
		Treatment Effect	-9.3±10.3	-4.1±7.5
D12 (%)	ΔEx test peak	Furosemide	-2.6±11.5	-7.3±15.5
		Saline	-3.8±16.1	1.6±9.2
		Treatment Effect	0.9±20.6	-8.3±18.1
	- Δ6MWT	Furosemide	-1.5±3.2	-4.6±5.6
		Saline	-3.5±7.1	2.3±3.0
		Treatment Effect	2.0±8.4	-6.2±6.6
	- ΔCPET	Furosemide	-0.3±5.2	-1.0±5.5
		Saline	1.2±2.8	1.2±2.2
		Treatment Effect	-1.3±6.6	0.2±5.1
Δ6MWT distance (m)		Furosemide	-0.2±20.1	-6.8±24.3
		Saline	2.0±23.1	15.0±38.6
		Treatment Effect	-2.2±38.5	-21.8±54.6
ΔCPET max WR (watts)		Furosemide	-0.8±5.6	0.5±5.7
		Saline	3.0 ±3.7	-0.7±3.4
		Treatment Effect	-3.8±6.8	1.2±3.5

5.3.3. Optimal exercise test for dyspnoea

Six patients performed the 6MWT and 6 patients performed CPET, on 8 occasions each (96 tests included in analysis). Patients reported the same level of exertion

whether they had done a maximal symptom limited CPET test (5.3±2.3 MBS units) or the 6MWT (5.2±1.9 MBS units, p = 0.97).

VAS increased to 56±17% from a resting level of 12% with 6MWT compared to increasing to 42±19% from a resting level of 18% with CPET (p=0.10). There was a trend for increased D12 scores at peak exercise at the end of CPET (27±22, %total±sd) compared to 6MWT (19±13, %total±sd) but this did not reach significance, p=0.06.

The averaged distance walked on the first 6MWT was 336±101m compared to 338±110m on the second test. There was no significant difference in the distance walked after nebulised furosemide compared to nebulised saline, -3.5m+19m, and 8.5m+22m respectively, p=0.44. The averaged maximal work rate achieved on the first and second CPET were identical (43±12watts) and the peak $\dot{V}O_2$ was similar (9.5±1.9 versus 9.8±2.1 ml/kg/min, mean±sd). The peak $\dot{V}O_2$ estimated from the distanced walked during the 6MWT was greater than the measured peak $\dot{V}O_2$ during CPET (12.7 vs 9.6ml/kg/min).

The reason given by the patient for stopping the CPET test is shown in Fig. 5.8. For the 6MWT, all patients completed the 6MWT and on only 6 of the 48 tests did the patient pause during the test. This was always due to dyspnoea. The frequency of stopping did not differ according to the mist inhalations.

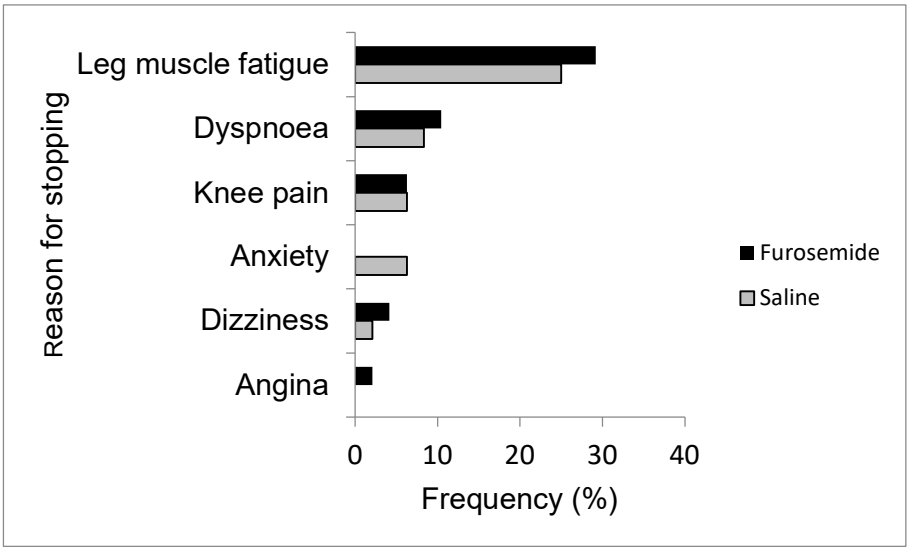


Figure 5.8 Reason for stopping CPET

6 participants each performed the test 8 times.

The CPET data averaged for all CPET tests is shown in Table 5.6, and this is separated into the pre-mist test and post mist test.

Table 5.6 CPET parameters

$\dot{V}O_2$ = oxygen consumption. AT = anaerobic threshold, RER = respiratory exchange ratio, WR = work rate, $\dot{V}E$ = minute ventilation, $\dot{V}CO_2$ = expired carbon dioxide, O_2 pulse = oxygen pulse, OUES = oxygen uptake efficiency slope

CPET parameter	All tests (mean±sd)	CPET Test 1 (pre-mist) (mean±sd)	CPET Test 2 (post-mist) (mean±sd)

$\dot{V}O_2$ peak	0.72±0.14	0.71± 0.13	0.73± 0.16
$\dot{V}O_2$ peak (ml/kg/min)	9.7±2.0	9.5±1.9	9.8±2.1
$\dot{V}O_2$ (% predicted)	54±15	53±15	54±16
AT	0.6±0.1	0.6±0.1	0.6±0.1
AT ml/kg/min	8±0.7	8±0.1	8±0.5
AT at % peak	78±4	79±4	78±7
AT at % ref	44±10	44±11	44±9
RER	0.97±0.08	0.99±0.08	0.96±0.08
WR (watts)	43±12	43±13	43±12
$\dot{V}E$ (l/min)	32.1±6.8	32.4±7.0	31.7±6.7
$\dot{V}E$ (% of M$\dot{V}V$)	41.2±2.8	41.3±12.3	41.1±13.4
$\dot{V}E/\dot{V}CO_2$ slope	45.6±9.0	45.8±10	44.9±8.2
$\dot{V}E/\dot{V}CO_2$ at peak ET	39.3±4.8	39.3±4.6	39.2±5.2
$\dot{V}E/\dot{V}O_2$ at peak ET	38.4±6.9	39.0±6.9	37.1±7.1
O_2 pulse	9±3	9±3	10±3
OUES	0.9±0.3	1.0±0.4	0.9±0.2

Effect of mist on CPET variables

Nebulised furosemide did not increase the maximal work rate achieved on CPET with furosemide inhalation resulting in a change of work rate of -0.17±1.5, compared to saline inhalation of 1.2±2.3 watts, p = 0.38. During CPET the change in the measured peak $\dot{V}O_2$ with exercise was identical for furosemide and saline, 0.2±1.0 and 0.2±0.7 ml/kg/min, respectively, p=0.93, Table 5.7. The change in the

estimated peak $\dot{V}O_2$ from the distance walked was not significantly difference for furosemide or saline.

Table 5.7 Change in peak $\dot{V}O2$ measured/estimated with furosemide and saline

	Furosemide	Saline
ΔCPET (measured peak VO_2 ml/kg/min	0.2±1.0	0.2±0.7
Δ6MWT (estimated peak $\dot{V}O_2$) ml/kg/min	-0.1±0.5	0.2±0.7

The RER was not altered by nebulised furosemide compared to nebulised saline (-0.02±0.02 versus -0.05±0.54, p= 0.104). In those that the anaerobic threshold was detected, it was increased by nebulised furosemide compared to saline, p=0.02, Table 5.8. Anaerobic threshold expressed as % peak $\dot{V}O_2$ showed no significant difference.

Table 5.7 Change in anaerobic threshold after each mist inhalation from baseline

AT = anaerobic threshold

	Furosemide	Saline	T.Test
ΔAT	0.05±0.05	-0.01±0.05	0.005
ΔAT ml/kg/min	0.6±1.11	-0.5±0.71	0.02
ΔAT of %peak VO2	1.5±10.6	-3.0±6.6	0.51

Nebulised furosemide did not affect the $\dot{V}O_2$ peak, $\dot{V}O_2$ ml/kg/min or $\dot{V}O_2$% reference value. Nebulised furosemide did not significantly affect the $\dot{V}E$ L/min, $\dot{V}E$

of %MV̇V, V̇E slope, V̇E/V̇CO_2 at peak ET or the V̇E/V̇O_2 at peak ET, Table 5.9. The change in O_2 pulse was also not altered by nebulised furosemide at 0.5±0.89 versus 0.92±0.38 for saline, p=0.29. The OUES was not changed by the nebulised furosemide.

Table 5.8 Change in ventilator parameters after mist from baseline

V̇E = minute ventilation, MV̇V = maximal voluntary ventilation, V̇O_2 = oxygen consumption, V̇CO_2 = expired carbon dioxide, ET = exercise tolerance

	Furosemide	Saline	T.Test
ΔV̇E L/min	0.07±2.4	-1.4±3.2	0.30
ΔV̇E of %MV̇V	0.9±3.2	1.4±7.1	0.89
ΔV̇E slope	0.6±7.9	1.7±12.5	0.89
ΔV̇E/V̇CO_2 at peak ET	0.4±1.3	-0.725±3.2	0.39
ΔV̇E/V̇O_2 at peak ET	0.5±1.6	-2.7±5.2	0.17

Effect of Mist on Perceived Exertion

There was no indication in this data that nebulised furosemide altered the level of perceived exertion at the end of exercise, and this was true for both CPET and 6MWT (-0.02±0.6 furosemide versus 0.09±0.4 saline, p=0.72)

5.3.4. Effect of Mist on Spirometry

Furosemide did not significantly change the spirometry data collected in this study compared to saline for any of the following parameters; FVC, FEV1, FEV1:FVC, PEF, MV̇V, Table 5.9. A significant difference was noted when furosemide was inhaled with slower slow inhalations compared to faster shallower inhalations; FVC

increased with slow breathing and reduced with fast breathing (p=0.001). Similar trend for FEV1 and FEV1:FVC but not significant. No change with PEF.

Table 5.9 Changes in spirometry with mist inhalations

Spirometry data from 6 patients (those allocated to CPET). Spirometry was performed immediately before the CPET test, before and after each mist inhalation (8 results per patient). FVC = full vital capacity, FEV1 = forced expiratory volume in 1 second, PEF = peak expiratory flow.

ΔFVC	Furosemide	0.02±0.13	p=0.26	Slow	0.05±0.13	*p=0.001
				Fast	-0.09±0.09	
	Saline	0.07±0.14		Slow	0.07±0.18	p=0.26
				Fast	0.08±0.10	
ΔFEV1	Furosemide	-0.01±0.14	p=0.48	Slow	0.06±0.16	p=0.102
				Fast	-0.08±0.08	
	Saline	-0.03±0.11		Slow	-0.07±0.13	p=0.48
				Fast	0.04±0.04	
ΔFEV1: FVC	Furosemide	0.10±3.35	p=0.15	Slow	0.80±4.66	p=0.494
				Fast	-0.60±1.52	
	Saline	-3.25±3.8		Slow	-4.80±4.09	p=0.15
				Fast	-0.67±1.53	

		5				
ΔPEF	Furosemide	-0.37±0.55	p=0.73	Slow	-0.45±0.69	p=0.514
				Fast	-0.29±0.42	
	Saline	-0.13±0.93		Slow	-0.41±0.97	p=0.73
				Fast	0.58±0.14	

5.3.5. Hypercapnic air hunger sensitivity

There was no evidence in this study of a treatment effect with nebulised furosemide in relief of AH, mean±sd furosemide vs saline, 2±14 vs 2±16, p=0.94, Table 5.11.

Table 5.10 Change in VAS with furosemide and saline during AH test

Difference in VAS before and after mist inhalation during AH steady state for all tests. The difference in the VAS ratings pre and post mist were averaged for the fast and slow breathing pattern for furosemide and saline inhalation.

Subject	**Furosemide** (Δ% full scale)	**Saline** (Δ % full scale)
1	28.75	10.45
2	16.15	21.75
3	-4.4	4.85
4	15.45	7.35
5	3	8.25
6	0.85	-6.25
7	-8.7	-14.35
8	-5.5	-3.3
9	-21.5	14.8

10	7.1	20.75
11	9.65	-33.2
12	-11.55	-6.7
Mean	**2.4±13.9**	**2.0±15.8**

Using only the data that met the inclusion criteria for a valid steady state ($ETCO_2$ values separated by <1mmHg from pre and post-test state, variation in $ETCO_2$ over the steady state level <2.5mmHg and no patient selection of 'extreme' on the VAS scale) there remained no significant difference in the relief of AH with furosemide compared to saline, -2±20 vs -1±20, p=0.94. Using this smaller dataset, the same pattern remained for when the furosemide was inhaled fast compared to slowly, with relief only seen with the fast inhalation, 3±24 vs -5±15, p = 0.62.

Nebulised furosemide did not affect the D12 score (or % change) after the AH test, compared to the pre-mist AH test. There was no significant difference whether furosemide was inhaled slowly or fast, p = 0.43. Nebulised furosemide did not alter the physical or affective components of dyspnoea differently for the AH test, although there was a trend for greater AH relief with saline for the physical element, p = 0.09, Table 5.11. Hypercapnic AH test in assessing dyspnoea using the D12 did not show any significant difference between nebulised furosemide and saline (-6±10 vs -9±10, p = 0.41).

Table 5.11 D12 after AH test

$D12_{AH}$ = D12 after the AH test (% full scale±sd), $D12_{AH\text{-}phys}$ = Physical components of D12 (% full scale physical±sd), $D12_{AH\text{-}emot}$ = Emotional components of D12 (% full scale emotional±sd)

	Change after furosemide nebuliser	Change after saline nebuliser	P-value
$D12_{AH}$	-6±10	-9±10	0.41
$D12_{AH\text{-}phys}$	-4±22	-15±24	0.09
$D12_{AH\text{-}emot}$	-3±7	-6±14	0.55

5.3.6. Urine output

The average urine output was 152±81ml after furosemide inhalation versus 122±89ml after saline inhalation. The difference in urine output with nebulised furosemide compared to nebulised saline was not statistically significant, p=0.18.

5.4. DISCUSSION

This study addresses the optimal dyspnoea measurement tool in heart failure and the impact of furosemide (VAS, MBS, D12), determines the optimal breathing frequency during nebulised furosemide and assesses the feasibility of CPET and air hunger tests in assessing nebulised furosemide for dyspnoea relief.

5.4.1. Optimal method for dyspnoea measurements

<u>D12 performance</u>

This pilot study is the first randomised clinical trial in heart failure patients using the multidimensional D12 questionnaire for assessment of dyspnoea. It demonstrates that the D12 is a stable measure of dyspnoea for use in heart failure and that, in this study, was the method that detected the greatest relief of dyspnoea at peak exertion by nebulised furosemide, Fig. 5.5.

D12 'these days' correlated modestly with the MLHFQ and this is likely because the MLHFQ has some questions relating to dyspnoea and its indirect effects, such as on activities of daily living, although it also covers other symptoms not related to dyspnoea such as cost of medical care. On one occasion a patient rated zero on the D12 but 50% on the MLHFQ for the physical aspects, this may be explained by the patient not experiencing dyspnoea (therefore rating zero on the D12) but experiencing other physical symptoms of heart failure (such as ankle swelling, poor sleep etc.)

The highest scores for both the D12 'these days' and MLHFQ were recorded on visit 1, compared to lower scores for the remaining 3 visits. The instruction for D12 'these days' is to rate dyspnoea over the previous 4 weeks. It is likely that patients did this for visit 1, however for visits 2, 3 and 4 their ratings may have relied only on the previous week's recall. It has been shown in previous studies that the patients remember the worst event when recalling symptoms[306]. It is also possible that patients' dyspnoea levels are reduced by the sequent visits becoming more familiar and ameliorating some of the associated anxiety.

Interestingly the D12 scores at peak exercise are only slightly higher than those recalled during the D12 'these days'. This has been noted before[53] and this

discrepancy is thought to arise from patients self-limiting their activity during exercise, inaccurate recall, recall bias (peak end rule) or may be due to the difference between a safe supervised clinical setting versus the unmonitored home environment[307]. Recalled sensations focus on the most meaningful or peak unpleasantness in order to avoid future similar sensations[306, 308]. Therefore, in the D12 'these days' patients may be remembering an event associated with peak exercise or recalling the worst event in the last few weeks.

Comparison of D12 compared to the original heart failure validation cohort

The D12 in this preliminary study showed a significant reduction in the emotional component for all D12 questionnaires - 'these days', peak exercise and AH 'steady state' - compared to the original cohort[7]. The reduction in the emotional component at peak exercise or AH 'steady state' may be explained by the fact that patients expect to get breathless during exercise or are warned that they will do so during the AH test, so therefore does not induce such an emotional response. The reduction in the emotion component for 'these days' is not as clear as selection of patients is from a similar pool of patients. However, the standard deviation is large in both the original cohort and this cohort and remains within the expected range. The total D12 score at steady state for AH was almost identical to the original cohort, however this was due to a significantly higher physical component. This may be due to AH being the most unpleasant sensation of dyspnoea[200].

Comparison of exercise dyspnoea ratings by the different tools

Dyspnoea ratings using three different dyspnoea tools (VAS, MBS and D12) in this preliminary study were significantly different for the same test condition, when

expressed as % of full scale. The VAS recorded the highest levels of dyspnoea followed by the MBS and D12, but this did not translate into the VAS detecting the greatest change in exertional dyspnoea ratings with an intervention (furosemide or saline mist.) Dyspnoea measurements were taken before and at the end of exercise and not throughout the test. Assessing dyspnoea during progressive exercise may have yielded some valuable data.

The VAS and MBS had a strong correlation and this has been documented previously[240]. Interestingly the correlation was stronger for CPET compared to the 6MWT suggesting that the scales are used differently depending on the nature of the exercise. The VAS scale is used over a wider range than the MBS and again this has been noted previously[240]. The word anchors on the MBS often limit patients from rating above 5 'severe', whereas on the VAS there were no word anchors in this study. The experience in this lab is that when individuals are given the choice of placing word anchors on the VAS scale on average they will place 'slight' 'moderate' and 'severe' equidistant along the scale, with severe at 75% full scale (unpublished data). This also explains the higher readings on the VAS compared to the MBS.

The D12 correlated only slightly with the VAS and MBS and had the lowest dyspnoea ratings overall. The D12 has 5 statements on the emotional aspects of dyspnoea, which rarely resulted in high scores after exercise, and therefore lowered the overall score. This may reflect the positive psychological benefits of exercise[309] which may diminish the affective domain of dyspnoea, particularly in a controlled environment. The correlation is strengthened when using only the physical components of the D12 to the VAS and MBS, suggesting that the VAS and MBS are

more focussed on the physical component of dyspnoea, with less emphasis on the emotional component.

Perceived level of exertion at peak exercise

Use of MBS to measure 'perceived level of exertion' correlated best with the use of the MBS to measure 'dyspnoea'. This is likely because they use the same word anchors on the scale. The 'MBS perceived level of exertion' also correlated to a lesser degree with the VAS measure of dyspnoea but not for the D12 measure of dyspnoea at peak exercise. This suggests that the D12 is not strongly related to the level of exertion as perceived by the patient. There was no association between the achieved work rate during CPET and the patients' reported level of exertion as each patient performed a maximal CPET test for their level of fitness/ability, resulting in different peak work rates for similar levels of exertion.

Effect of nebulised mist on exertional dyspnoea measured using different tools.

Preliminary analysis of this pilot study shows that dyspnoea ratings at peak exercise were not significantly improved by nebulised furosemide. When assessing the change in dyspnoea ratings with the nebulised furosemide the D12 had the greatest change. This is below the minimally clinically important difference (MCID) of 9.7%[7] suggesting that this change would not be detected by the patient. The VAS and MBS were also below their MCIDs. The rate of recovery of dyspnoea, recorded on the VAS and MBS, after exercise did not show any difference according to mist inhalation or whether the mist was inhaled with a slow or fast breathing frequency.

5.4.2. Optimal breathing pattern

During nebulisation patients were successful in targeting their breathing frequency to the fast and slow rates that were set by the metronome. The patients spontaneous baseline level of tidal volume was not recorded in this pilot study (respitrace was only applied during nebulisation with targeted breathing). If the tidal volume were to appropriately compensate for the deviation of the respiratory frequency from the resting level during targeted breathing one would expect ventilation to be matched between slow and fast targeting. However, ventilation was not matched (10±4 and 8±4l/min for the fast and slow rates respectively) and this discrepancy increased over the nebulisation period. Therefore, faster mist inhalations were associated with higher overall ventilation which would increase the amount of furosemide delivered to the lungs. This has implications for comparing dyspnoea relief with different breathing patterns of inhalation. For example, a slower rate of mist inhalation would result in lower overall ventilation and therefore, less furosemide delivered to the lungs which may reduce the effect of the furosemide on dyspnoea relief.

<u>Breathing frequency of nebulised furosemide – effect on relief of exertional dyspnoea</u>

In this preliminary study the VAS score demonstrated that nebulised furosemide inhaled using a higher breathing frequency resulted in a reduction in exertional dyspnoea, for both 6MWT and CPET. There was no difference in the distance walked or the peak work rate achieved so this cannot account for the difference

seen. However, the D12 score showed that the slower breathing frequency of nebulised furosemide resulted in a greater furosemide treatment effect. This was only true for the 6MWT and not CPET, and this may be explained by the reduction in the distance walked during the second 6MWT. This was a pilot study for descriptive analysis and was not powered to show any statistical significance. Analysis of the results was performed to assess if there were any clear signals. However, lack of statistical significance does not imply a lack of effect, rather that it is underpowered to show any significant difference. Taking this into account, analysis showed that none of these effects were statistically significant, thereby suggesting that nebulising furosemide at different rates does not show a clear signal that it alters the effectiveness of dyspnoea relief. However, the standard deviations for these results were large, and this may explain the lack of significant findings. Larger datasets from bigger, powered studies might be worthwhile to confirm the trends seen.

5.4.3. Optimal exercise test for inducing dyspnoea.

CPET and 6MWT are both valid methods of inducing dyspnoea (see chapter 2 - methods for details). Dyspnoea was greatest on the 6MWT compared to CPET, despite CPET being intended as a maximal test and 6MWT as a sub-maximal test. The patients perceived level of exertion was similar suggesting the 6MWT was a maximal test for some patients, or that some patients chose to stop CPET earlier before they had reached their peak. However, experimenter observation of the 6MWT suggested that in some patients the 6MWT was a maximal test, with some patients stopping during the test due to dyspnoea, prior to being able to continue to complete the full 6 minutes.

Walking involves a larger muscle mass and more work against gravity compared to cycling and consequently, peak $\dot{V}O_2$ is 5–10% higher on the treadmill than on a cycle ergometer[310]. This may explain why the 6MWT is more dyspnoea inducing than CPET. On the other hand, the D12 score taken at peak exercise was highest for CPET compared to 6MWT. This may be due to the inclusion of the emotional aspects of dyspnoea that are recorded as part of the D12, with patients finding CPET more 'emotionally' challenging than walking, which they might be expected to be more familiar with and therefore have less anxiety attached to the test. The peak $\dot{V}O_2$ estimated from the 6MWT distance was greater than the measured peak $\dot{V}O_2$ during CPET. This calculation can be used on a group wise analysis[303], it is intended to create a generalised equation that can be used to predict peak $\dot{V}O_2$ amongst patients with diverse cardiopulmonary disorders. In this preliminary analysis neither nebulised furosemide nor saline significantly altered the peak $\dot{V}O_2$ (measured or estimated).

The distance walked during the 6MWT in this study was similar to previous studies in heart failure[207, 311]. No improvement in the distance walked was seen with furosemide nebulisation. A systematic review of 6MWT in randomised blinded pharmacological studies in patients with chronic heart failure showed that only 9 of 47 studies showed a significant increase in 6MWT distance (2 for ACE inhibitors, 3 for beta blockers, 1 for digoxin, 1 for ibopamine, 1 for L-arginine, 1 for beriberine) and this correlated with an improvement in symptoms. It is also noted that trials showing a significant improvement were more likely to be seen in patients with more severe heart failure compared to those with milder symptoms. However, this study had patients with severe heart failure but no increased trends were seen in

distance walked suggesting that maybe the furosemide does not result in an improvement in exercise capacity. The distance walked in the second exercise was slightly longer than the first test which is surprising as one might expect the fatigue to be greater. This effect has been seen in previous studies and is thought to be a learning effect[312]. The slight increase seen was related to an increase in the distance walked following saline inhalation.

The main reason for stopping the CPET test was leg fatigue, followed by dyspnoea. Other reasons included knee pain, anxiety, dizziness and angina. Patients paused during six 6MWT tests and this was always due to dyspnoea. This fits with the result discussed above showing that the 6MWT is more dyspnoea inducing than CPET.

In this pilot study, there was no suggestion of a treatment effect seen with nebulised furosemide compared to nebulised saline according to CPET variables. The anaerobic threshold was not able to be determined in all patients (missing in 27% of results) so this result needs to be interpreted with caution as the numbers are small. The anaerobic threshold was significantly higher for nebulised furosemide compared to saline. However, the anaerobic threshold expressed as percentage of peak VO_2 was not significant, indicating that the difference in the AT between furosemide and saline is related to the difference in the peak VO_2 achieved. This suggests that oxygen delivery may be improved with nebulised furosemide although these are patients on high levels of systemic furosemide and any additional diuresis is likely to be negligible. An alternative mechanism may be via nebulised furosemide causing bronchodilation[173].

As this study included patients with advanced chronic heart failure, a maximal CPET was not possible and no patients were able to perform a true $\dot{V}O_2$ max test (see methods chapter for criteria) and peak $\dot{V}O_2$ was used instead. Most patients performed a submaximal exercise test with an RER on average <1.0. The RER was slightly lower for the second exercise test compared to the first which is likely to be due to fatigue. $\dot{V}E:\dot{V}CO_2$ slope and OUES are useful markers when the test is submaximal [313]. Peak $\dot{V}O_2$ is also a prognostic indicator irrespective of the RER[225]. None of these changed with the intervention. As expected the peak $\dot{V}O_2$ was markedly reduced compared to reference values[314]. The percent predicted value was 54±15% which is severely reduced. A $\dot{V}O_2$ peak <10ml/kg/min is classed as severe disability. A $\dot{V}O_2$ peak <15ml/kg/min is associated with NYHA class IV[315]. In this study they were in NYHA III but had a $\dot{V}O_2$ peak <10ml/kg/min which suggests the CPET has over-estimated the severity of their heart failure (or they have done a suboptimal test), or they were classified in the wrong group clinically[316]

The absence of any trends/suggestions in this pilot study of nebulised furosemide for dyspnoea relief in patients with heart failure may be due to the type of dyspnoea induced during exercise. It is likely that a large component of dyspnoea during exercise is due to the work/effort component [317] and a recent previous study has shown that nebulised furosemide only relieves the air hunger component of dyspnoea[276].

Spirometry

In this pilot study, patient spirometry preceded each CPET test. When combining the fast and slow breathing frequency of inhaling the furosemide and saline mist, there was no difference seen in spirometry values. However, a significant increase

in FVC was seen when the furosemide mist was nebulised with the slow breathing frequency compared to the fast breathing frequency. This may be due to bronchodilation and a reduction in atelectasis[173] or may be due to chance as there were no other significant changes on spirometry with nebulised furosemide

5.4.4. Hypercapnic air hunger sensitivity

Air Hunger Test

The AH test was a challenge for these patients to complete. As a result, there was limited meaningful data from this test. It was noted during the study that some of the patients were hyperventilating with low $ETCO_2$ and this has been shown previously[318]. This may have accounted for some of the difficulties associated in this test as the test relies on a steady baseline ventilation at the start of the test. This could make comparison of the ratings before and after the mist inhalation less reliable.

No relief of AH was noted with nebulised furosemide compared to saline when comparing the dyspnoea ratings for the same fixed level of $ETCO_2$ (steady state). The steady state was difficult to achieve in these patients with heart failure. The test was often aborted early due to the patient reaching 'extreme' on the VAS scale whilst trying to achieve a steady state or for other reasons such as a poor steady state including $ETCO_2$ values separated by >1mmHg from pre and post-test state or the variation in $ETCO_2$ over the steady state level was >2.5mmHg. However, the results did not alter whether analysing only those tests that met the criteria for a steady state or including all test results.

Low resting $ETCO_2$ in patients with heart failure has been show in previous studies[81, 319]. This was also noted in this study this may explain some of the challenges in performing the AH test in this patient population. In animals, hyperventilation (and hypocapnia) occurs via stimulation of vagal afferents due to increased pulmonary venous pressure[320]. Patients with high pulmonary wedge pressures have lower arterial CO_2 levels than those with normal wedge pressure[321]. In heart failure arterial oxygen is usually normal so hypoxia is not driving the hypocapnia[322]. A recent study by Rocha et al 2017 that showed that patients with resting hypocapnia had higher VE/VCO2 (ventilatory inefficiency) which was related to capillary CO_2 and not to VD/VT[81]. The increased neural drive leads to an increased in ventilatory response which is above what is required to overcome 'wasted ventilation', resulting in hypocapnia. However, the hyperventilation did result in better arterial oxygenation, which in the case of heart failure with a poor cardiac output is likely to be aiming to improve oxygen delivery, although at the detriment of worsening dyspnoea and poorer exercise tolerance.

In this preliminary analysis, the D12 score performed at the end of the AH test was not affected by nebulised furosemide. There was a trend seen for greater AH relief in the physical component of dyspnoea with nebulised saline compared to nebulised furosemide. This may be due to the action of saline itself, such as moistening the airways, or may be due to chance due to the low power in this study.

5.5. CONCLUSIONS

The D12 varies consistently with the other measures and is at least as stable a measure of dyspnoea, albeit at a lower level. It can be used as a fast and efficient baseline measure of dyspnoea in patients with heart failure. The VAS and MBS compared well in the assessment of exertional dyspnoea and either could be used in future clinical trials with nebulised furosemide. The D12 appeared to be the most sensitive for detecting change in dyspnoea with nebulised furosemide and this finding suggest that in future clinical trials of nebulised furosemide it may be beneficial to include the D12

Nebulising furosemide with different breathing frequencies does not appear to enhance the relief of dyspnoea and therefore does not need to be manipulated in future trials of nebulised furosemide. There is a slight suggestion that a greater relief of AH occurs when furosemide is inhaled with a faster (shallower) breathing pattern. This may be explained by increased furosemide delivery due to the greater overall ventilation, or because less furosemide is absorbed systemically if it is being preferentially deposited in the conducting airways by the quick inhalation. However, this preliminary finding needs to be verified by targeting tidal volume as well as frequency to ensure better matching of overall ventilation.

Both exercise and hypercapnic air hunger test are able to elicit dyspnoea. The air hunger test proved difficult in this patient population, although resulted in the higher D12 scores compared to the exercise tests. Since the D12 takes account of both emotional and physical aspects of dyspnoea this might suggest that hypercapnic air hunger test has a greater affective valence compared to exercise.

Nebulised furosemide is specific to air hunger relief and it is possible that the patients are experiencing work/effort more than air hunger. CPET and 6WMT both elicited dyspnoea, with the greatest dyspnoea seen with 6MWT, with similar levels of perceived exertion. Breathlessness was the predominant cause for stopping exercise with the 6MWT, whereas for CPET it was leg fatigue. Given these points and the ease and simplicity of the 6MWT, in comparison to CPET and the AH test, this suggests it may be the optimal method to elicit dyspnoea to detect a change with intervention (nebulised furosemide) in future clinical trials.

This is explorative data. Such measures typically require larger datasets. The trends reported suggest that larger, powered studies are warranted to endorse or refute these results. One thing that has not been done previously is to see how measures of dyspnoea relief relate to exercise induced changes in cardiac biomarkers which is addressed in the next chapter.

6. CARDIAC BIOMARKERS WITH EXERCISE AND NEBULISED FUROSEMIDE

6.1. INTRODUCTION

6.1.1. Cardiac biomarkers

The main cardiac biomarker used in heart failure is the B-type naturetic peptide (BNP) or N-terminal pro-BNP (NT-proBNP). BNP is strongly linked to heart failure and can be used to discriminate between acute dyspnoea caused by heart failure, and that caused by primary lung disease[244]. It has a high negative predictive value and is therefore helpful in excluding a diagnosis of heart failure when patients present with dyspnoea. It is also useful for prognosis and monitoring response to heart failure treatment[323].

Troponins (Troponin T (TnT) and Troponin I (TnI)) are primarily used to diagnose a myocardial infarction[250] by using a single cardiac troponin value above the 99th centile and a significant time-dependent change in the cardiac troponin concentration in the presence of clinical symptoms and signs[250]. The magnitude of the concentration change used to diagnose a myocardial infarction (i.e. the δ value) is not clear. The European Society of Cardiology (ESC) recommends a change of 20-50% depending on whether the baseline troponin is below or above the 99th percentile.

Advances in immunoassay techniques have led to a 100-fold reduction in the limit of detection of troponin from 500ng/L to 6ng/L (high sensitivity troponin, hsTn). hsTnI has been shown to be associated with increased mortality in those presenting to the emergency department with acute dyspnoea[324]. The use of hsTnI has

significantly increased the number of patients with heart failure with detectable troponin to 92%[245, 246]. Measuring hsTn over a few months strongly predicts all-cause mortality (HR 1.88)[247].

6.1.2. Effect of Exercise on Cardiac Biomarkers

Exercise reduces morbidity and mortality for patients with heart failure with reduced ejection fraction[248, 249]. Primary data studies, in both healthy volunteers and patients with heart failure, have shown conflicting evidence in cardiac biomarker response to exercise, Table 6.1. However, a meta-analysis of 45 studies in healthy volunteers showed an increase in cardiac biomarker release after strenuous exercise[325]. Performing exercise in heart failure may be a confounding factor to diagnosing an MI using the Tn δ value and can increase BNP[326]. In another patient cohort (pulmonary arterial hypertension) hsTnT levels increased after maximal physical exercise whilst NT-pro-BNP remained constant[327]. One study showed a positive correlation between BNP and dyspnoea using the New York Heart Association (NYHA) function class as a scale of dyspnoea[328].

Table 6.1 Studies assessing BNP response to exercise

Study	N	Year	Participant type	Protocol	Result
McNairy et al.[326]	30	2002	Heart Failure Healthy volunteers	Submaximal cycle ergometer - 75% of maximal heart rate	NYHA I-II ↑30%, ↑3.5% at 1 hr NYHA III-IV ↑16%, ↑15% at 1hr
Kruger et al.[329]	57	2004	Chronic heart failure and healthy volunteers	Maximal cycle ergometer	BNP levels were not significantly altered by vigorous exercise
Krupicka et al.[330]	15	2010	Healthy volunteers	Maximal cycle ergometer	Fast and transient rise of plasma BNP, remained well within normal range
Zdrenghea et al.[331]	87	2014	Heart failure	Cycle ergometer 6MWT 400mWT	BNP ↑ both during maximal and submaximal exercise testing
Aengevaeren et al.[332]	191	2017	Cardiovascular disease (CVD) and healthy volunteers	Prolonged moderate intensity walking	Small ↑ BNP in CVD but not in healthy volunteers.

6.1.3. Biological Variation of Cardiac Biomarkers

The biological variability from repeated measurements of BNP and hsTnI has been studied in patients with heart failure[333-337] but not in relation to changes with repeated exercise. The within-person variation (CVi) for BNP has been shown to be between 35 and 60% in healthy volunteers, and 20-40% in heart failure patients with a between-person variation (CVg) of 40-60% in healthy volunteers, and 40-120% in heart failure[336, 338, 339]. Biological variability in patients with chronic disease is critical for interpretation and analysis of both BNP and hsTnI to understand their utility in clinical situations. The interpretation of cardiac

biomarkers in relation to exercise allows further insight into the normal variability within each individual.

6.1.4. Systemic Absorption of Nebulised Furosemide

Nebulised furosemide has been used for over 20 years in research and is thought to act via a direct action within the lungs and not via a systemic effect. This is supported by studies in rats which show that modulation of lung receptors only occur when inhaled and not when given intravenously[30], and in studies showing that the beneficial effects of furosemide are only observed when inhaled and not via tablets[282]. This theory is also supported by the lack of haemodynamic changes when inhaled[290]. However, until recently the amount of furosemide absorbed into the circulation when inhaled was unknown. In the recent study by Morélot -Panzini et al. (2018) absorption efficiency was up to 30% of the inhaled dose[185] with controlled delivery that avoided loss of furosemide to the atmosphere by separating inspiratory flow from expiratory flow. Analysis of the blood samples from this data adds to the field by determining an absorption efficiency for inhaled furosemide when delivering the aerosol via an open facemask.

It is not known whether existing blood assays for furosemide are adequately sensitive to detect small increments in blood levels of furosemide following a nebulised dose of 40mg, on top of high levels of systemic furosemide in patients with heart failure. One of the aims of this study was therefore to assess whether a change in furosemide concentration could be detected with a nebulised dose of 40mg and if possible, to compare with cardiac biomarker release during exercise.

Specific aims:

1) Establish a correlation between cardiac biomarkers and exertional dyspnoea in this patient cohort. It was hypothesised that there would be a positive correlation.

2) Verify the cardiac biomarker response to exercise and compare this between exercise types (cardiopulmonary exercise testing-CPET and 6minute walk test-6MWT). It was hypothesised that cardiac biomarkers increase with exercise and return to baseline within 60 mins, and this would not affected by exercise type.

3) Determine the biological variation in BNP and hsTnI with exercise over time. It was hypothesised that there would be minimal biological variation during a single visit and over the duration of the study (~1 month)

4) Assess the furosemide absorption efficiency and compare with cardiac biomarker release during exercise. It was hypothesised that furosemide absorption efficiency would be <30% as an open facemask is used in this study. It was also hypothesised that cardiac biomarker release with exercise would be inversely correlated with furosemide absorption.

6.2. METHODS

6.2.1. Participants and Blood Protocol

The 12 patients participated in this randomised double-blind placebo-controlled crossover presented in Chapter 5 had multiple blood samples taken which are analysed to address the aims of this study. All patients performed two exercise tests on 4 separate visits, the exercise type (CPET or 6MWT) remained the same for individuals over the course of the study. A simplified protocol is shown in Fig. 2.11 in the Methods chapter, to highlight the timing of blood samples. The 4 samples taken each day were all taken within 3 hours. The time between paired samples

was 65±22mins (mean±SD, end of first pair to start of second pair). This was repeated on 4 visits, obtained over a period of at least 21 days (mean±SD 26.9±10.2days, range 22 to 53days).

Two blood samples were taken from each patient for the furosemide assay, one before the mist inhalation and one within 5 minutes after the mist inhalation.

Full details of the protocol are described in Chapter 5. Ethics approval was covered by the approval in the study described in Chapter 5.

6.2.2. Blood sampling technique

Blood were taken via an antecubital venous cannula inserted at the start of each visit, 10ml of blood was discarded prior to each blood collection and 5ml saline was flushed into the cannula after each sample to maintain cannula patency. 12ml of blood was taken for samples A, D and E (troponin and BNP) – see Fig. 2.11. 4ml was taken for sample C (furosemide assay), and 16ml was taken for sample B (troponin, BNP and furosemide assay).

6.2.3. Data analysis

All participants had all samples taken, 16 samples collected per patient for cardiac biomarker analysis, 4 per visit for 4 visits, and 8 samples per patient for furosemide analysis. The furosemide assay analysis was undertaken in 4 patients (see Chapter 2-methods for specific details).

Linear regression was used to compare mean BNP and hsTnI level with dyspnoea ratings at the end of exercise for visual analogue scale (VAS), modified Borg scale (MBS) and Dyspnoea12 (D12). Due to the significant differences in baseline

measurement of BNP and Troponin, percentage change from the average of the first sample (A) taken each visit (1-4) was used for analysis and taken to be 100%.

Repeated measures ANOVA was performed on the cardiac biomarker response to exercise, using SPSS Version 25.

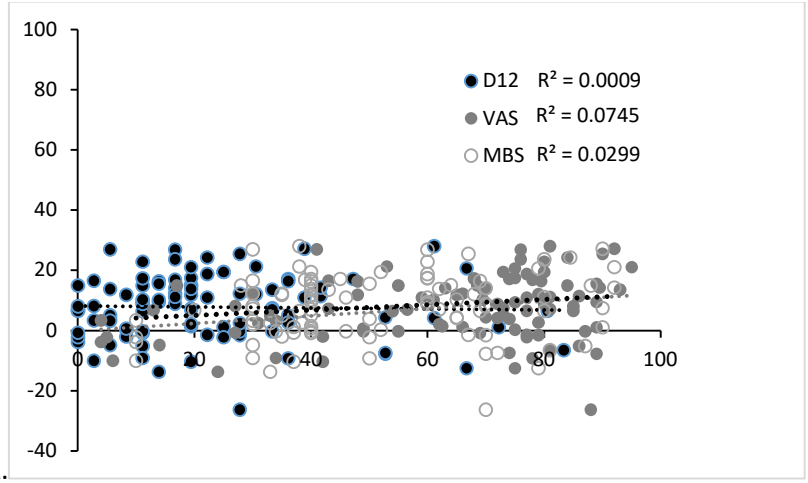
The biological variation profiles of cardiac biomarkers, BNP and Troponin I, in individuals before and after exercise (CPET and 6MWT) in patients with advanced heart failure were determined. CVi and CVg were calculated using SPSS Version 25. The biological variation profiles of change in BNP and Troponin I in individual after CPET compared to after 6MWT in patient with advanced heart failure were analysed.

6.3. RESULTS

6.3.1. Correlation between cardiac biomarkers and exertional dyspnoea

There was a poor correlation (R^2 <0.1) between the change in cardiac biomarkers (BNP and hsTnI) with exercise and all ratings of dyspnoea (VAS, MBS and D12) at peak/end exercise, Fig. 6.1. There was also no correlation between the resting baselines levels of BNP and hsTnI and exertional dyspnoea ratings.

A.



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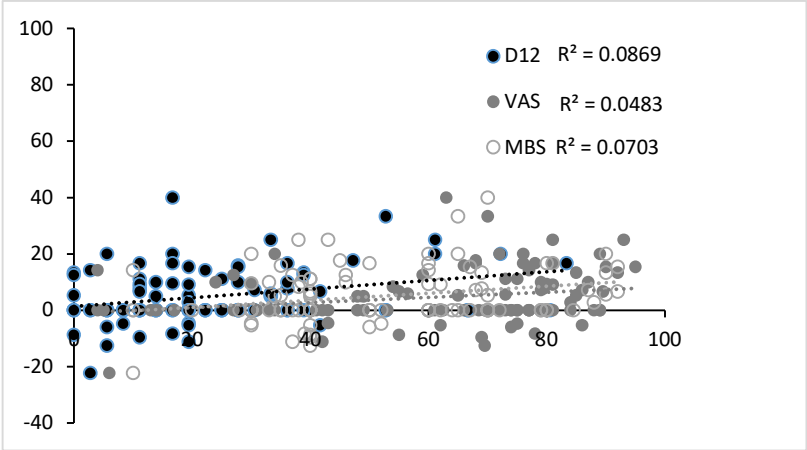

Figure 6.1 Correlation of dyspnoea ratings with cardiac biomarkers

A: Change in peak exertional dyspnoea, using the D12, VAS and MBS, at the end of exercise (CPET or 6MWT) did not correlate with BNP (% change from baseline after exercise). B: Change in peak exertional dyspnoea at the end of exercise did not correlate with hsTnI. D12 = Dyspnoea-12, VAS = Visual Analogue Scale for dyspnoea, MBS = Modified Borg Scale for Dyspnoea, BNP = B-type naturetic peptide, hsTnI = high sensitivity Troponin I

6.3.2. Effect of exercise on BNP and Troponin

BNP varied widely from 8.7 to 1096.2ng/L across all data points pooled from all the subjects. The range for hsTnI was 4 to 42ng/L. Within each subject the BNP varied by 4.2 to 289.1ng/L, and troponin varied by 2 to 17ng/L, with up to 28% change with exercise for BNP and up to 40% change with exercise for hsTnI. There were significant increases in cardiac biomarkers, BNP and hsTnI, (both p=0.001) before and after exercise, using all pooled data, Fig. 6.2.

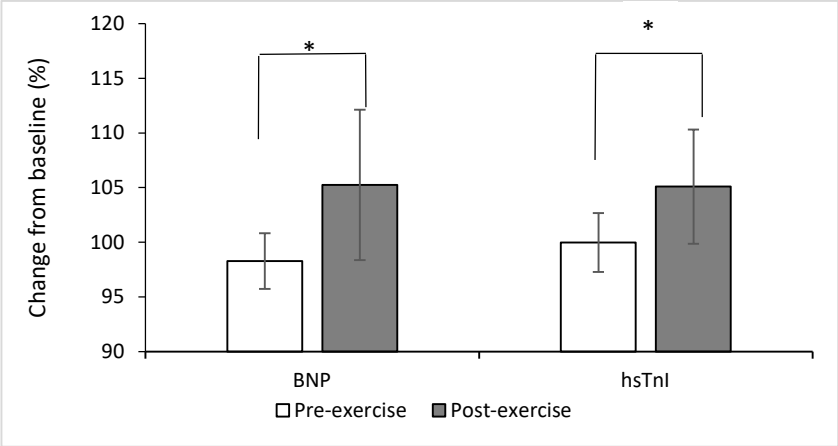

Figure 6.2 Change in cardiac biomarkers before and after exercise

*BNP and hsTnI before and after exercise, taken as a percent change from baseline (average of sample A on all 4 visits). * p=0.001 BNP= B-type naturetic peptide, hsTnI = high sensitivity troponin I*

The response was not different if analysed separately for pre and post mist; the increase in hsTnI response to exercise before and after mist inhalation remained constant at 5% and the change in BNP remained similar at 8% and 7%, Table 6.2. BNP dropped significantly (p=<0.001), to below baseline, 60 min after exercise with similar increase on second test. hsTnI returned to baseline (no overshoot) 60 min after exercise, Fig. 6.3. Separating the mists into furosemide and saline showed the same pattern, Table 6.2, with an increase in BNP and hsTnI response to exercise. BNP was also significantly reduced within 60 minutes of finishing exercise, for both furosemide and saline (p=0.01 and 0.004, respectively). There were no significant differences between furosemide and saline and response to exercise in cardiac biomarkers (p=0.74 and p=0.13, BNP and hsTnI, respectively). The hsTnI response to exercise tended to be smaller before and after the saline mist (3 and 4%, respectively) compared to the furosemide mist (7 and 8% respectively) but this difference was not seen for BNP.

Table 6.2 BNP and Troponin change with exercise before and after mist inhalation.

BNP (ng/L) = B-type naturetic peptide. hsTnI (ng/L)= high sensitivity troponin I, Ex = Exercise (cardiopulmonary exercise testing or 6-minute walk test). Diff = change in cardiac biomarkers (%) after exercise – before exercise.

	Pre-mist				Post-mist			
	Pre-Ex	Post-Ex	Diff	P value	Pre-Ex	Post-Ex	Diff	P value
BNP (%)	100±0	108±7	8	*0.02	96±5	103±6	7	0.07
hsTnI (%)	100±0	105±6	5	*0.06	101±6	106±6	5	*0.001
	Pre-Furosemide mist				Post-Furosemide mist			
	Pre-Ex	Post-Ex	Diff	P value	Pre-Ex	Post-Ex	Diff	P value
BNP (%)	100±0	108±12	8	0.18	97±13	105±14	8	*0.01
hsTnI (%)	100±0	107±15	7	0.24	99±13	107±15	8	*0.02
	Pre-saline mist				Post-saline mist			
	Pre-Ex	Post-Ex	Diff	P value	Pre-Ex	Post-Ex	Diff	P value
BNP (%)	100±0	108±9	8	*0.02	95±10	102±14	7	0.08
hsTnI (%)	100±0	103±9	3	0.84	102±13	106±12	4	0.15

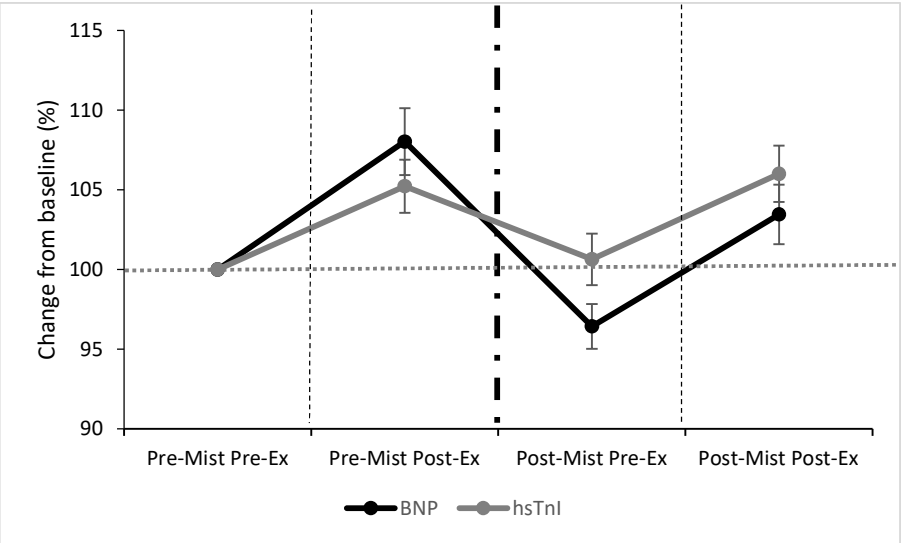

Figure 6.3 Change in cardiac biomarkers with exercise

BNP and hsTnI increase with exercise, before and after mist inhalation. hsTnI returns to baseline prior to the start of the second exercise test, within 60 minutes. BNP falls below baseline within 60 minutes. Mean %change from baseline±sem. Dash-dotted line marks mist inhalation. Light dashed lines = exercise (either CPET or 6MWT). BNP= B-type naturetic peptide, hsTnI = high sensitivity troponin I, Ex = exercise. CPET = cardiopulmonary exercise test. 6MWT = 6-minute walk test.

There were no significant differences in the response to exercise for BNP or Troponin for either of the two different modes of exercise tested (CPET or 6MWT), p=0.347, Table 6.3. BNP increased by 10% after the 6MWT compared to 6% with CPET. hsTnI remained constant with an increase of 5% for both CPET and 6MWT. Before and after mist inhalation did not significantly alter these results.

Table 6.3 Cardiac biomarkers change with two different exercise types

BNP and Troponin (% from baseline on visits 1-4) increases in response to a submaximal (6MWT) and maximal (CPET) exercise test. 6MWT = 6-minute walk test, CPET = Cardiopulmonary exercise test.

Exercise type	Cardiac biomarker	Pre-Ex	Post-Ex	Diff	P value

6MWT	BNP (%)	98±1	108±5	10	*0.006
	Trop (%)	100±3	105±4	5	*0.04
CPET	BNP (%)	98±3	104±7	6	0.06
	Trop (%)	101±3	106±7	5	*0.03

6.3.3. Biological variability of BNP and hsTnI

The laboratory analytical variation (CVa) is up to 12% for BNP and up to 10% for hsTnI. Outliers were removed using Cochran C (one subject was removed for BNP and another subject removed for hsTnI). Normality test (Shapiro Wilk) were met. The within subject coefficients of variation (CVi) is 20% and 10% for BNP and hsTnI, respectively. The within group coefficients of variation is 80% and 50% for BNP and hsTnI, respectively.

6.3.4. Furosemide absorption efficiency

Blood concentrations of furosemide were increased after a 40mg nebuliser of furosemide, Fig. 6.4. The absorption of furosemide ranged from undetectable (<0.03mg/L) to 0.17mg/L (median 0.085). The maximal absorption efficiency from nebulised furosemide was 2.1% (median 1.1%). This was calculated from the difference in furosemide concentration of the blood sample taken immediately prior to furosemide nebulisation and that taken within 5 minutes of completing it. If all 40mg was absorbed into the blood and assuming that the average volume of distribution is approximately 5 litres, the concentration in the blood would be 8.0mg/L. The absolute value of maximal absorption of furosemide in this study was 0.17mg/L (0.25mg/L detected post furosemide nebulisation minus 0.08mg/L

furosemide detected at baseline, immediately prior to nebulisation). As a percentage of blood volume this results in 2.1% maximal absorption efficiency.

Ideally, multiple blood samples would have been taken after completion of the nebuliser to determine the area under the curve and half life but due to time constraints this was not possible. A single dose was taken within 5 minutes of completing the nebuliser as this has been shown in other studies of nebulised substances (such as salbutamol) to show the peak plasma concentration[340]. However, this is an assumption and further studies are warranted to investigate this further.

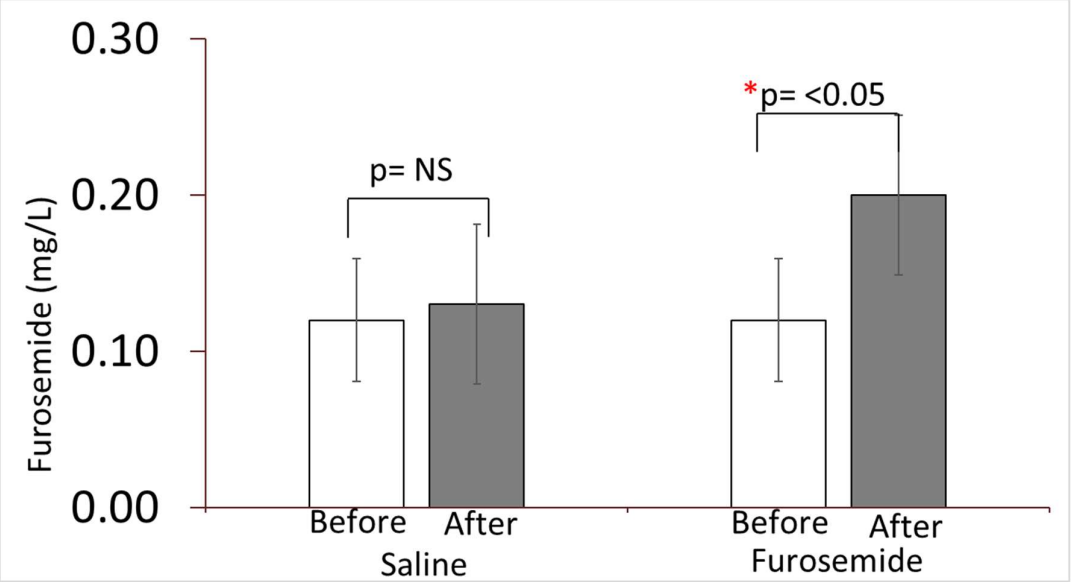

Figure 6.4 Blood furosemide assay

Blood assay results before and after 4ml nebulised saline (left) or 40mg nebulised furosemide (right). An increase in the blood levels of furosemide was detected after a 40mg dose of nebulised furosemide. No change in furosemide level was seen with saline inhalation. Data pooled from 4 visits in each of 4 patients (S2, S3, S8 and S9).

BNP response to exercise correlated poorly with the amount of furosemide absorped (R^2 =0.03, Fig. 6.5A). However, if the clear outlier was removed the

correlation became strong with an R^2 = 0.6 (p=0.04, Fig. 6.5B) indicating that the greater the absorption of furosemide from a nebulised dose the greater the BNP response to exercise. One patient had a small reduction in blood furosemide following nebulised furosemide which was just above the limit of detection for the furosemide assay; this was taken to be zero absorption assuming that the elimination of systemic furosemide does not exceed the absorption from the lungs over the period of inhalation. There was also a correlation seen with hsTnI and furosemide mist absorption (R^2 = 0.3) with no outliers identified.

A B

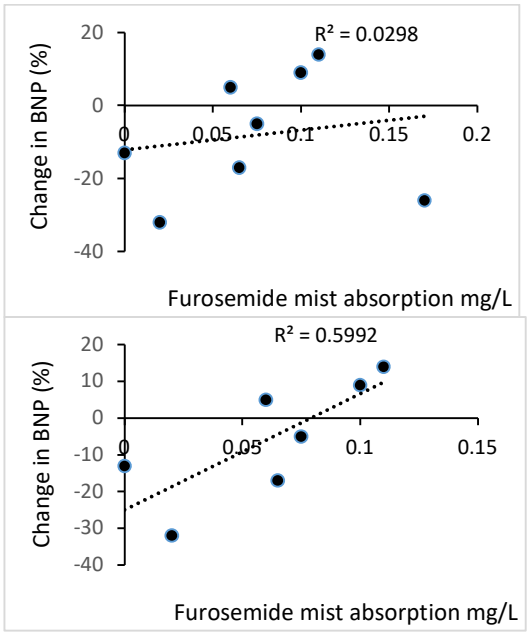

Figure 6.5 BNP response to exercise with furosemide mist absorption

A. Change in systemic furosemide levels before and after furosemide inhalation for 4 patients, on 2 visits each. B. Same plot with one outlier removed produced a strong correlation (R^2 = 0.6) indicating the more furosemide that is absorbed systemically into the blood stream from a nebulised dose of furosemide the greater the BNP response to exercise.

6.3.5. Absorption of furosemide and relief of Air Hunger

Nebulising furosemide in different breathing patterns did not significantly alter the air hunger (AH) ratings, however there was a trend for more furosemide absorbed with faster shallower inhalations compared to slower deeper inhalations, 0.1±0.05 vs 0.03±0.08 mg/L±sd. The relief of AH was not significantly altered by the systemic absorption of furosemide (8±29 vs 7±13 %VAS full scale±sd). There was a weak correlation showing the greater the furosemide absorption the greater the relief in AH, Fig. 6.6.

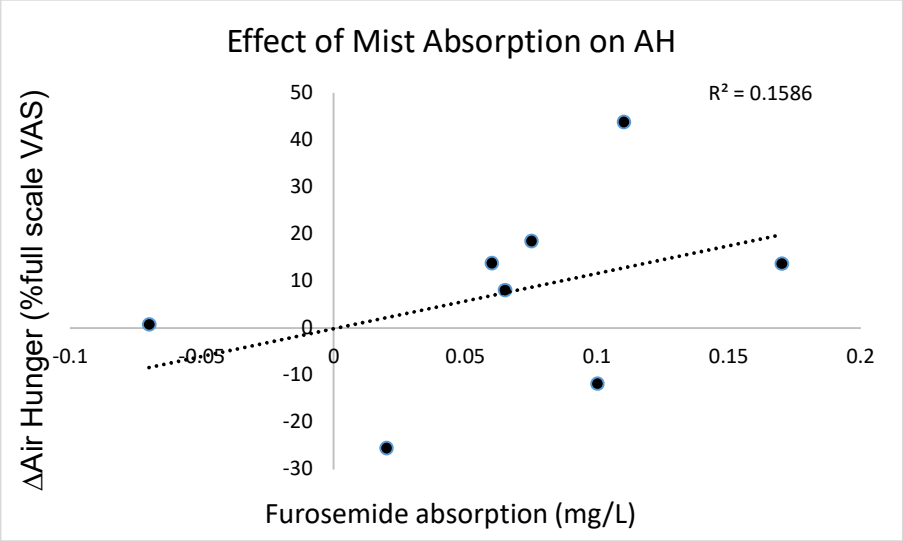

Figure 6.6 Correlation between furosemide absorption and AH relief

6.4. DISCUSSION

The main findings of the present analysis were as follows:

1) Dyspnoea ratings did not correlate with cardiac biomarker release with exercise refuting the initial hypothesis.
2) Cardiac biomarkers increased with exercise irrespective of exercise type or mist inhalation.

3) Analysis of biological variation produced lower CVi for hsTnI compared to BNP indicating that the former may be more suitable for patient follow up and determining therapeutic strategies from repeat measurements of cardiac biomarkers.
4) Furosemide assay was sensitive enough to detect small but significant changes in blood levels of furosemide following inhalation of 40mg furosemide. BNP response to exercise significantly correlated with furosemide absorption.

6.4.1. Lack of correlation of exertion dyspnoea with cardiac biomarkers

Maximal exercise is associated with dyspnoea. Exertional dyspnoea is not usually the exercise limiting factor in healthy individuals but is often the limiting factor in respiratory disease (such as COPD). BNP and hsTnI increase in response to ventricular wall stress[341, 342]; during maximal exercise this is thought to be due, in part, to increased end diastolic volume with increased stretch. Although it would be reasonable to speculate that there would be a correlation between peak exertional dyspnoea ratings and change in cardiac biomarkers response to exercise in heart failure; this may not be the case since there are many factors such as fatigue of exercising muscle that may precede any ventilatory limitations[343].

This is the first study to look at the correlation between the cardiac biomarker response to exercise and exertional dyspnoea. This was not detected for either the change in the actual BNP/hsTnI value or the percent change from the pre-exercise measurement. This may be because the increased in wall stretch (and therefore cardiac biomarker release) is only one small component to the changes that occurs

with exercise that result in dyspnoea. It does not take into account the many other changes that occur with exercise such as the increased heart rate, increased lactate, diaphragm effort, muscle fatigue etc. that also play a part in the sensation of dyspnoea. The sensation of dyspnoea is also subjective and covers a range of distinguishable subjective experiences such as work/effort, air hunger and chest tightness which are not detectable by the tools used to measure dyspnoea in this study. It may be that the BNP is linked to an individual component of dyspnoea that the VAS, MBS or D12 are not sensitive enough to detect. The timings of blood sampling may also have affected the results, although most previous studies have followed a similar protocol, with sampling done immediately prior to exercise and immediately after exercise[326]. This has been shown to be able to detect a change in BNP with exercise, and if the sample had been delayed by 1 hour the BNP would have returned close to, or below, baseline.

Resting cardiac biomarker levels also did not correlate with dyspnoea ratings. This indicates that the resting BNP or hsTnI level does not predict who will experience increased levels of dyspnoea during exercise testing.

6.4.2. Expected increase in cardiac biomarkers with exercise

Following exercise BNP increased by 8% and hsTnI by 5%. This BNP increase is lower than has been reported in some other studies, which reported increases of 15-37%[326, 344]. This difference may be explained by the patient population as in this study they were stable chronic patients with advanced heart failure on optimal medical therapy, whereas in McNairy et al. (2002) study it is not mentioned that they are stable so they may have included patients with a recent diagnosis. This is

supported by the higher baseline BNP reading in the McNairy et al. (2002) compared to this study. For those with positive response to exercise, the raw baseline level is correlated to the increase in BNP response with exercise (R^2 =0.6). The higher the baseline BNP value the greater the response to exercise. It may also be due to 'noise' as the numbers are small in both studies. This cardiac biomarker response was seen during both the maximal (CPET) and submaximal (6MWT) exercise test. The largest response (10%) was seen for BNP in response to the 6MWT. This is surprising as one might expect the largest response to be seen during a maximal exercise stress test, however in this study the response for a maximal stress test was lower at 6%. This may be due to the fact that the CPET was performed on a cycle ergometer and the participants were free to stop at their own will, although they were encouraged to do as much as possible. This is supported by an RER <1.1 in the majority of patients indicating a submaximal test. Therefore, CPET is not a true maximal test in this cohort. The 6MWT, on the other-hand, involved walking (can be more metabolically demanding than cycling slowly) and was stopped after 6 minutes. This may be close to a maximal exercise test for many of the participants with advanced heart failure and may have led to greater stress on the heart with greater release of cardiac biomarkers. Within an hour of stopping exercise hsTnI had returned to baseline, and in the case of BNP significantly below baseline and this was irrespective of type of exercise or mist inhalation (furosemide or saline). This decrease was not seen in a previous study of same cohort of patients (NYHA III-IV) where they still had a 15% increase detected at 1 hour [326], or in a cohort of healthy volunteers at 3 hours[345]. This may again be explained by the potential difference in the stability of patients in the McNairy study and this study,

with this study being of chronic stable patients on optimal therapy, whereas this is not stated in their study, or it may again be due to 'noise' due to the small numbers in each group.

Many studies have looked at the change in BNP with exercise, both in patients with heart failure and healthy volunteers (Table 6.3) although few have studied repeated exercise tests within the same patients. Sedaghat-Hamedani et al. (2013) performed a meta-analysis of 45 studies and assessed the increase in biomarker after strenuous exercise. 83% of healthy volunteers had results above the 99% centile after prolonged exercise[325]. It can therefore be thought of as a physiological response to exercise but in the case of patients with advanced heart failure this may occur after shorter, less intensive exercise.

6.4.3. Biological variation suggests that hsTnI is more suitable for monitoring heart failure

The biological variation of cardiac biomarkers within each day and across all 4 visits within each subject was smallest for hsTnI, with a slightly higher CVi for BNP (10% vs 20%, respectively). The CVg was very large for BNP (80%) and hsTnI (50%). The CVi for BNP and hsTnI are within the same ranges as previously quoted in the literature[336, 338, 346].The response to exercise was consistent during each day and across visits. The use of hsTnI to diagnose a heart attack in heart failure should not be significantly affected by preceding activity and any change >20%, above 99th centile, should be considered significant[250]. Given the smaller CVi for hsTnI this may be more suitable for monitoring heart failure than BNP. BNP increases with exercise but returns to below baseline within 60 minutes. BNP changed by a

maximum of 28% in this study and hsTnI increased by up to 40% with exercise so caution must be taken when assessing BNP or hsTnI results if the patient has rushed to clinic and had a blood sample taken immediately when being compared to their baseline readings.

6.4.4. Systemic absorption of furosemide is detectable and correlates with BNP response to exercise.

The amount of furosemide absorbed into the systemic circulation from a 40mg nebuliser is minimal, with a maximal absorption efficiency at 2.1%. This is much less than Morélot -Panzini et al. (2018) had detected in their recent study (30%) and this is likely to be due to the different delivery method used. In their study, they used controlled delivery so that the furosemide mist was only activated on inspiration; rather than in this study where the nebulisation ran continuously through an open facemask. As approximately two thirds of the breathing cycle is spent in expiration some of the furosemide mist is expelled into the environment and not inhaled.

This study suggested that increased systemic absorption of furosemide may enhance the BNP response to exercise with an $R^2 = 0.6$ if the outlier is removed. It is unclear why there was an outlier. Work rate, distance walked, and change in heart rate (as a percentage of maximal heart rate according to age) were not different in this outlier and these factors did not correlate with BNP response, before and after furosemide inhalation. It may represent a laboratory analytical error in BNP measurement, was it was not identified as an outlier for hsTnI. The reason underlining the greater BNP response to exercise with increased furosemide

absorption is uncertain. It could be argued that one would expect to see the opposite effect if the furosemide is reducing preload (via diuretic action on the kidneys), and therefore reducing wall stretch and BNP release. However, one study has investigated the possibility that nebulised furosemide could affect preload (Newton et al., 2012) but this study did not find any change[290]. Alternatively, if the furosemide inhalation was enabling patients to do more exercise (increased 6MWT, increased peak workload) then one may expect a greater increase in BNP/hsTnI in response to exercise but this increase in work capacity was not seen. One possible explanation could involve the reduction in tidal volume seen after furosemide inhalation[27] which is likely to be attenuated with greater absorption of furosemide from the lungs. Higher tidal volumes would be associated with greater venous return and could therefore result in a higher BNP release, potentially explaining a correlation between furosemide absorption and BNP response to exercise. Further studies to address this preliminary finding are required. In an on-going study, baseline respiratory frequency is recorded prior to mist inhalation. This shows the majority of patients with heart failure are hyperventilating. Sheikh et al. (2013) showed a reduction in respiratory frequency by 7bpm (from 24 to 19bpm) with nebulised furosemide [174]. If the hyperventilation seen in heart failure reduces after furosemide, it may reduce the respiratory sinus arrhythmia, and with deeper, slower breathing, there would be greater venous return, increased end diastolic volume and therefore BNP release.

The trend seen for greater furosemide absorption with fast shallow breathing compared to slower deeper breathing may be due to the higher ventilation during fast shallow breathing patterns, see previous Chapter, Fig. 5.6. The weak

correlation between the amount of furosemide absorbed and relief of AH is explorative and requires further study. It is possible that the more furosemide is absorbed, the greater the action on the $Na^{+}K+Cl-$ channels in the lungs and this enhances the action of removing any residual fluid locally.

6.4.5. Limitations

The furosemide analysis was performed in 4 of the 12 patients. 4 samples were initially analysed to see if it was feasibly possible to detect a change in furosemide blood levels after furosemide inhalation in patients already on oral furosemide tablets. It was possible to detect a significant change in furosemide but due to analytical problems, the laboratory was not able to offer further analysis on the remaining samples. Other laboratories were contacted but their sensitivities were not low enough to be able to detect the changes identified by the first laboratory. The 32 samples processed on their High-Performance Liquid Chromatography (HPLC) method posed a challenge analytically as there were multiple other peaks present in all samples. They were unable to report what these were but suggested that they were possibly related to other drugs administered to the patients. For most samples, this did not interfere with the peaks of interest. However, for eight samples there was interference on their internal standard (IS). Five of these samples did not have detectable furosemide peaks therefore these have been reported as below their lower limit of quantification. 3 samples had detectable furosemide but had interference. These three results were calculated on external standard. This is not usual procedure; however, the quality control was acceptable on external standard.

6.5. CONCLUSIONS

Dyspnoea ratings did not correlate with cardiac biomarker release with exercise. Cardiac biomarkers increased with exercise irrespective of exercise type or mist inhalation and returned to baseline or below baseline within 60 minutes. The intra-individual biological variation of BNP and hsTnI is small, however there is wide inter-individual variation. hsTnI had the lowest CVi and is therefore more suitable than BNP for patient follow up and to detect true changes in the clinical picture. Furosemide assay was sensitive enough to detect small but significant changes in blood levels of furosemide following 40mg inhalation. Furosemide absorption correlates with cardiac biomarker response to exercise. This interesting finding requires further study to elicit the reasons for this.

Given the findings of this study it would be useful to assess the furosemide assay in more detail and assess the biological variation. This would require sampling blood levels at different time points i.e immediately at the end of nebulisation, at 1 minute, 2, minutes, 5 minutes, 10minutes and 30minutes. This would give a more accurate and detailed understanding of the pharmacokinetics of nebulised furosemide.

7. CONCLUSIONS

The primary purpose of this thesis was to investigate the mechanisms and management of dyspnoea in advanced heart failure. Dyspnoea mechanisms in heart failure are not fully understood and treatment options are limited, often with patients remaining breathless despite optimal medical treatment. Furosemide as a tablet or injection is a key component of heart failure treatment to relieve congestion and thereby ease symptoms. However, increasing doses of furosemide are required over time, which can lead to renal impairment preventing further up-titration and leaving patients symptomatic. Nebulised furosemide offers an attractive treatment option that may modulate the afferent signals reporting the prevailing level of dyspnoea without appreciable addition to the systemic load. There is also the potential of targeting any residual pulmonary congestion via direct actions on the lung Na^+, K^+, Cl^-channels; studies suggest that cardiogenic lung oedema is driven by Cl- channels which are inhibited by nebulised furosemide[347].

Nebulised furosemide has been shown to relieve dyspnoea, although the variability in response has limited its transfer to clinical practice[185, 276, 295]. This thesis has; i) enhanced the understanding of the underlying mechanisms of dyspnoea, ii) addressed potential sources of variability seen in previous studies, iii) provided new evidence that dyspnoea relief by nebulised furosemide acts via a direct effect on the lungs and not via absorption into the systemic circulation. Another novel aspect of this thesis was the use of a recently developed and validated multi-dimensional dyspnoea questionnaire (D12) and the hypercapnic air hunger test, which have not previously been used in any clinical trials of heart failure. The general aims identified in the introduction are discussed in light of the findings.

7.1. General aim 1: Address a key outstanding question with regard to dyspnoea relief by nebulised furosemide

A key question that was previously unanswered is whether nebulised furosemide specifically targets a certain quality of dyspnoea. This has been addressed by this thesis and the findings recently published[276]. By studying healthy volunteers, it was possible to verify that nebulised furosemide relieves dyspnoea but also prove for the first time that nebulised furosemide specifically relieves the 'air hunger (AH)' component of dyspnoea and does not affect the 'work/effort (WE)' component within the same individuals. These results were consistent with the theory that different neural pathways result in distinguishable qualities of clinical dyspnoea[11-13] with air hunger being the most unpleasant of these[200]. Since air hunger is modulated by the activity of pulmonary stretch receptors, it is scientifically plausible that nebulised furosemide acts to relieve dyspnoea via altering the sensitivity of these receptors. The thesis also uncovered a beneficial cumulative effect in dyspnoea relief from repeated furosemide nebulisations which may inform dosing regimens should nebulised furosemide be accepted into clinical practice

The mechanism of action of nebulised furosemide was proved not to act via systemic absorption but due to a direct action within the lungs, likely via the vagal afferents on the lung stretch receptors which are involved in the sensation of 'air hunger' but not work/effort. This is consistent with studies in rats showing that direct exposure of furosemide to lung tissue results in modulation of PSR afferent activity but not when administered intravenously[30]. It also agrees with studies in humans showing beneficial effects of furosemide only when inhaled, not when

administered as a tablet[282], and an absent haemodynamic response to nebulised furosemide[290].

This study suggests that patients will derive the most benefit from nebulised furosemide when AH predominates the symptom burden. This can be detected using multi-dimensional questionnaires, such as the multidimensional dyspnoea profile (MDP)[243] which was designed to individually measure three dimensions of dyspnoea; intensity, quality and unpleasantness. Nebulised furosemide may significantly alter the symptom burden profile of heart failure patients towards a less unpleasant experience.

7.2. General Aim 2: Determine the symptom burden of dyspnoea among heart failure patients in the community setting.

Dyspnoea is accepted to be multi-dimensional, similar to pain[348]. A recently validated multi-dimensional questionnaire, the Dyspnoea-12 (D12), was used in this thesis to determine the extent of dyspnoea prevalence within patients with heart failure living in the community. The prevalence of dyspnoea within the community setting has been studied in a variety of different patient groups[349-351], but none have been performed using the D12. The D12 had not previously been used in clinical trials of patients with heart failure, despite this cohort being included in the original validation of this instrument[7]. The D12 score reflects both the physical and emotional aspects of dyspnoea. Compared to other methods for measuring dyspnoea, such as the visual analogue scale or modified Borg scale, the D12 has the advantage of also determining whether the dyspnoea comprises of mainly a physical or an emotional component.

Chapter 3 involved a D12 postal survey which provided preliminary data on dyspnoea in the local heart failure community which, although underpowered, confirmed that dyspnoea is a prevalent symptom of heart failure. In this preliminary analysis, there was a suggestion that the prevalence of dyspnoea was higher in patients with a preserved ejection fraction compared to those with a reduced ejection fraction, although the levels of dyspnoea recorded on the D12 were similar. Gender and age did not appear to affect the D12 score in this limited analysis.

Dyspnoea in heart failure is usually thought to occur only on exertion (until the end stages of the disease), however this study provided preliminary data showing that almost half the patients experienced some dyspnoea at rest. This has not been previously reported in the literature. Unlike in chronic obstructive pulmonary disease (COPD)[263], there was no obvious variation during the day or over the week. Although underpowered, and as to be expected, the presence and level of dyspnoea on the D12 correlated with the New York Heart Association (NYHA) class, with the higher NYHA score associated with the higher D12 scores. Many patients were classed as NYHA III. The description of this class states that patients are 'comfortable at rest', however this study showed that substantial numbers within this class of patient were dyspnoeic at rest. It is possible that patients learn to tolerate their dyspnoea and report being comfortable at rest, despite still being symptomatic.

7.3. General Aim 3: Lay the groundwork for a future clinical trial of nebulised furosemide for dyspnoea relief as an adjunct to treatment of chronic heart failure

Previous studies have shown variability in the extent of dyspnoea relief with nebulised furosemide[29, 276, 295]. The sources of this variability are not well understood. In Chapter 5 a number of potential sources of this variability were explored. This study was a pilot study and although statistical analysis was performed, this needs to be treated with caution as the results are underpowered to draw conclusions. The following conclusions based on the preliminary analysis are intended to be hypothesis generating.

The preliminary study reported in chapter 5 was unable to detect a clear effect on dyspnoea relief by changing the breathing frequency when inhaling furosemide. However, in this study tidal volume was not controlled and did not fully compensate for changes in breathing frequency so that overall ventilation was not well matched between conditions. Thus, this study cannot fully discount breathing pattern of inhalation as a potential cause of variability in dyspnoea relief with nebulised furosemide. However, two recent studies of dyspnoea relief with nebulised furosemide used a different delivery method (via a mechanical ventilator) and also found that this did not affect the variability in response[185, 295].

Two different methods were used to induce dyspnoea - exercise and hypercapnia. While exercise is an established dyspnoeic stimulus that has been widely used in clinical practice, the hypercapnic air hunger test has not previously been used to experimentally induce dyspnoea in patients. The AH test was difficult to deliver in

this patient population, with many results having to be excluded due to not reaching the requirements of a reliable test. The criteria for unreliable data was 1) scoring 'extreme' on the visual analogue scale (VAS) scale whilst trying to achieve a steady state, 2) targeted $ETCO_2$ levels differing by >1mmHg between the last minute of pre- and post-nebuliser tests and 3) targeted $ETCO_2$ fluctuating by more than 2.5mmHg during the steady state.

In addition to the above, on-going studies in our lab show that high numbers of patients with heart failure exhibit hyperventilation at rest (hypocapnia). This has been previously noted in the literature[81, 322] with high pulmonary pressures being the proposed mechanism as discussed in Chapter 5[319]. Another notable finding in patients with heart failure is oscillatory breathing. This is thought to arise from enhanced carotid body chemoreflex sensitivity with surges of sympathetic nerve activity and hyperventilation causing disruption to the respiratory control system. The brainstem integrates the peripheral afferents and controls the respiratory and sympathetic nerve activity[352]. This in turn activates the sympathetic nervous system via respiratory-sympathetic coupling which leads to a reduction in the vagal efferent signals to the heart and increased sympathetic activity. This causes a worsening of cardiac function. The kidneys are also susceptible to the increase in sympathetic nerve activity via the carotid body reflex, resulting in reduced renal perfusion and activation of the renin-angiotensin-aldosterone system, with a decline in renal function. The carotid body mediated respiratory sympathetic coupling effect on the heart and kidneys is known as cardiorenal syndrome[353]. The hyperventilation makes the air hunger test challenging to control, particularly when trying to match pre- and post- mist conditions. This suggests that the air

hunger test needs to be modified if it is to be used in future clinical trials to try to achieve the a more reliable data set. This could be achieved for example, by including the use of a ramp protocol before and after the mist, rather than aiming for a 'steady state'.

The utility of various rating scales of dyspnoea were tested in this thesis. Whilst the D12 has been translated into many different languages, validated and compared with other tools, it has not previously been employed in a randomised controlled trial. The data showed good concurrent validity with the other dyspnoea measurements used in this study. The D12 was able to detect the greatest change in exertional dyspnoea with nebulised furosemide, although this was below the minimally important clinical difference (MCID)[354] so is a change that may not be appreciated by the patient.

As expected, cardiac biomarkers increased acutely after exercise in this study with a small intra-individual and wide inter-individual biological variation of high sensitivity troponin I (hsTnI) and brain naturietic peptide (BNP). They returned to baseline within 60 minutes. This was irrespective of exercise type (cardiopulmonary exercise test-CPET or 6minute walk test-6MWT) and there was no correlation between exertional dyspnoea ratings and cardiac biomarker response to exercise.

Analysis of furosemide detected in blood samples from 40mg nebulised furosemide showed variability in the amount absorbed from the lungs, with a maximal absorption efficiency of 2%. This thesis showed an association between the systemic absorption of furosemide and the BNP response to exercise. The reasons for this are unclear and this interesting finding requires further study to elicit the

reasons for this. In another recent study[185] furosemide absorption from an inhaled dose noted that there was an inverse correlation between the amount of furosemide absorbed and the dyspnoea relief. This might imply the longer the furosemide remains in contact with the pulmonary stretch receptors the greater the dyspnoea relief.

CPET and 6MWT were both found to be valid methods of inducing dyspnoea. Preliminary analysis did not show any significant improvement in the distance walked or the work rate achieved with nebulised furosemide. All patients were able to perform CPET despite being in the advanced stages of heart failure, however CPET is more time consuming and resource heavy with significant technical skill required for interpretation. Therefore, on balance, 6MWT is preferable in this patient population as it is simple, inexpensive and all patients were able to perform the test.

7.4. CONCLUDING REMARKS

The healthy volunteer study in this thesis provides the most convincing evidence of a treatment effect of nebulised furosemide for dyspnoea relief to date, and supports the theory that it acts via the manipulation of pulmonary stretch receptors. Furthermore, the doubling of dyspnoea relief with the second dose might inform future dosing regimens should nebulised furosemide enter clinical practice. Due to the nature of the condition, it was reasonable to expect that advanced heart failure patients would show a pronounced dyspnoea relief with nebulised furosemide. The preliminary study reported in this thesis was not able to support this belief. The low $ETCO_2$ (hyperventilation) seen in some of the patients

may have amplified the 'work/effort' component of their dyspnoea which, as shown by the healthy volunteer study, is not relieved by nebulised furosemide. While this study in heart failure provides useful data for the design of future clinical trials with nebulised furosemide this was a pilot study and the trial was insufficiently powered to generate concrete conclusions regarding the treatment effect of nebulised furosemide for dyspnoea relief. The survey of patients with heart failure within the community showed a high prevalence of low levels of dyspnoea at rest. Despite the excellent safety record of nebulised furosemide, the minimal side effects, broad beneficial local effects within the lungs and the scientific plausibility in its mechanism of action; there remains considerable doubt about its clinical utility. The thesis sends a strong signal that if nebulised furosemide is harnessed correctly it could form a viable option for dyspnoea relief in a specific cohort of patients. Future research within this field is therefore warranted. A further study that is powered to assess the effects on nebulised furosemide on dyspnoea relief in patients with heart failure is currently on-going. The addition knowledge gained in this thesis has been used to inform and simplify this current study. This includes the use of the VAS to assess dyspnoea, and not the MBS. Additionally, no specific breathing pattern during nebulisation is required. This current study will be published in a peer-review journal.

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