An Evaluation of Central and Cerebral Haemodynamic Interactions in Chronic Obstructive Pulmonary Disease

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2021

Thesis submitted for the degree of Doctor of Philosophy

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Abstract

Background:

Chronic obstructive pulmonary disease (COPD) is characterised by airflow obstruction, an independent predictor of increased cardiovascular risk including stroke. Cardiovascular risk can be predicted by central haemodynamics including arterial stiffness as measured by aortic pulse wave velocity (aPWV) and central pulse pressure (CPP) in addition to cerebral haemodynamics as measured by middle cerebral artery pulsatility index (MCAPI). Arterial stiffness is increased in COPD, which may be linked to the cerebral haemodynamics and risk of stroke. This study aimed to investigate the associations between central and cerebral haemodynamics and measures of health status in patients with COPD compared to aged-matched controls.

Method:

The thesis comprises of three studies:

- A longitudinal study included three assessment timepoints (baseline, 2 and 6 years) in patients with COPD and controls. Detailed arterial assessments included aPWV and CPP using the SphygmoCor system. Lung function was assessed using forced expiratory volume in the first second/ forced vital capacity (FEV_1/FVC) measured using spirometry. Health status included the measurement of physical function using the validated Time-Up-and-Go test (TUG) and the Six-minute Walking Test (6MWT), as well as subjective measures including the Comprehensive Geriatric Assessment (CGA), the COPD Assessment tool (CAT) and the St George's Respiratory Questionnaire (SGRQ).
- A cross-sectional study investigating the differences in patients with COPD and controls included all above assessments, with the addition of middle cerebral artery pulsatility index (MCAPI) using transcranial Doppler ultrasound.
- A cross-sectional study investigated differences in cerebral volumes (white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF) volumes) using MRI in patients with COPD and controls.

Results:

- The longitudinal study included 75 patients with COPD and 71 controls. At each assessment time-point, aPWV remained significantly higher in COPD patients than controls but both groups showed a similar change in aPWV. After 6 years, patients with COPD had lower FEV_1/FVC and physical function as measured by the TUG, 6MWT and CGA, all were related to aPWV (all p<0.05).
- The cross-sectional study included 45 patients with COPD and 50 controls. MCAPI was similar in COPD and controls. In COPD, MCAPI was significantly associated with CPP (r=0.433, P=0.003) and FEV_1/FVC (r=0.330, P=0.027), but not aPWV. In controls, MCAPI was associated with CPP (r=0.601, P=0.001) and aPWV (r=0.452, P=0.001). In COPD, using Stepwise Multiple Regression Analysis, CPP remained an independent predictor of MCAPI (P=0.003).
- Pilot data from 6 patients with COPD and 6 aged and gender-matched controls showed no difference in cerebral volumes between the groups. MCAPI was associated with GM and CSF volumes, with no association between aPWV or CPP and cerebral volumes.

Conclusion:

Patients with COPD have increased arterial stiffness, cardiovascular comorbidities and poorer health status compared to matched controls. The chronically increased and maintained higher levels of aPWV suggests premature vascular ageing and increased cardiovascular risk in COPD patients. Although patients with COPD had increased aPWV, only CPP was independently associated with cerebral flow pulsatility, suggesting the utility of CPP to potentially indicate and monitor cerebrovascular changes throughout disease. The relationship between cerebral pulsatility and cerebral volumes measured by MRI suggests a potential role of MCAPI in cardiovascular risk prediction. However, further large-scale studies are needed to improve understanding of the systemic mechanisms underlying cardiovascular risks and their interaction with comorbidities to improve outcomes in COPD.

Acknowledgements

First and foremost, I am extremely grateful to my supervisors, Dr Nichola Gale and Dr Barry McDonnell for their invaluable advice, continuous support, and patience during my PhD study. Their insight, knowledge and plentiful experience steered me through this research. Their guidance and encouragement have been beyond valuable for both the work of this thesis and my professional development. It was a real privilege and an honour to work with both of you.

My sincere gratitude to all participants that took part in the study, who were so generous with their time and enabled this research to be possible. I also would like to thank the following people, without whom I would not have been able to complete this research:

- Maggie Munnery for training me to use the SphygmoCor system and taking part in the inter-rater reliability of aPWV as proficient user. I thank her for allowing me to shadow her and for providing guidance on clinical aspects encountered during data collection.

- Laura Watkeys for her input in the training of using TCD and taking part in the inter rater-reliability of MCAPI measurement as proficient user. Also, for coordinating access to the Health Assessment Suite and for helping with technical aspects of this research.

- Dr Eric Stohr for his valuable consultation, advice, and practice with regards to the study of validation of measurement positions of MCAPI.

- Professor John Cockcroft for his input and valuable discussions.

- Cardiff University Brain Research Imaging Centre team led by Professor Kevin Murphy for their invaluable part in the MRI study. A great thanks to Dr Jessica Steventon for her role in screening participants for MRI, data acquisition and analysis. Thanks also goes to Dr Marcello Venzi and Joseph Whittaker for their assistance in data acquisition and analysis.

- The research support office at the school of HCARE, Cardiff University, for their continuous support.

- The staff at the front reception, Cardiff Met University, Llandaff campus, for their generous support with participants bookings.

- The Ministry of Higher Education, Research and innovations, Oman for their sponsorship of this PhD through the National Scholarship program. Thanks to the Ministry of Health, Oman for providing support.

A heartfelt thanks to my husband and two sons, father, siblings, friends and colleagues, who have been by my side throughout this PhD. Without their tremendous understanding and encouragement in the past few years, it would be impossible for me to complete my study.

List of publications

Published papers:

McDonnell, B. Beaney, T. **Al Shezawi, M.** Cockcroft, J. et al, on behalf of the International Society of Hypertension and British and Irish Hypertension Society, May Measurement Month 2018: an analysis of blood pressure screening results from the UK and the Republic of Ireland, *European Heart Journal Supplements*, Volume 22, Issue Supplement H, August (2020), Pages H132–H134, https://doi.org/10.1093/eurheartj/suaa047

Published abstracts:

Al Shezawi, **M**. Munnery, M. Cockcroft, J. Watkeys, L. Stohr, E. Gale, N. McDonnell, B (2020). P80 Predictors of Middle Cerebral Artery Pulsatility Index in Chronic Obstructive Pulmonary Disease and Healthy Controls; Data from the ACRADE Study. *Artery Research* 25(Supplement 1), pp. S123-S123. doi: https://doi.org/10.2991/artres.k.191224.110

Brown, C. **Al Shezawi, M**. Watkeys, L. Munnery, M. Pugh, C. Stöhr, E. McDonnell, B (2020). P59 Marked Differences in Cerebral Haemodynamics Obtained with Transcranial Doppler vs. 2-D Angle-corrected Ultrasound. Artery Research 25(Supplement 1), pp. S100-S100. doi: https://doi.org/10.2991/artres.k.191224.090

Gale, N. **Al Shezawi, M**. Munnery, M. McDonnell, B. Cockcroft, J (2018). P72 Aortic Pulse Wave Velocity in Patients with Copd: 5-Year Data from The Arcade Study. *Artery Research* 24(C), pp. 99-100. doi: https://doi.org/10.1016/j.artres.2018.10.125

Al Shezawi, M. Cockcroft, J. Munnery, M. Watkeys, L. Gale, N. McDonnell, B (2018). P93 Relationship Between Aortic Pulse Wave Velocity and Mid Cerebral Artery Pulsatility Index In Patients With Chronic Obstructive Pulmonary Disease; Pilot Data From The Arcade Study. Artery Research 24(C), pp. 105-106. doi: https://doi.org/10.1016/j.artres.2018.10.146

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List of abbreviations

aPWV	Aortic Pulse Wave Velocity
aSBP	Aortic Systolic Blood Pressure
ACE	Angiotensin Converting Enzyme
ARBs	Angiotensin Receptor Blockers
AIx	Augmentation Index
BMI	Body Mass Index
BP	Blood Pressure
CVD	Cardiovascular Diseases.
CUBRIC	Cardiff University Brain Research Imaging Centre
c-f PWV	Carotid-Femoral Pulse Wave Velocity
CIMT	Carotid Intima-Media Thickness
CPP	Central Pulse Pressure
CBF	Cerebral Blood Flow
CBFV	Cerebral Blood Flow Velocity
CSF	Cerebrospinal Fluid
CVA	Cerebrovascular Accidents
CVR	Cerebrovascular Reactivity
CKD	Chronic Kidney Disease
COPD	Chronic Obstructive Pulmonary Disease
CDI	Colour Doppler Imaging
CGA	Comprehensive Geriatric Assessment
CAT	COPD Assessment Test
CHD	Coronary Heart Disease
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
EDV	End-Diastolic Velocity
FLAIR	Fluid Attenuated Inversion Recovery

FAST	FMRIB's Automated Segmentation Tool
FEV1	Forced Expiratory Volume in One Second
FVC	Forced Vital Capacity
GM	Grey Matter
HR	Heart Rate
ICV	Intracranial Volume
ICC	Interclass Correlation Coefficient
IHD	Ischemic Heart Disease
MRI	Magnetic Resonance Imaging
MAP	Mean Arterial Pressure
MCA	Middle Cerebral Artery
MCAPI	Middle Cerebral Artery Pulsatility Index
MI	Myocardial Infarction
PSV	Peak Systolic Velocity
PI	Pulsatility Index
PP	Pulse Pressure
PWV	Pulse Wave Velocity
QoL	Quality of Life
RI	Resistive Index
SVD	Small Vessels Diseases
SD	Standard Deviation
SGRQ	St George's Respiratory Questionnaire
SBP	Systolic Blood Pressure
SPV	Systolic Peak Velocity
TAWF	Temporal Acoustic Window Failure
TCD	Transcranial Doppler
TIA	Transient Ischemic Attack
TUG	Timed Up and Go Test
TNFα	Tumour necrosis factor-α
WMHs	White Matter Hyperintensities

List of appendices

Chapter 1

Background and Literature Review

Chapter 1 : Introduction and literature review

1 Background

Chronic Obstructive Pulmonary Disease (COPD) is chronic inflammatory condition, characterised by poorly reversible airflow obstruction. COPD is a progressive disease were, in most cases, symptoms manifest during mid-life or later. It is estimated that the worldwide prevalence of COPD is 12% (Varmaghani et al. 2019) and in the UK, around 1.2 million people are currently diagnosed with COPD. However, the number of undiagnosed cases is estimated to be closer to 2 million. It is the fifth most common cause of mortality and accounting for nearly 30,000 deaths every year in England (British Lung Foundation 2017; Resource impact report NICE 2018) and is associated with a substantial economic burden (Iheanacho et al. 2020).

COPD is an umbrella term that includes two primary conditions; these are chronic bronchitis and emphysema. Both conditions affect the lung in a different way where chronic bronchitis is a disease of airway, whereas emphysema affects the alveoli, the small air-sacs of the lung. Each condition has distinct characteristics including a productive cough in chronic bronchitis and air-trapping in emphysema. However, in most cases, they can present together. Nonetheless, both conditions share similar outcomes including impaired gas exchange, dyspnoea and exercise intolerance (Kim and Criner 2013). In addition, asthma and COPD can coexist in many patients. Asthma is defined by reversible airflow obstruction; it is usually considered as a separate clinical entity however if poorly controlled and over time, airflow obstruction may become irreversible and may fit the definition of COPD (GOLD 2018).

Damage to the lungs is predominantly caused by smoking; however, exposures to harmful dust, chemicals, and wood smoke have also been associated with the development of the disease especially in developing countries (Devine 2008). In rare cases affecting only 1-2% of patients with COPD, deficiency of the alpha-1 antitrypsin protein exposes the lungs to proteases such as neutrophil elastase. Except for alpha-1 antitrypsin, exposure to toxic particle leads to an exaggerated response in COPD (Devereux 2006).

Inflammation, oxidative stress, protease–antiprotease imbalance, are key pathogenic triad of COPD (Figure 1.1). Inflammation in the small airways of the lung is a normal protective response to inhaled toxins results from smoking or chemicals. This process is exaggerated in COPD and leads to an increase in inflammatory cells, including neutrophils, macrophages, and T lymphocytes in the airway, which release cytokines and inflammatory mediators and enzymes that lead to tissue destruction (Fischer et al. 2011). Furthermore, inflammatory cells can also increase oxidative stress where oxidative stress markers, including hydrogen peroxide and 8-isoprostane, are increased in sputum and systematic circulation in patients with COPD. The increase of oxidative stress can lead to anti-proteases' inactivation, a protective mechanism that prevents alveolar wall destruction and mucus gland hyper-stimulation. Also, there is a reduction in a transcription factor that regulate many antioxidant genes leading to a reduction in endogenous antioxidants. The extent of the inflammation is related to the degree of the airflow obstruction and can participate in the disease process (MacNee 2006; Malhotra et al. 2009; Barnes 2016). Also, Protease-antiprotease imbalance is considered an essential process in the development and progression of COPD. Many of these proteases are involved in the destruction of elastin fibres (Pandey et al. 2017).

Figure 1.1 : Pathogenic triad of COPD: oxidative stress, protease–antiprotease imbalance, and inflammation [1]

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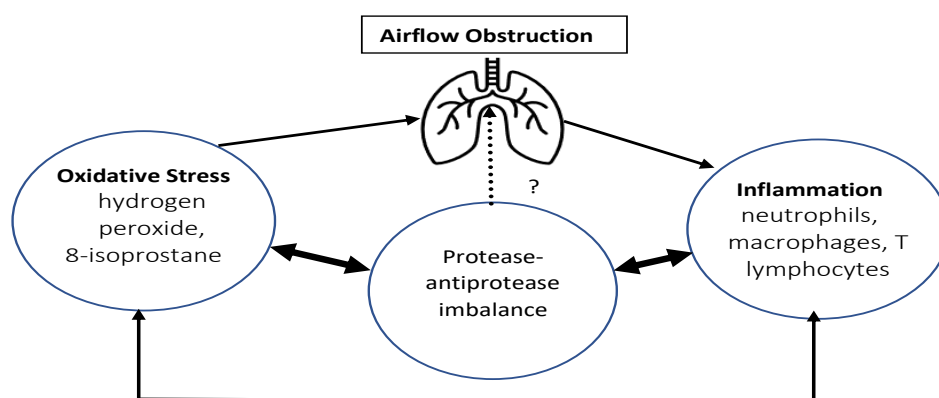
[1]Adapted from (Fischer et al 2011)

These pathological changes lead to physiological dysfunction in the form of airflow obstruction caused by progressive airway obstruction, overstimulation of mucus glands, air trapping, gas exchange abnormalities, pulmonary hypertension (MacNee 2006), and skeletal muscle dysfunction (Maltais et al. 2014). As a result, patients with COPD present with symptoms such as dyspnoea, chronic cough, excess sputum production, fatigue, and exercise intolerance. In addition, patients can also go through periods of acute worsening in symptoms known as COPD exacerbations that are triggered by viral or bacterial infection, or exposure to dust or chemicals which requires intensive treatment by antibiotics, steroids and, in severe cases hospitalisation, ventilatory support (Rabe et al. 2007).

Spirometry is the gold standard for diagnosing COPD to establish the presence of airflow obstruction that is not fully reversible, however the identification of symptoms and associated risk factors also contribute to the diagnosis of COPD. Poorly reversible airflow obstruction is a key characteristic of COPD and is used to diagnose and categorise the severity of COPD. A post-bronchodilator Forced Expiratory Volume in 1 second (FEV_1) /Forced Vital Capacity (FVC) <0.70 confirms the presence of airflow obstruction. Also, this ratio and percentage of predicted FEV_1 is used to classify COPD severity based on four main categories as recommended by the Global Initiative for Chronic Obstructive Lung Disease (GOLD 2018). These are:

- GOLD 1—mild: $FEV_1 \geq$ 80% predicted
- GOLD 2—moderate: 50% $\leq FEV_1 <$ 80% predicted
- GOLD 3—severe: 30% $\leq FEV_1 <$ 50% predicted
- GOLD 4—very severe: $FEV_1 <$ 30% predicted (Rodriguez-Roisin et al. 2017).

Although an accelerated deterioration of lung function over time is expected as disease progress (D'Amato et al. 2016), evidence shows that the progression of COPD is very heterogeneous with some patients having rapid deterioration in lung function, others maybe more stable over time (Casanova et al. 2011b).

The management of COPD consists of a combination of smoking cessation, pharmacotherapy, and pulmonary rehabilitation. These together aim to relieve symptoms, slow the disease progression, reduce the rate, and the extent of acute exacerbations in addition to improving exercise tolerance (Rodriguez-Roisin et al. 2017). The main pharmacotherapy agents used in the management of COPD are maintenance bronchodilators such as β_2-agonists, anticholinergics, mucolytic agents, and inhaled corticosteroids (Harrison and Kim 2019).

1.1 COPD and comorbidities

It is well established that COPD is frequently associated with numerous extra-pulmonary conditions which include conditions affecting other body systems beyond the lungs. The presence of these conditions has a significant impact on the health status of these patients in addition to increased mortality risk. Some of these conditions include Cardiovascular Disease (CVD), weight loss, nutritional abnormalities, skeletal muscle dysfunction and cerebrovascular diseases such as cerebral small vessel disease, stroke, and cognitive impairment (Sin et al. 2006; Cavailles et al. 2013). The multisystem nature of the disease represents an increasing burden on healthcare resources. However, early diagnosis and treatment can help to reduce the impact of the disease and improve the quality of life of individuals living with the disease (Duerden et al. 2015; British Lung Foundation 2017).

The presence of additional co-existing disease in an individual is frequently referred to as "comorbidity," which was first proposed by Feinstein (Feinstein 1970). Comorbidity, also referred to as multi-morbidity, is when two or more diseases affecting different organ or system and has distinct aetiology and pathophysiology are present in one individual (Krueger and Markon 2006). However, the specific definition and use of the term lacks an agreed consensus. Comorbidity may be linked to frailty which is defined as an age-associated decline in reserve and function across multiple physiologic systems (Valderas et al. 2009). However, these substitutions incorporate numerous theoretical concepts such as co-occurrence or correlation, where the existence of the two diseases may happen by chance as in co-occurrence, or the two diseases may be related as in correlation (Krueger and Markon 2006). Nevertheless, the term comorbidity also has elements of co-occurrence but also

acknowledges a possibility of underlying causality between the two diseases; hence it is the more accepted term in the medical field (Boyd et al. 2016).

One of the most common comorbidities in COPD is Cardiovascular Disease (CVD), which encompasses the diseases of heart and blood vessels and includes Coronary Heart Disease (CHD) and Cerebrovascular Accident (CVA) (Schwab et al. 2017). In CVA, there can be a permanent disruption of the blood supply into the brain such as ischemic or haemorrhagic stroke or temporary disruption of blood supply in case of Transient Ischemic Attack (TIA). It is estimated that unspecified CVD is prevalent in 28% to 70% of COPD patients and accounts for 50% of deaths (Mullerova et al. 2013). In a systematic review and meta-analysis including COPD patients with varied age range and disease severity, COPD patients were found to present with 2-5 times greater risk of developing different forms of CVD compared to matched controls (Chen et al. 2015).

A potential mechanism explaining comorbidity/multi-morbidity in COPD is that of premature biological ageing. Biological ageing brings about anatomical, physiological, and immunologic changes to individuals that may result in structural or functional deficits, which can lead to frailty (Agustí 2005). Frailty is a state of increased vulnerability caused by the ageing-associated decline in reserve and function at multiple body systems (Fried et al. 2001). COPD has been associated with alterations in markers related to ageing such as shorter Telomere length, decreased anti-ageing molecules sirtuin 1,total klotho, and soluble klotho in addition to reduced Ku70, a DNA repair gene. However, Telomere length remained the only ageing marker which independently associated with lung function leading to accelerated biological ageing in these patients. Based on these ageing markers, patients with COPD were found to be biologically approximately 7.5 years older than similar age healthy individuals (Rutten et al. 2016).

The role of age in accelerating vascular changes had been recognised since the 17th century by Thomas Sydenham, who claimed: "a man is as old as his arteries" (Brew and McArthur 2020). The health/age of arteries is reflected in the function or stiffness of arteries which may be associated with CVD and has been found to be altered in COPD with evidence of increased

arterial stiffness (Mills et al. 2008). Several mechanisms are proposed to explain the relationship between COPD and increased arterial stiffness beyond the age-related changes in the arteries. However, the literature lacks consensus, and further work is required to establish the extent of the effect of these factors in the progression of arterial stiffness.

Arterial stiffness can increase due to arteriosclerosis, a disease of the muscular medial layer of the artery rather than atherosclerosis, a condition of the intimal layer of the artery. It results from vascular smooth muscle hypertrophy and increased medial and adventitial collagen deposition (Vlachopoulos et al. 2015), leading to reduced effectiveness of the elastic reservoir of the aorta.

Independent of chronological ageing, the elastic properties of arteries act as a safeguard to excessive flow and pressure outputs generated from ventricular ejection. The high pulsatile flow is buffered initially in the aorta through the Windkessel effect, in which the aorta distends and recoil, regulate and determine the appropriate pulsatile blood pressures and flow being transmitted down the arterial tree to sensitive peripheral end organs (Nichols 2011). Compliant arteries are highly distensible, allowing a small increase in systolic pressure with a relatively significant increase in volume leading to low aortic wall tension and low left ventricular workload. However, as the arteries age and become stiff, this protective mechanism is impaired considerably (Izzo and Shykoff 2001; Boutouyrie et al. 2009; Cockcroft and Mancia 2012), leading to alterations in pressure, blood flow and arterial diameters with each heartbeat (Townsend et al. 2015). Mechanistically, arterial stiffness is the resistance of the arterial wall to expansion when arterial volume increases (Gavish and Izzo 2016). The expansion in the diameter of the artery wall in relation to pressure, known as distensibility, is determined by the relationship between the proportion of collagen and elastin recruited in the vasculature (Barksdale and Logan 2009). This is vital in buffering pressure profiles and flow with periodic variations, known as pulsatility (Gavish and Izzo 2016). As consequences of increased stiffness, the pulse wave propagates and travels through the artery at a much higher speed than a less stiff arterial segment, hence increased velocity of the pulse wave is considered as a marker of arterial stiffness (Vlachopoulos et al. 2015) and an independent marker of CV risk (Ben-Shlomo et al. 2014).

1.2 Pathophysiology of arterial stiffness

Inflammatory changes and increased oxidative stress in COPD have also been linked to systematic manifestations, including cardiovascular disease (MacNee 2006; Sin et al. 2006). Prolonged low-grade inflammation leads to endothelial cells dysfunction that causes alterations in the arterial wall structure. These alterations can result from the loss of protective mechanisms against atherosclerosis and arteriosclerosis caused by increased oxidative stress. This can lead to arterial stiffness, a key risk factor for CVD development (Maclay and MacNee 2013; Castellon and Bogdanova 2016). Also, a reduction in the levels of oxygen known as hypoxia, is a common characteristic of COPD and was associated with CVD development. Hypoxia impacts the vascular tone by increasing oxidative stress and ROS production, affecting the regulation of vascular ion channels and cell signal pathways leading to vascular dysfunction (Abe et al. 2017). In addition, hypoxia increases heart rate and cardiac output via β-adrenergic stimulation resulting in higher blood pressure BP, impacting vascular function, and predisposing individuals to increased CVD risk (Brassington et al. 2019).

1.3 Measurement of arterial stiffness

Arterial stiffness can be determined by measuring the velocity of the arterial pressure wave using a formula developed by Moens and Korteweg more than 100 years ago (Gavish and Izzo 2016). Arterial stiffness can be accurately measured using an invasive pressure catheter. However, catheter based measurements are not always feasible due to the invasive nature of the method, the high cost, and risks of infection involved. Thus, there is increasing interest in measuring arterial stiffness using non-invasive tools, which can be used as a risk indicator in many populations. (Davies and Struthers 2003). Some of the most commonly used non-invasive techniques include tonometry (measuring of pressure using strain gauge pressure sensor), oscillometry (assessing the curve between cuff pressure and arterial volume), ultrasound (where arterial stiffness is directly determined from changes in pressure that dictate volume fluctuations) , and Magnetic Resonance Imaging (MRI) (that accurately acquire blood flow velocity and virtually measure the aortic length) (Townsend 2017). Pulse

Wave Velocity (PWV) recorded by applanation tonometry is a commonly used, simple and reproducible method to determine arterial stiffness (Laurent et al. 2006) which can be measured either through the carotid-femoral or brachial-ankle pulse wave velocity. However, the carotid-femoral (c-f PWV), also commonly referred as aortic Pulse Wave Velocity (aPWV), is the most widely used index of arterial stiffness and has been recommended as a clinical marker for cardiovascular risk stratification (Boutouyrie et al. 2009). It was estimated that 1 m/s increase in aPWV corresponded to an increase of 14%, 15%, and 15% in total CV events, CV mortality, and all-cause mortality, respectively (Vlachopoulos et al. 2010). In addition, a number of studies have evaluated inter and intra reliability of aPWV in a variety of populations which showed a high reproducibility as shown by ICC's of 0.95 (Sigrist et al. 2010), 0.87 (Rodriguez et al. 2016) and 0.75 (Sima et al. 2016).

The schematic diagram of hemodynamic changes in arterial stiffening (Figure 1.2) shows (A) Aortic blood pressure waveform of a healthy, normotensive person. The forwards traveling wave precedes the (backward traveling) reflected wave. (B) Aortic pressure waveform of a person with arterial stiffness. Due to increased pulse wave velocity, the forward traveling wave and reflected wave are summated leading to augmented pulse pressure (van Varik et al. 2012).

Figure 1.2: Carotid pressure waveform as recorded by applanation tonometry [2]

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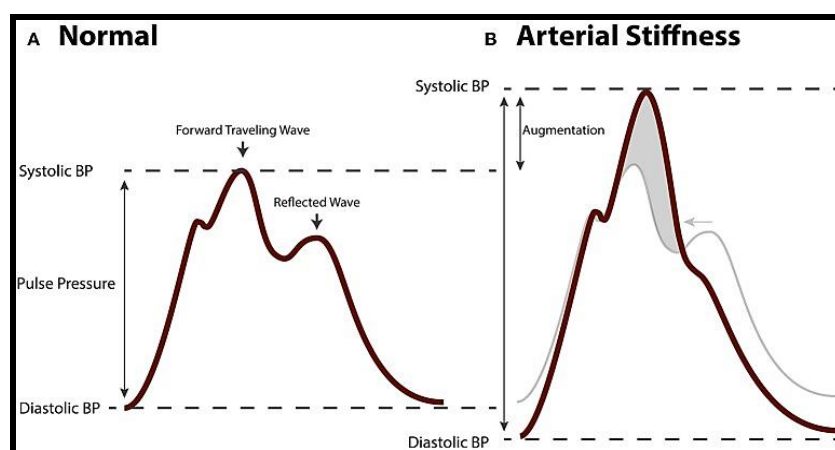
[2] van Varik,et al. (2012)

1.4 **Clinical benefits of measurement of arterial stiffness**

The measurement of arterial stiffness by aPWV has demonstrated its predictive value for cardiovascular events and cardiovascular and all-cause mortality independent of classical risk factors in several large population longitudinal studies. This suggests it's use as a novel and clinically relevant marker beyond traditional risk factors, and its use has been supported by several guidelines (Laurent et al. 2006; Boutouyrie et al. 2009; Van Bortel et al. 2012; Townsend 2017). The European Society of Cardiology Working Group on peripheral circulation has recommended the use of aPWV as it meets most of the nine essential criteria for a clinical surrogate endpoint. These are proof of concept, prospective validation, incremental value, clinical utility, clinical outcomes, cost-effectiveness, ease of use, methodological consensus, and the availability of reference values (Vlachopoulos et al. 2015). The result of a meta-analysis of observational data from 17 cohorts that included a total of 17,635 subjects from the general population also supported that aPWV is a predictor of a cardiovascular events and stroke independent of traditional risk factors (Ben-Shlomo et al. 2014). Similarly, Vlachopoulos et al conducted a meta-analysis that included 17 longitudinal studies involving 15,877 subjects from the general population, hypertensive and patients with end stage renal disease followed for a mean of 7.7 years. They showed that the risk of cardiovascular CV events, CV mortality, and all-cause mortality in subjects with increased aPWV, is almost twice as high compared with the risk in subjects with lower aPWV. It was estimated that for every 1.0 m/s increase in aPWV, there is a 14% increase in the risk of cardiovascular events (Vlachopoulos et al. 2010).

There is evidence of the utility of aPWV in hypertensive patients, as aPWV was found to predict coronary events (Boutouyrie et al. 2002) and all-cause and cardiovascular mortality (Laurent et al. 2001). Recently, the European Society of Cardiology/European Society of Hypertension Guidelines has concluded that the use of aPWV for the assessment of hypertensive organ damage may be considered in the management of arterial hypertension (Williams et al. 2018).

Aortic PWV measurement was also deemed beneficial in reclassifying the level of CV risk in several populations beyond traditional risk stratification. For instance, in the Framingham study that included 2232 participants (mean age 63 years), 15.7% of patients at intermediate risk could be reclassified into a higher (14.3%) or lower (1.4%) risk category when accounting for aPWV value (Mitchell et al. 2010).

Nonetheless, establishing reference values for aPWV is crucial to make it clinically meaningful and to recognise other factors that can influence this value such as age and blood pressure (Vlachopoulos et al. 2015). A consensus expert report recommended a cut-off point of 12 m/s was proposed indicating subclinical organ damage that was later changed to 10m/s (Collaboration 2010; Van Bortel et al. 2012).

1.5 **Indices derived from aPWV measurement.**

The waveform from the aorta can be analysed to evaluate arteries' function, which provides a more detailed representation of pressure variability with every cardiac cycle (Barksdale and Logan 2009). Pulse wave analysis allows the estimation of central pressure indices and other potential indicators of arterial function (Gavish and Izzo 2016). Central hemodynamic indices including central BP, pulse pressure PP, and wave reflection measures such as Augmentation Index (AIx) can be extracted from the captured pulse waves (Figure 1.2 A). Pulse Pressure, defined as systolic minus diastolic pressure, is considered a measure of pulsatile hemodynamic stress and a surrogate marker of arterial stiffness (Muntner et al. 2019). The peripheral blood pressure indices are likely to underestimate central haemodynamic changes. Therefore, central BP indices are more relevant for cardiovascular risk assessment where organs including the heart, brain, and kidney are directly affected by them (Namasivayam et al. 2009; Vlachopoulos et al. 2015). However, the aPWV waveform is altered with arterial stiffness. High pulse pressure (PP) in relation to the mean arterial pressure represents a typical waveform of stiffened arteries (Figure 1.2 B). Increased arterial stiffness can reduce the aortic artery's compliance and increases the characteristic impedance (deformability of the artery), causing an increase in the pulse pressure.

Characteristic impedance represents the wave travelling in one direction in the absence of reflections in the other direction. It is determined by the geometry and the characteristics of the arterial wall. However, it is crucial to consider that aortic input impedance can be affected during transmission to the brachial artery by changes in the pressure wave's amplitude and shape. Hence, the peripheral arterial pressure waveform may not fully represent the aortic impedance (Nakayama et al. 2000; Nishijima et al. 2001).

Assessing arterial stiffness through the measurement of aPWV using c-f PWV has its own notional and technical issues that should be considered. The calculated value of c-f PWV is a combined measurement of pulse wave velocities through the aorta, carotid, and femoral arteries, which are structurally different (Gavish and Izzo 2016). Likewise, how distance is measured is equally important because the calculated PWV depends on the travelled distance, and there can be up to 30% variations in the measured PWV when using different path length measurement methods (Salvi 2017). Measuring the distance by subtracting carotid-suprasternal notch distance from suprasternal-notch to femoral distance has been linked to the best agreement with invasive measurement making it a widely accepted method (Weber et al. 2009). Also, Mean Arterial Pressure (MAP), representing the vessel distending pressure, can significantly influence aPWV. There is a non-linear relationship between arterial stiffness and increased MAP; hence, adjusting for MAP is essential when calculating aPWV (Kim et al. 2007).

1.6 Consequences of arterial stiffness

In health, the normal protective gradient between the heart and the periphery is reduced because of aortic stiffness. In age and pathology, aortic stiffness makes the waveform reflect faster and later in systole, producing a higher aortic systolic blood pressure (aSBP), a lower aortic diastolic blood pressure (aDBP) and a wider PP with no change to MAP (Izzo 2004; Nichols 2011; Meani et al. 2018).

Central Pulse Pressure (CPP) has been identified as an independent risk factor for cardiovascular events and clinical outcomes in several large population studies (Williams et

al. 2006; Glasser et al. 2014; Selvaraj et al. 2016). Although PP is proposed as a marker of arterial stiffness, it is essential to note that this variable can be affected by several factors, most importantly cardiac output and the stiffness of the large arteries (Levy 2018). A PP of 40 mmHg is considered normal in a person with a blood pressure of 120/80 mmHg (120-80=40mmHg). Importantly, a narrow PP suggests low aortic wall tension and low left ventricular workload (Izzo and Shykoff 2001). However, in contrast, a high PP (>40mmH) reflects decreased arterial compliance (due to deterioration of extracellular matrix of the arterial walls) often observed in the elderly and patients with CVD (Selvaraj et al. 2016). When PP increases, coronary artery perfusion is reduced, thereby leading to increased risk of myocardial infarction (Barksdale and Logan 2009). In addition, both the increase in systolic BP and the decrease in diastolic BP increase cardiovascular risk by causing isolated systolic hypertension (ISH), which has been shown to increase risk of developing heart failure and myocardial ischemia, through the mechanisms previously discussed (Cockcroft and Mancia 2012; Gavish and Izzo 2016).

1.7 **Interplay between central haemodynamic and cerebral haemodynamic**

There is increasing evidence to support the connection between central haemodynamic and cerebral circulation. Due to the differences in the wall properties and diameter of peripheral vasculature, impedance mismatch can occur when the wave transmits distally. This is explained as with each heartbeat, pressure and flow waves travel toward the periphery, and because of points of bifurcations, these waves are reflected to the heart. The reflected waves are collide with the antegrade wave resulting in amplification of waves (Avolio Alberto et al. 2009). In health, this variability is beneficial as the amplification of the waves raises the diastolic pressure and augments the coronary perfusion (Mayet and Hughes 2003). The waveform reflection benefits the microcirculation by transmitting less pulsatile energy (Pearson et al. 2019). Central arteries are viscoelastic; hence, they can smooth out the pulsatile pressure resulting from each heartbeat and undergo a small systolic pressure increase with volume increase resulting in a reduced PP (Vlachopoulos et al. 2015). Cerebral

autoregulation through microvascular vasodilation and vasoconstriction facilitates the delivery of oxygen and nutrient to the microvasculature at the local level of the brain. The vasomotor effectors that regulate cerebrovascular resistance achieve this equilibrium (Aaslid et al. 1989; Jefferson et al. 2018).

The relationship between central and cerebral haemodynamic can be compromised by central arterial stiffness which results in the origin of the increased pulsatile stress transmitted into the cerebrovascular circulation (Singer et al. 2014). When central arteries are stiff, they are unable to accommodate the volume increase without a substantial increase in pressure. High blood pressure results in cerebral vessels failing to adopt to functional changes, yet this can be controlled by anti-hypertensive medications (Zhang et al. 2006). Elevated PP may damage the microcirculation by changing cerebral perfusion pressure that alters cerebral blood flow and increases resistance to mean flow. As a result, capillaries of organs with high flow and low impedance such as the brain become exposed to high-pressure pulsatility, making them susceptible to pulsatile damage (Aaslid et al. 1989; Izzo and Shykoff 2001; Mitchell 2008; Avolio Alberto et al. 2009). It can lead to microcirculatory dysfunction and impaired tissue perfusion that limit the ability of the tissue to adapt to ischemic stress (Laurent et al. 2003) and reduce the efficiency of the delivery of oxygen and nutrient to the cerebrovascular vessels (Gavish and Izzo 2016). This can be further augmented if haematocrit level is deranged, a key determinate of blood viscosity associated with arterial wall shear stress (Kwater et al. 2009). Figure 1.3 summarises the role of vascular mismatch in reducing pulsatility and enhancing cardiac reperfusion (A) and the complaint aorta that facilitates continuous blood flow (B), leading to optimum blood flow controlled by cerebral autoregulation (C). However, when the aorta is stiff, impedance mismatch reduces, which increases the transmission of pulsatile energy (D) and altering the flow dynamics that potentially reduce perfusion in the brain (E). This may alter vascular resistance, perfusion pressure, and blood-brain barrier integrity (F).

Figure 1.3: Theoretical model of cerebral damage induced by aortic stiffening [3]
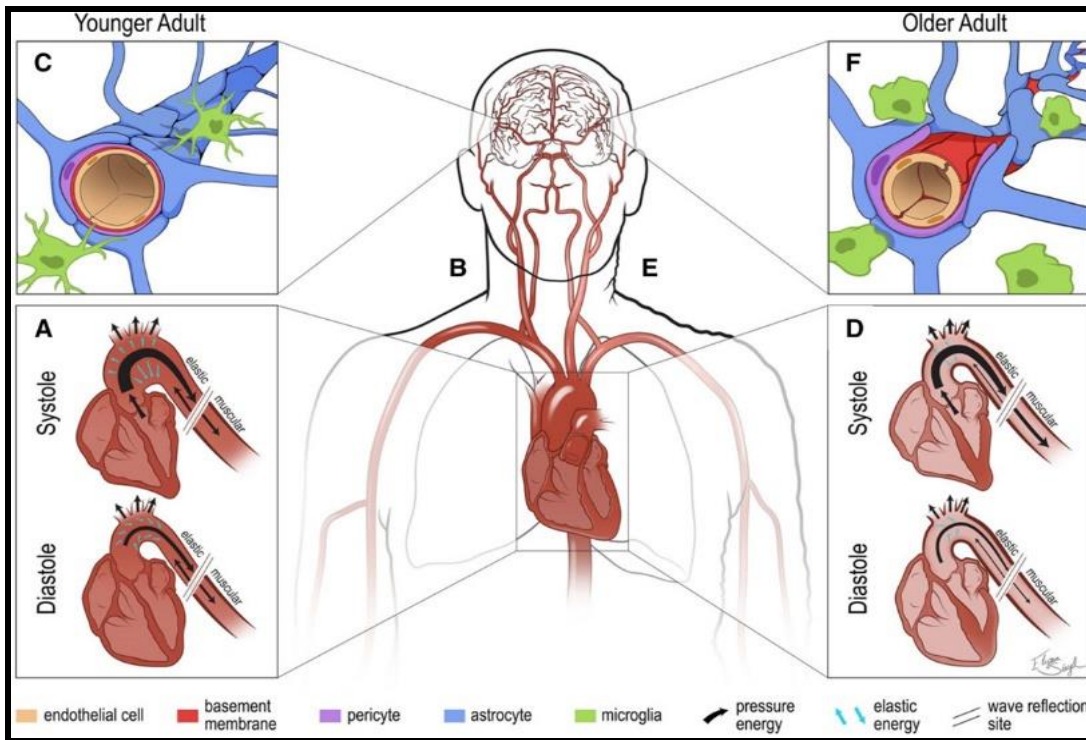
Higher pulsatile energy transmitted to the brain increases the risk of stroke, with parameters obtained via transcranial Doppler shown to predict this risk. Pulsatility Index (PI) of the Middle Cerebral Artery (MCA) is frequently measured to establish the degree of flow pulsatility within the cerebral circulation, considering that MCA is one of the biggest arteries supplying around two-thirds of blood flow to the brain (Harris et al. 2018). In addition, a measure of arterial stiffness, such as aPWV and other central blood pressure measures, have also been associated with measures of cerebral haemodynamics such as PI (Kwater et al. 2009; Pearson et al. 2019). A number of studies have evaluated the inter and intra-rater reliability of MCAPI which showed a high short term reliability as shown by ICC's of 0.90 (Kaczynski et al. 2018) and 0.75 (Venturelli et al. 2015), and a fair to good long term reliability as shown by ICC of 0.63 (Ortega-Gutierrez et al. 2014).

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[3] Jefferson et al. (2018)

1.8 **The link between MCAPI and aPWV**

Evidence supports a connection between cerebral haemodynamic and central arterial stiffness in which PI reflects an increase in pulsatile pressure in the systemic circulation (Webb et al. 2012; Kim et al. 2015). For example, pathological changes in the small cerebral vessels has been linked to reduced distensibility of large arteries (Silva and Faraci 2020). In addition, MCAPI has been shown to correlate with the measure of central arterial stiffness including aPWV and CPP (Xu et al. 2012). It was also independently associated with aPWV in patients with Diabetes mellitus (Sanahuja et al. 2016) and patients with leukoaraiosis (Webb et al. 2012). The above evidence supports the interplay between central and cerebral haemodynamic and the impact of the loss of large arterial compliance on the downstream flow dynamics.

There is substantial research supporting the use of non-invasive measures of arterial stiffness in both health and disease. CVD is particularly prevalent in COPD; hence a literature review is required to explore the connection between arterial stiffness and CVD in this population.

1.9 **Aim of the literature review**

This review aims to provide a current and critical synthesis of the literature describing the relationship between COPD and arterial stiffness as well as COPD and stroke. It seeks to explain the interconnections between central and cerebral artery haemodynamics and the impact of COPD in this relationship.

1.10 **Search strategy**

A literature search was conducted using AMED, CINHAL, Medline, EMBASE, Pubmed, Google Scholar, Cochrane, and the Web of Science databases from 2000 to 2020. The search was limited to articles in the English language, with further references identified through secondary citations. Despite focusing the search on articles from 2000 onward, some older

literature related to fundamental theories and mechanisms were retrieved from secondary citations; and also included. The search was divided into two sections, reviewing literature examining the association between COPD and arterial stiffness and literature examining the association between COPD and stroke.

The objectives of the literature search were:

- To investigate the relationship between COPD and arterial stiffness, the following keywords were used and linked with "AND" or "OR":

"COPD" OR "CAD" OR "CLD" OR "chronic bronchitis" OR "emphysema" AND "Arterial stiffness" OR "CVD" OR "Vascular Diseases" OR "PWV" OR ""aPWV" OR "c-f PWV" OR "carotid-femoral PWV"

- To investigate the relationship between COPD and stroke, the following keywords were used and linked with "AND" or "OR": "COPD" OR "CAD" OR "CLD" OR "chronic bronchitis" OR "emphysema" AND "Stroke" OR "Cerebrovascular accident" OR "transient ischemic attack (TIA)" OR Pulsatility Index " OR "MCAPI".

The search resulted in numerous papers that were screened for relevance by title and abstract, sub-categorised, summarised, evaluated using appropriate appraisal tools including the Critical Appraisal Skills Programme and the Specialist Unit for Review Evidence tools (Critical Appraisal Skills Programme 2018; Specialist Unit for Review Evidence 2018) (Table 1.1). In addition, a search for the relationship between arterial stiffness and cerebral haemodynamics in healthy populations and in some conditions including hypertension, diabetes, rheumatoid arthritis and renal disease were also conducted to explore these relationships and provide a context for the main aim of the research.

Table 1.1: Outcomes of database search

Databases	COPD and arterial stiffness (n=)	COPD and stroke (n=)
CINAHL	2	14
EMBASE	344	9
PubMed	93	83
Cochrane	46	24
Web of sciences	90	42
Google scholar	7260	15230
AMED	1	6
Total after removing duplicates	5346	7130
Total after screening titles/abstracts	314	127
Total used in the review	88	31
Abbreviations: n= number of papers		

1.11 Chronic Obstructive Pulmonary disease (COPD) and Cardiovascular Diseases (CVD)

There is accumulating evidence of both structural and functional changes in the vasculature of patients with COPD (Agustí 2005) which may contribute to increased cardiovascular risk. In a cross-sectional, observational analysis of 514 clinically stable COPD patients with moderate to severe airflow obstruction, 54% had one or more echocardiographic abnormalities, whereas 23.5% had two or more abnormalities. These abnormalities include left ventricular (LV) hypertrophy, increased right ventricular systolic pressure (RVSP) and impaired LV ejection fraction. However, these abnormalities were not previously reported in about 64% of those patients who shared a similar risk of cardiovascular diseases, such as being older, male, and with a higher Body Mass Index (BMI) (Houben-Wilke et al. 2017). In line with this, cardiac chamber size and stroke volume were reported to be reduced in a small number of COPD patients who presented with lung hyperinflation compared to healthy controls (Khanji et al. 2016). This is further supported by the work of Barr and colleagues where impaired left ventricular filling, reduced stroke volume, and lower cardiac output has been associated with severe airflow obstruction and a higher degree in emphysema (Barr et al. 2010). Therefore, suggesting that the cardiac system in patients with COPD is structurally and functionally compromised and may potentially contribute to the development of CVD commonly seen in COPD.

There has been extensive debate in the literature about the relationship between COPD and the development of CVD, as shown by a systematic review and meta-analysis (Chen et al. 2015; Morgan et al. 2017; Kim et al. 2018). There was a two-fold increase in the odds of having any CVD in patients with COPD compared to individuals without COPD, as per a meta-analysis of observational studies (Chen et al. 2015). Equally, patients with COPD had increased risk of stroke (HR, 1.30; 95% CI, 1.18-1.43; $p<0.001$) as per a recent meta-analysis (Kim et al. 2018). In addition, the risk of stroke is increased in patients with COPD exacerbation with an adjusted hazard ratio of 1.28 (95% CI,1.03±1.59) in a large sample in

addition to increased mortality post-stroke compared to individuals with stable COPD or without COPD (Lin et al. 2017; Morgan et al. 2017; Kim et al. 2018).

However, the mechanisms linking COPD and stroke remain unclear. Factors including increased age, smoking, sedentary lifestyle, and low socioeconomic class were identified traditional risk factors associated with the development of both COPD and CVD, making it difficult to distinguish between the mechanisms linking the two conditions (Maclay and MacNee 2013). It has been proposed and shown that increased systemic inflammation and increased oxidative stress markers were associated with vascular dysfunction. Suggesting that this association could potentially explain some of the excess cardiovascular morbidity and mortality associated with COPD (Mills et al. 2008).

1.12 Measures of vascular stiffness in COPD

Measures of vascular stiffness including aPWV, Carotid Intima-Media Thickness (CIMT), arterial wall distensibility and endothelial function are adversely affected in COPD. Carotid Intima-Media Thickness CIMT is a measure of the thickness of the inner two layers of the carotid artery, the intima and media. It is used to identify early vascular wall damage, and the progression of vessel structural thickness as surrogate marker of atherosclerosis and is the first manifestation of organ damage in older individuals (Park et al. 2019). Endothelium-derived mediators markedly influence arterial stiffness due to their role in regulating vascular tone and structure (Correia and Haynes 2007).

In a study including 86 male COPD patients, and 86 age-matched healthy controls free from CVD, increased CIMT was present in 64% of COPD patients versus 8% healthy controls. The increased CIMT was associated with an 8- and a 6-fold higher risk of developing atherosclerosis in patients with hypoxemia and hypercapnia, respectively and CIMT predicted the occurrence of CVD. However, more evidence is required, as this study was limited by the heterogeneity of smoking status and COPD stages in the studied group (Hafez et al. 2016).

In another study, including 61 healthy middle-aged smokers with airflow obstruction compared to 122 age-matched smokers and a similar number of non-smokers controls, CIMT was increased in smokers with airflow obstruction. The increased levels of atherosclerotic plaque highly associated with lower FEV_1 predicted in the group with airflow obstruction. This association suggests that COPD may be susceptible to vascular atherosclerosis. However, the cross-sectional nature of this study limits causality. In addition, it included middle-aged men; hence results may not apply to females. Besides, COPD diagnosis was not confirmed by post-bronchodilator spirometry. Nonetheless, the presence of atherosclerotic changes in middle age individuals with airflow obstruction suggests that vascular abnormalities start early in the course of COPD disease (Iwamoto et al. 2009).

In line with this, a systematic review of 32 studies that included 3198 patients with COPD and 13867 controls also concluded that measures of vascular stiffness are increased in COPD. Patients with COPD had increased aPWV, increased CIMT, and compromised endothelial function as expressed by reduced Flow-Mediated Dilatation (FMD) in those patients who are free from CVD. These changes are evident in moderate stages of airflow obstruction supporting the existence of atherosclerotic changes earlier in the disease course (Wang et al. 2017b). Interestingly, a study by a group of investigators found no difference in the aortic distensibility between high-risk CVD patients with normal lung function and COPD patients with low-risk CVD. The risk was classified by QRISK, an algorithm for predicting cardiovascular risk using traditional risk factors for CVD including age, type 2 diabetes, hypertension, rheumatoid arthritis, renal disease, atrial fibrillation, sex, socioeconomic deprivation, and ethnicity. Age was identified as the only factor to significantly predict aortic elasticity independent of other cardiovascular risk factors in both groups, and older age was strongly correlated with the distensibility of the abdominal aorta (Khanji et al. 2015). The Anglo-Cardiff Collaborative Trial (ACCT), a large population study including about 4000 healthy participants, also reported that age, MAP, HR, female gender were predictors of aPWV. Moreover, age-related changes in aPWV were non-linear and more marked in subjects over 50 years (McEniery et al. 2005).

In a study including 18 male patients with COPD and 17 healthy comparators matched for age and smoking history, patients with COPD had increased arterial stiffness as measured by aPWV. However, there was no evident impairment in the endothelial function in patients with COPD when compared to controls. Therefore, increased arterial stiffness may be implicated in the systemic manifestations of COPD. However, this conclusion is to be taken with caution as the study size was small and study population was limited to men (mean age 65 years) where the effects of age may have superseded any effect of COPD on endothelial activity. Nonetheless, the results suggest that abnormalities of the vascular extracellular matrix may be an independent systemic feature of COPD (Maclay et al. 2009).

The previous evidence supports the importance of endothelial function, which may be linked to vascular ageing, and has a vital role in an increased CVD risk. As patients with COPD explicit a premature ageing process at a faster rate than non-COPD, they may be at higher risk for CVD (Divo et al. 2018). However, caution must be exercised when interpreting data from studies limited by being cross-sectional, including small sample size and single gender, and therefore limiting generalisability.

1.13 Severity of COPD and CVD

Despite a well-established relationship between arterial stiffness and risk of CVD and mortality (Ben-Shlomo et al. 2014; Vlachopoulos et al. 2015; Townsend 2017), and the connection between COPD and CVD (Chen et al. 2015; Morgan et al. 2017; Kim et al. 2018), the degree of airflow obstruction and its influence on this risk lacks consensus. This highlights the lack of clarity in the literature about the specific role of COPD severity in increased arterial stiffness and its impact on CVD. There is evidence from large population studies supporting a link between airflow obstruction and CVD risk and mortality. A Scottish prospective population study including 15,411 participants also reported that reduced FEV_1 accounted for one-quarter of the attributable risk for mortality from Ischemic Heart Disease (Hole et al. 1996). Similarly, a group of researchers reported a strong association between COPD severity and the risk of developing Myocardial Infarction (MI) (OR 3.00, 95% CI 1.53–5.86) after adjusting for smoking in 35,772 patients from UK General Practice Research

Database (Schneider et al. 2010). More recently, The Prospective Urban Rural Epidemiology study, a large prospective, international, community-based cohort (126,359 participants, age range 35-70 years) from 17 countries studied the effect of airflow obstruction as measured by country-specific predicted FEV_1 values on the risk of CV mortality. The result showed a significant and graded relationship between decreasing baseline FEV_1 % and increased risk of mortality and CVD. The study also reported that airflow obstruction contributed to around a quarter of deaths and a sixth of CVD events, a higher contribution than other major risk factors such as hypertension, previous CVD, or tobacco use. Interestingly, the groups with mild to moderate impairment in FEV_1% had a more substantial contribution to the risk of mortality and CVD than severe FEV_1% impairment (FEV_1% <-2 SD from population mean) despite having similar shared risk factors such as age (Duong et al. 2019). The study highlights the importance of airflow obstruction as a risk factor for predicting mortality and CVD risk beyond the traditional risk factors and powered by including diversity of study sample allowing for generalisability. In addition, the Lung Health Study, a multicentre clinical trial of smoking intervention and inhaled bronchodilator in middle-aged smokers with mild to moderate COPD (n= 5,887) reported that cardiovascular mortality rose by 28% for every 10% decrease in FEV_1 (Anthonisen et al. 2002). However, a meta-analysis included 29 datasets found no association between COPD severity and Ischemic Heart Disease (IHD), also refer to as coronary heart disease that occurs when the blood flow to the heart muscle is reduced because of a partial or complete blockage of the arteries (Chen et al. 2015). The data suggest that COPD severity does not necessarily lead to increased risk, compared to what has previously reported in large population studies. It implies that mortality and CVD risk are high even in those with subclinical airflow obstruction, making identifying those at risk difficult. A possible explanation for this that patients with severe FEV_1 impairment (defined as 30% ≤ FEV_1 < 50% predicted) with increased cardiovascular comorbidities may benefit from the use of cardiovascular acting medications that provide some protection against the increased risk of mortality (Yang et al. 2020).

Despite the lack of consensus on the relationship between airflow obstruction and risk of CVD and CVD mortality, there is more agreement on the relationship between airflow obstruction and arterial stiffness in COPD. A study including 62 stable COPD reported

increased aPWV in patients with severe COPD compared to patients with mild or moderate COPD (Cinarka et al. 2014). Similarly, a group of researchers investigated the association between arterial stiffness and lung function in 827 individuals at midlife and compared it to later life. They concluded that lung function at midlife and later-life was inversely associated with aPWV with lung function at midlife being a strong risk factor for increased arterial stiffness in men (Bolton et al. 2009). In addition, a cross-sectional study in 157 patients with COPD reported an association between increased aPWV and airflow obstruction as measured by FEV_1 % of predicted. Also, aPWV was associated with the severity of emphysema independent of markers of COPD severity such as low BMI, airflow obstruction, and six-minute-walk distance. This association is proposed to be connected to systemic elastin degradation in COPD (McAllister et al. 2007). A similar association was also reported between airflow obstruction as measured by FEV_1 percentage or L and other measures of arterial stiffness, including the Augmentation Index (AIx) in 41 patients with COPD (Costanzo et al. 2017). The study by Costanzo et al may be criticised for not measuring aPWV, as the gold standard method used in assessing cardiovascular risk. Although both aPWV and AIx are measures of arterial stiffness, AIx is affected by height and heart rate (Papaioannou et al. 2008) and plateau's after the age of 55 years (Fantin et al. 2006), unlike aPWV, making the later a better marker of arterial stiffness in older individuals (McEniery et al. 2005; Chen et al. 2015). In addition, the assessment of brachial-ankle PWV in 1356 COPD patients indicated an increase in the prevalence of arterial stiffness, and this increase is particularly significant in patients with moderate to severe COPD (Oda et al. 2015). However, the brachial-ankle PWV is not the gold standard measure of aPWV as it reflects the mechanical properties of a combination of both central elastic and peripheral muscular arteries (Yu et al. 2008).

The results of the previous studies support that COPD is associated with increased arterial stiffness independent of other risk factors. In addition, the evidence discussing the relationship between COPD and CVD risk has considerable heterogeneity between study results, likely due to methodological differences such as the use of different measurement tools, the small sample size in some of the studies and different comparison groups. Evidence is extensive but challenging to establish a consensus; hence, more robust studies are required to establish this link fully.

1.14 **COPD exacerbation and CVD**

The in-hospital mortality from COPD exacerbation varies between 10% and 60%, with higher death rates occurring in the more severe COPD (Seemungal et al. 2009). Greater mortality rates from cardiovascular events happen during the period of acute exacerbation (Fabbri et al. 2011). A possible explanation for the increased risk is that airway infection which provokes an exacerbation in COPD, may give rise to systemic inflammation (Donaldson et al. 2010). Exacerbation may impose a risk of developing a cardiovascular event as a result of the increased systemic inflammation. Thomson and co-workers demonstrated that levels of systemic inflammatory markers, including C-reactive protein, fibrinogen and leukocyte count were associated with increased risk of having exacerbations in 61,650 patients with COPD from the Copenhagen City Heart prospective cohort study (Thomsen et al. 2013). Through this period, cardiac troponin levels were increased and correlated positively with the severity of exacerbation and strongly predicted 30 days mortality (Chang et al. 2011). Similarly, in a small study, platelet monocyte aggregates, which exert a pro-inflammatory action, were reported to be increased during this stage (Maclay et al. 2011). During 5 days following the onset of exacerbation, COPD patients had 2.27 fold increased risk of MI (Donaldson et al. 2010). During COPD exacerbations, cardiac function may be impaired by several factors, including severe hypoxemia, pulmonary hypertension, and systemic inflammation. However, the mechanism behind this relationship is not fully identified (Chang et al. 2011). In the London COPD cohort, patients with stable COPD and IHD presented with more dyspnoea, reduced exercise capacity, and poor health status compared to COPD patients free from IHD. The combination of both diseases also resulted in the addition of five extra days of recovery time during exacerbation onset but did not have an effect on the frequency of exacerbation (Patel et al. 2012). It is worth noting that these patients were prescribed three or more cardiovascular medications in addition to the COPD medications hence medications interactions and its influence in the relationship between IHD and exacerbation frequency and severity cannot be excluded. In this study, exacerbation frequency and severity were self-reported by patients, and this can impose some risk of overestimation or under presentation, however, the authors also analysed the health care utilisation in relation to the events of exacerbation including the hospitalisation and

antibiotics or corticosteroids prescription to confirm these events and their degree. This may suggest that the combination of both diseases does not necessarily trigger exacerbations but may have a role in prolonging recovery from exacerbations and negatively affecting cardiovascular outcomes in COPD.

1.15 Arterial stiffness and medications

As discussed previously, comorbid patients with COPD and CVD are often prescribed several medications to treat CVD in addition to COPD medications. The most common reported CVD is high blood pressure (Mannino et al. 2008; Stridsman et al. 2013; Vanfleteren et al. 2014) and therefore, patients with COPD are commonly prescribed several antihypertensive medications. These include angiotensin-converting-enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), beta-blockers, calcium channel blockers, and diuretics. These medications mostly affect the dynamic component of arterial stiffness by reducing blood pressure rather than to the structural element for arterial wall remodelling (Janic et al. 2014; Ye et al. 2018).

In the Multi-Ethnic Study of Atherosclerosis cohort that included 1206 hypertensive participants without evident CVD followed at 5-time points over ten years, BP control was associated with slower progression of arterial stiffness as measured by distensibility of the right common carotid artery regardless of the type of anti-hypertensive medications (Gepner et al. 2017). However, there may be a risk of survival bias as data from individuals who survived and completed the 5th measurement were used in the analysis. In addition, BP measurement was taken five times in 10 years, which may not represent the whole period as BP can be influenced by several factors, including lifestyle and medication compliance. Furthermore, there may be a challenge in standardising the vessel tree's measurement point at the follow-up visits, but reproducibility data by the investigator showed good reproducibility.

However, a meta-analysis including 28 high quality randomised control trials investigated the impact of different antihypertensive medications on arterial stiffness as measured by PWV.

The study showed that these medications influenced reducing SBP but had no effect on reducing arterial stiffness. The authors used network analysis to include studies that measured the effect of different types of antihypertensive medications on one outcome, which was arterial stiffness. However, this meta-analysis maybe limited by selective reporting bias and the inclusion of some studies with small sample size (Ye et al. 2018).

Furthermore, evidence from the Framingham study, a longitudinal community-based cohort study measured vascular stiffness and blood pressure progression at two-time points, baseline and seven years showed that antihypertensive medications decrease arterial stiffness by reducing SBP, reducing vascular filling, and reducing the arterial wall load. However, an opposite relationship may also be possible where SBP is reduced due to reducing arterial stiffness. In addition, the study showed that initial blood pressure was not independently associated with the progression of aortic stiffening (Kaess et al. 2012).

As COPD is an inflammatory condition by its nature, evidence and guidelines recommend anti-inflammatory drugs for controlling the disease (Beeh and Glaab 2009). Systematic inflammation may be the causal link between COPD and increased CVD risk, hence reducing inflammation may be vital in modifying the risk of CVD (Van Eeden et al. 2012). A review aimed to explore the studies investigating the role of anti-inflammatory drugs in arterial stiffness reduction in several populations with chronic inflammatory diseases concluded that these drugs positively affected reducing arterial stiffness (Maki-Petaja and Wilkinson 2009). In addition, a study which included 17 patients with rheumatoid arthritis, a systemic inflammatory condition, reported a reduction in aortic stiffness after 8 weeks of anti-tumour necrosis factor-α (TNFα) therapy (Maki-Petaja et al. 2012). Although both are inflammatory conditions, it has not been shown that anti TNF has similar impact on COPD (Barnes 2007).

However, there is some evidence linking anti-inflammatory medications to increased susceptibility to a small chance of increased cardiac risks including cardiac arrhythmias in patients with COPD who have underlying CVD. More evidence is needed as the current data may be influenced by selective bias from a COPD trial paradox, where patients with COPD presenting with CVD are usually excluded from trials (Lahousse et al. 2016).

1.16 Mechanisms linking COPD to arterial stiffness

There are several potential mechanisms linking COPD and arterial stiffness. The most common mechanisms include smoking, inflammation, lifestyle and body composition.

1.16.1 Smoking

Evidence shows that smoking has been associated with increased arterial stiffness, even in healthy individuals. A study reported a significantly higher aortic systolic pressure and AIx in a small number of young, healthy smokers compared with non-smokers (Mahmud and Feely 2003). On the contrary, Camplain and colleagues reported no association between increased central arterial stiffness and smoking in older individuals at the Atherosclerosis Risk in Communities cohort study (Camplain et al. 2016). The different outcomes in these studies may relate to the studied age group as smoking may influence arterial stiffness in the younger groups differently than the older group. In addition, the former study used different arterial stiffness measures than aPWV and had a small sample size hence more evidence is needed to confirm this relationship.

Although smoking is recognised as one of the main risk factors for COPD and also shown to associate with systemic inflammation in those patients (Liew 2015), the notion that COPD by itself is an instigator for increased arterial stiffness is debatable. Studies reporting such connection were limited by comparing COPD smokers or past smokers with smokers' controls making the exclusion of smoking effect uncertain in this population. For instance, the research by Cinarka et al found no significant association between systemic inflammation and smoking when measuring C-reactive protein in 62 stable COPD patients (Cinarka et al. 2014).

To overcome the methodological limitation of previous studies, a recent cross-sectional study measured aPWV in 355 patients with COPD and 4310 controls that included two subsets, smokers versus never smoked to exclude the influence of COPD combinations and smoking. They concluded that smoking is a risk factor for increased aPWV and that increased aPWV

cannot be explained purely by COPD (Soumagne et al. 2020). However, considering that the nature of the connection between COPD and smoking is not indistinct, linking the development of cardiovascular disease in COPD patients to lung disease solely may require more evidence (Maclay and MacNee 2013).

1.16.2   Systemic inflammation

Systemic inflammation in COPD has been associated with the development of cardiovascular disease (King 2015). However, this association is disputed in several studies. An earlier systematic review and a meta-analysis included 14 studies demonstrated that reduced lung function is associated with increased levels of systemic inflammatory markers including CRP, fibrinogen and leucocytes (Gan et al. 2004). Several cross-sectional studies reported an increase in the inflammatory markers in COPD patients including interleukin 6, interleukin 8, TNFα and fibrinogen (Maclay et al. 2007), CRP, interleukin 6 and interleukin-1 receptor antagonist (Johansen et al. 2012), interleukin 6 and TNFα soluble receptors 1and2 (Sabit et al. 2007) and Homocysteine levels (Avci and Avci 2016). In the same way, endothelial micro-particles were elevated suggestive of endothelial cell apoptosis even in individuals with mild COPD (Thomashow et al. 2013). However, McAllister and colleagues found no association between PWV and high level of CRP or between emphysema and high level of CRP in 157 COPD patients (McAllister et al. 2007). In agreement, a study including 1534 participants from the general population found no association between inflammatory markers such as interleukin 6 and CRP and carotid-radial PWV, although these markers were inversely related to FEV_1 in a multivariable-adjusted model (van Rooyen et al. 2016). The authors propose that factors other than inflammation may initiate increased arterial stiffness in this population. The available evidence linking inflammation and increased arterial stiffness is limited by the nature of cross-sectional studies, which do not determine causality in addition to selective bias of the studied sample that limit generalisability. In addition, the measurement of PWV was assessed using carotid-radial PWV and not the gold standard c-f PWV. This suggests that the link between inflammation and increased arterial stiffness is complex, and future specific markers of inflammation are needed.

1.16.3   Lifestyle

Measures of health status including physical, and patients reported measures are negatively affected in COPD and have been related to increased arterial stiffness (Stickland et al. 2013; Sievi et al. 2015). Evidence supports that lifestyle factors, including physical inactivity and sedentary lifestyle, were associated with vascular ageing in older adults (Karimi et al. 2016; Nadruz et al. 2017; Orkaby et al. 2019; Gomez-Sanchez et al. 2020). Such a relationship may be attributed to the progressive loss of pressure absorption effect in the vasculature due to increased arterial stiffness and hence affecting the microcirculation of different body organs including the skeletal muscles leading to impairments in physiologic reserve (O'Rourke and Hashimoto 2007). Furthermore, the anti-inflammatory effect of exercise training may be an important factor in reducing BP by inducing nitric oxide release and antioxidant enzymes (Tsukiyama et al. 2017). Additionally, the progression in airflow obstruction may lead to increased symptoms of dyspnoea that result in avoidance of activities and hence cause further deterioration in physical activity and function.

1.16.4   Body composition

Body mass index (BMI) has been linked to the risk of all-cause mortality in patients with COPD. Two meta-analyses that included 17 observational studies involving 30,182 COPD patients with COPD and 22 studies comprising 21,150 patients with COPD showed that being overweight is associated with a lower risk of all-cause mortality and underweight is associated with a higher risk of all-cause mortality (Cao et al. 2012; Guo et al. 2016).

The reported effect of body composition on arterial stiffness in patients with COPD is controversial, and the variations suggest a lack of consensus on the exact role of body composition in this population. Central obesity was a strong predictor of aPWV in 3769 individuals from the Whitehall II Study, a large longitudinal study (Johansen et al. 2012). However, a study by McAllister et al reported an association between increased aPWV and low BMI (McAllister et al. 2007). In addition, another study reported no relationship between

Fat-Free Mass Index and either aortic PWV or AIx (Sabit et al. 2007). The relationship between measures of body composition and aPWV may relate to the shared risk factor in which obesity is associated with CVD, and increased aPWV is associated with CVD. These results should be interpreted with caution when relating them to COPD. The Whitehall II study included healthy middle-aged participants who may not represent the COPD population, been older, and usually with comorbid diseases. In the same way, BMI should not be compared to central obesity as they represent different elements of body composition. Furthermore, studies were limited by small sample size and the cross-sectional design except for the Whitehall II Study.

1.17 COPD and cerebrovascular disease

Despite the availability of several lines of evidence linking COPD to increased risk of stroke, the exact mechanisms that explain this link remains unclear. However, evidence supports changes in the brain's structure and function in COPD, as shown in the following section.

1.17.1   Changes in structure and function of the brain in COPD

The most commonly known lesions affecting the white matter is known as white matter hyperintensities (WMHs), also referred to as leukoaraiosis and can be detected in MRI or CT scans (Wardlaw et al. 2015). Alteration in the WM in the brain is a sign of cerebral small vessels diseases (SVD), and a major cause of stroke (Mitchell et al. 2011). Although WM occupies 50% of the total brain volume, it has a similar metabolic rate to that of GM (Dewar et al. 1999; Goldberg and Ransom 2003). It also receives a disproportionately small blood supply and little collateral circulation, making it susceptible to ischaemic damage (Dewar et al. 1999; Arai and Lo 2009) and has been associated with triple the stroke risk (Debette and Markus 2010). These lesions can result from degenerative or inflammatory processes as well

as ischemia related to reduce blood flow leading to permanent demyelination and axonal destruction (Pantoni and Garcia 1997; Yamamoto 2017).

White matter hyperintensities are associated with vascular risk factors including increased age, with a prevalence of 10% - 20% in individuals aged 60 years old, however, this has been shown to increase up to almost 100% in individuals older than 90 years (Smith et al. 2017). Moreover, WMH are also associated with hypertension, but this relationship is controversial as it is not clear if hypertension affects the brain directly or indirectly through systemic vascular stiffening that affects brain white matter (Brisset et al. 2013). Evidence also suggests that the impact of WMHs extend to other structures in the cortex, including the grey matter. New research showed that local WMH influence the grey matter in remote areas leading to secondary brain atrophy (Wang et al. 2020).

Recent literature has focused on highlighting connections between COPD and different elements of brain structure and function. The reported association between COPD and brain anomalies range from changes in perception, memory, cognition, white matter integrity and grey matter function to more life-threatening events such as TIA and stroke (Rusanen et al. 2013; Singh et al. 2014; Liao et al. 2015).

Higher risk of CVD was linked to increased cerebral blood velocities and pulsatility in several studies (Bos Michiel et al. 2007; Kwater et al. 2009; Xu et al. 2012; Robertson et al. 2019). As shown by the Rotterdam Study, a large population-based study, including 2022 participants (mean age 70 years), a strong and independent association between higher Middle Cerebral Artery (MCA) blood flow velocity and increased risk of stroke was reported. These data reported that for every 1 SD increase in the cerebral blood velocity at MCA, there was a 38% increase in the risk of ischemic stroke (Bos Michiel et al. 2007). In a cross-sectional study involving 165 participants with high risk of CVD, increased aPWV correlated with MCAPI (p= 0.007), in adjusted multivariate regression analysis. Besides, PP was related to MCAPI (p=0.001) (Kwater et al. 2009). Likewise, Xu et al studied the association between indices of arterial stiffness and MCAPI in 334 individuals (mean age 51 years; age ranged from 19 to 81 years). The result showed that MCAPI increased with age and was closely related to central

PP (p<0.001) and weakly related to aPWV (p=0.036). However, when both PP and PWV were added into the model, aPWV was no longer significantly related to PI (Xu et al. 2012). Similarly, a study included 61 participants (mean age 74 ± 6 years) also reported that carotid PP and CIMT were independently associated with increased PI after adjusting for age and sex and that they both accounted for more than a third of the variance in cerebrovascular pulsatility (Robertson et al. 2019). In addition, a study including 99 healthy individuals reported a positive relationship between MCAPI and CPP (p=0.003) but not with aPWV (Pearson et al. 2019).

Previous evidence suggests that stiffness of the central arteries results in the widening of PP that impacts the cerebral circulation haemodynamics. It also supports the utility of cerebral blood flow parameters in predicting risk for cerebral vascular disease. However, most of these studies demonstrate associations rather than causation, and the inclusion of a relatively small sample and younger or middle-aged participants may have influenced the results. In addition, employing different methodologies for measuring stiffness is a point to consider when interpreting these data as they may represent different vascular segments; hence their interactions with cerebral haemodynamic may vary accordingly.

A number of studies have investigated the connection between central arterial stiffness and measures of cerebral structural changes including white matter hyperintensities (WMH) and Silent Brain Infarcts. For example, data from the Atherosclerosis Risk in Communities cross-sectional study investigated the connection between aPWV and markers of structural brain damage measured by MRI in 1255 older participants (mean age 76 years). The result showed that higher aPWV was related to higher white matter hyperintensities WMH compared with those with lower aPWV (P=0.03). However, the level of aPWV in this group did not influence the odds of lacunar infarct or cerebral microbleeds (Palta et al. 2019).

In addition, in a retrospective hospital-based study, Lee and colleagues reported that age and MCAPI were significantly correlated with moderate to severe WMH (both at p < 0.001), in 83 participants with acute small subcortical infarcts (mean age 61.5 ± 11.4). Unlike the positive relationship between aPWV and WMH reported by (Palta et al. 2019), Lee et al found no relationship between aPWV and severity of WMH in this group. However, arterial stiffness

measurement was obtained by measuring brachial-ankle PWV, which measures different element of arterial structure and is not comparable to aPWV, where the later provide a better representation of stiffness of the aorta. Hence, this conclusion may not represent the complete central-cerebral hemodynamic interaction (Lee et al. 2018).

Recently, Matsumoto and colleagues studied the relationship between peripheral and central BP parameters and silent cerebral diseases where they examined silent brain infarcts and WMH using MRI in 993 participants from a population-based cohort of stroke-free participants. The result showed that central SBP and PP were more strongly associated with the silent cerebrovascular disease compared to peripheral BP. However, both peripheral and central PP were independently associated with silent brain infarcts (both $P<0.05$) after adjusting for age, sex, hypertension, atrial fibrillation in multivariate analysis. In addition, older age and hypertension were significantly associated with the upper quartile of WMH (Matsumoto et al. 2020).

A further cross-sectional case-control study examined the white matter integrity of 20 older male smokers with a diagnosis of COPD who were free from neurological diseases and compared them to 18 healthy male non-smoker volunteers. They found that the magnitude of structural alterations within brain tissue parts was significantly higher in patients with COPD compared to the controls. However, this observation did not correlate with Arterial Blood Gases, FEV_1, or the severity of the disease as measured by spirometry and classified by the GOLD stages (Shehata et al. 2016). A similar alteration in cerebral white matter causing a reduction in the connectivity between cortical regions in 31 patients with COPD (mean age 68 years, mean FEV_1 52% predicted) compared to 23 healthy controls was also found using whole-brain deterministic tractography (Spilling et al. 2015). In agreement with the previous findings, Dodd et al also reported a reduction in the white matter integrity of all brain areas in addition to interruption in functional activation of grey matter in 25 stable non-hypoxemic patients with COPD (Dodd et al. 2012). Similarly, cortical thinning was present in 25 patients with stable COPD who were not on long term O_2 therapy (Chen et al. 2016). The above evidence suggests that COPD is associated with alterations in cerebral structure, but the exact mechanism that links these alterations to COPD is not fully understood.

Several lines of evidence examined the relationship between central stiffness and measures of cerebral blood flow velocities. Kwater and colleagues studied the connection between parameters of arterial structure and function and measures of the cerebral circulation. They measured systolic and diastolic BP, MAP, PP, aPWV, CIMT, and mean Cerebral Blood Flow Velocity (CBFV) at MCA in 165 middle-aged individuals (mean age 57±12 years). The measured aPWV was 12.7±3.3 m/s, which is considered high for this age; however, 63% of participants had hypertension that may explain the high aPWV value. Interestingly, they found no relationship between SBP, DBP, PP, or MAP and mean CBFV. However, higher aPWV and greater CIMT were associated with lower mean CBFV, but when accounted for possible cofounders, aPWV was no longer associated with mean CBFV. Although participants in the study were middle age, the percentage of hypertension was high, and most participants (87%) were on at least one medication that may have influenced the nature of the relationship between the central and cerebral measures (Kwater et al. 2014). An important consideration when interpreting this study is that the study did not measure the pulsatile components of the flow velocities and may have seen an increased PI in this case despite having low mean CBFV.

In addition, Jefferson and co-workers investigated the relationship between arterial stiffness and resting CBFV and cerebrovascular reactivity (CVR) in 155 participants with normal cognition and 115 with mild cognitive impairment from the Vanderbilt Memory and Ageing Project (mean age 72±7 and 73±7 years respectively). They measured aortic stiffness and CBFV using MRI. Increased aPWV was related to reducing frontal lobe CBFV in participants with normal cognition, whereas it did not relate to CBF of any brain regions in participants with mild cognitive impairment. In the same way, higher aPWV was related to higher CVR in the whole brain in participants with normal cognition but unrelated to CVR in all brain regions. The nature of the association in the participants with normal cognition can be explained by the fact that those participants were normotensive who appropriately responded to the changes in partial pressure while measuring CVR, suggesting that they have complaint cerebrovascular accommodating any increase in cerebral blood volume. It also implies that older individuals with hypertension are more likely to be exposed to long-term

high pressure that may potentially affect cerebrovascular integrity and reactivity (Jefferson et al. 2018).

This conclusion was examined by the work of Zhang and colleagues who investigated how blood pressure, hypertension duration, and antihypertensive treatment correlate to systolic blood flow velocities at major cerebral arteries, including the MCA in 1294 individuals (age range 42–73 years). After controlling for confounders, higher SBP was independently associated with an increase of maximal CBFV in MCA by 1.63 cm-1. The team also reported no difference in blood flow velocities between medicated or non-medicated hypertensive patients and in those with shorter or longer hypertension duration. It suggests that blood pressure influences blood flow velocities at the MCA largely. It also highlights the importance of antihypertensive medications in maintaining normal blood flow velocities within cerebral arteries by lowering BP (Zhang et al. 2006).

This suggests that stiffness of the central arteries results in the widening of PP that impacts the cerebral circulation haemodynamics. It also supports the utility of cerebral blood flow parameters in predicting risk for cerebral vascular disease in combination with or independently of blood pressure. The results support previous evidence which link risk factors including age and hypertension to cerebral abnormalities. in addition, the literature largely supports the role of using central blood pressure indices in predicting CVD than peripheral blood pressure parameters.

It is postulated that factors including age, hypoxemia, duration of the disease, presence of other comorbidities, and disease severity may increase the risk of developing these alterations. In the same way, inflammation is commonly seen in patients with COPD who also have increased oxidative stress that may lead to structural remodelling of cerebral vessels and disruption in the blood-brain barrier (Brassington et al 2019). However, the consistency in the literature remains a problem and further research is warranted.

1.17.2   COPD and stroke

Recent literature has highlighted the association between COPD and the prevalence and risk of stroke in different large population cohort studies from several communities. Key results of studies that met the inclusion criteria of the literature search are summarised below (Table 1.2 and 1.3).

A systematic review and a meta-analysis that included eight studies (7 nested case-controls and 1 prospective cohort) concluded that COPD had a 30% increased risk of stroke compared to non-COPD individuals, independent of other shared CVD risk factors. However, lack of detailed information on lifestyle risk factors including smoking, alcohol consumption, or level of activity in addition to insufficient data on medication effect in some studies prevented further stratified analysis to establish the exact influence of these factors (Kim et al. 2018). In addition, another systematic review investigated the relationship between COPD and CVD but included five studies relating COPD to stroke had concluded that the prevalence of stroke in patients with COPD was from 6.9% to 9.9%, and the adjusted RR for stroke ranged from 1.0 to 1.6 (Mullerova et al. 2013).

The systematic review by Morgan and colleagues including 30 studies, mainly cross-sectional, examining the influence and prevalence of stroke in COPD, also found that the prevalence and incidence of stroke are increased in COPD compared to the general population. However, the high degree of heterogeneity in the included studies did not allow pooling of estimates. Nevertheless, the magnitude of the association invariably reduced when adjusting for smoking. As smoking is a shared risk factor in COPD and stroke, it is possible that the link between COPD and stroke may be influenced by these risk factors other than by COPD. In addition, two included studies investigated the association between COPD severity and risk of stroke, but such association was not confirmed. The review also concluded that COPD plays an essential role in this association in specific subsets of patients with COPD and certain stroke subtypes with the higher relative risk for haemorrhagic stroke compared with ischemic stroke (Morgan et al. 2017).

The association between stroke and reduced lung function was further supported by prospective population-based longitudinal studies in long term follow-up of 26 year (Söderholm et al. 2012) and 40 years (Gulsvik et al. 2012) highlighting the role of reduced lung function in increasing the risk of stroke in those patients. However, Rothnien and colleagues did not find such association, but their cohort were COPD patients with exacerbations who exhibit different manifestations than those with a stable period of the disease (Rothnie et al. 2018). The design of the study, being a self-controlled case series, may have impacted the result as authors measured the outcome of the interest, and therefore, the absolute rate of stroke was undetermined.

There is also growing interest in reviewing the connection between increased risk of stroke and COPD exacerbation, yet results are controversial and inconclusive. COPD exacerbation increases susceptibility to cardiovascular events because of an increase in blood viscosity, infections and inflammation (Truelsen et al. 2001). Evidence from the prospective population-based Rotterdam Study showed that the risk of stroke is approximately seven-fold higher in the weeks following severe exacerbation (Portegies et al. 2016). Likewise, in 16,485, participants followed up for 1.5 years, COPD exacerbations increased the risk of subsequent CVD events within the first 30 days after an exacerbation in those who have a risk of CVD. There were 18% stroke and 5% TIA cases in individuals having COPD exacerbation (Kunisaki et al. 2018). This is further supported by another large case series study, including 3010 patients with COPD in which there was a 51% increase risk of ischemic stroke in the 91 days after an acute exacerbation and the peak risk occurring at 4–7 days after an acute exacerbation (Rothnie et al. 2018). The increased risk of ischemic stroke reported in this study is unlike what was previously reported by the review of (Morgan et al. 2017), where COPD was more associated with haemorrhagic stroke.

However, a large retrospective cohort study (n=1918 patients with COPD with exacerbations, 3836 adults with COPD with no exacerbations and 7672 participants without COPD) in Taiwan reported an increased incidence of stroke in patients with COPD with or without exacerbations, but a stroke in patients with previous exacerbation strongly linked to more comorbidities and increased mortality (Lin et al. 2017). In addition, a study including 6441

patients with COPD and 19323 age matched controls from the UK Clinical Practice Research Datalink concluded that the odds of stroke were not affected by exacerbation frequency. Nonetheless, exacerbation frequency was strongly associated with haemorrhagic stroke but not with either ischemic stroke or TIA. In addition, reduced lung function in patients with COPD, as indicated by low FEV_1 and low FEV_1 /FVC ratio, was also associated with stroke. The study also found a strong association between the COPD GOLD stage (GOLD 2018) and stroke, with the odds of stroke decreasing with increasing COPD severity (Windsor et al. 2016). A plausible explanation for this might be that treatment for frequent exacerbations causing a reduction in stroke risk.

Several mechanisms have been suggested to explain the connection between COPD and stroke. COPD patients can present with hypoxemia and hyperventilation, more so in severe cases, causing a metabolic disturbance that can contribute to arterial wall changes. The metabolic disturbance may also induce cardiac arrhythmias such as atrial fibrillation, a condition that predisposes individuals to higher risk stroke and is prevalent in COPD (Kim et al. 2018). In addition, the higher risk of haemorrhagic stroke compared to other types of stroke is in line with reports of increased cerebral small vessel disease seen in COPD where hypoxia and increased oxidative stress level play an essential role in the development of haemorrhagic stroke (Dodd et al. 2012; Austin et al. 2016). Therefore, evaluating the small vessels, haemodynamic may provide an insight into the nature and impact of COPD manifestation in cerebral circulation and potentially, provide an early warning of adverse outcomes.

Despite the availability of several lines of evidence linking COPD to increased risk of stroke, the exact mechanisms that explain this link remains unclear. There is now increasing interest in studying the lung-brain interaction using neuroimaging technologies such as magnetic resonance imaging (MRI) that enable a detailed examination of the structure and function of the brain. This technology allows for the quantification of several metrics associated with cerebrovascular health, including the measurement of cerebral volumes such as White Matter (WM), Grey Matter (GM) and Cerebrospinal Fluid (CSF). In addition, it allows for

evaluations of outcomes related to stroke including white matter lesions, lacunar infarcts and microbleeds (Lahousse et al. 2015).

Table 1.2: Reviews of Prevalence and Incidence of Stroke in COPD

Authors and Study Design	Included Sample	Main Result
Kim et al 2018 Systematic review and meta-analysis	8 studies (n= 198,867 patients with COPD; 1,366,757 controls) • Nested case-control: 7 • Prospective cohort: 1	• COPD increases risk of stroke (hazard ratio, 1.30; 95% confidence interval, 1.18-1.43) • The increase in the risk is independent of other shared CVD risk factors
Morgan et al 2017 Systematic review	30 studies Prevalence studies: 21 (N=263,868 patients with COPD) • Cross-sectional: 14 • Matched cohort: 6 • Case-control: 1 Incidence studies: 11 studies (N=231,435 patients with COPD) • 7 Cohort :7 • Matched cohort: 4	• Prevalence and incidence of stroke are increased in people with COPD • Adjustment for smoking invariably reduced the magnitude of the associations • COPD is an independent risk factor for stroke • COPD is causal in certain subsets of patients with COPD and for certain stroke subtypes
Mullerova et al 2013 Systematic review	25 studies N= 1,606,496 patients with COPD. 1,417 controls COPD and CVD: 25 studies COPD and Stroke: 5 studies	• The prevalence of stroke in patients with COPD was from 6.9% to 9.9% • The adjusted RR for stroke ranged from 1.0 to 1.6 • The RR for hospitalization due to stroke was from 1.2 to 1.3 in patients with COPD vs matched cohorts without COPD

Table 1.3: Cohort Studies of Prevalence and Incidence of Stroke in COPD

Authors and Study Design	Included Sample	Main Result
Lin et al 2017 Cohort study	COPD with exacerbation: n=1918 COPD without exacerbation: n=3836 Age and gender match controls N= 7672	• Increased stroke incidence in COPD with exacerbation, adjusted HR of 1.28 (95% CI, 1.03–1.59). • COPD with exacerbation were associated with post-stroke mortality • COPD exacerbation is an important independent risk factor of stroke and post-stroke adverse events
Kunisaki et al 2018 a post hoc cohort analysis	N= 16,485 participants with COPD follow-up time was 1.5 year from the SUMMIT cohort	• In individuals with combined onset of exacerbation and CVD event, 18% had stroke and 5% had TIA • COPD exacerbations increased risk of subsequent CVD events in individuals who have risk factors for CVD • Risk was higher in hospitalized patients and within the first 30 days after exacerbation
Rothnien et al 2018 self-controlled case series	N=3,010 patients with COPD From Clinical Practice Research Datalink (CPRD)	• Risk of ischemic stroke were increased by 51% in the 91 days after an acute exacerbation • 1.7 times higher risk after severe exacerbation and 1.4 higher risk after moderate exacerbation compared with stable period • The peak risk of ischemic stroke was in the 4–7 days after acute exacerbation • No association between stroke risk and GOLD stages

Table 1.4: Cohort Studies of Prevalence and Incidence of Stroke in COPD (continued)

Study and design	Included Sample	Main Result
Windsor et al 2016 Cohort study	N=6,441 patients with COPD Age matched controls:19,323 UK Clinical Practice Research Datalink	• A strong association between GOLD stage and stroke (p=0.002). • Odds of stroke is not affected by exacerbation frequency • Strong association between exacerbation frequency and haemorrhagic stroke but not with either ischemic stroke or TIA •
Soderholm et al 2012 Prospective cohort study.	N- 27,771 patients with COPD (mean age, 44 years) Mean follow up 25.7±6.5 years	• low FEV_1 or FEV_1/FVC, is a risk factor for SAH, independently of smoking.
Gulsvik et al 2012 Cohort study	N= 5617 patients with COPD Mean follow up of 40 years The Bergen Clinical Blood Pressure Survey	• lung function is consistently, independently, and persistently associated with the risk of fatal stroke

1.18 **Theoretical framework**

The previously discussed evidence supports that patients with COPD represent a complex clinical population who present with higher comorbidities. The concept of comorbidity was previously described (Feinstein 1970) and acknowledges that the existence of two diseases that has distinct aetiology and pathophysiology in an individual may occur by chance or have elements of co-occurrence that acknowledges a possibility of underlying causality between the two diseases (Krueger and Markon 2006). The multi-morbidity conceptual framework adopts the notion of patient-centred approach where a specific disease, co-occurrence diseases, patients physical, social, and psychological status are all taking into account (Boyd et al. 2016).

Four etiological models of comorbid diseases were proposed to explain the nature of the relationship between chronic diseases when comorbidity or multi-morbidity is in place. These models are the direct causation, associated risk factors, heterogeneity, and independence (Valderas et al. 2009). To explain the link between COPD and CVD discussed by this review, the heterogeneity model may be the best to illustrate this relationship. In the heterogeneity model, the risk factors for each disease are not correlated, but each one of them can cause either disease (Figure 1.5). For example, risk factors such as age and smoking are not related; however, both can be associated with COPD or CVD (Morgan et al. 2018).

Figure 1.4: The heterogeneity model of comorbid diseases [4]

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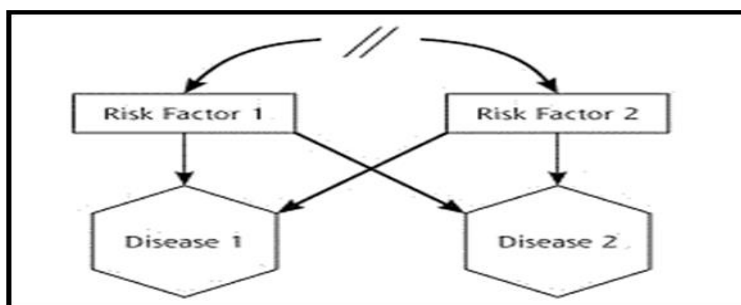
[4] Valderas et al. 2009

A potential mechanism explaining the link between COPD and CVD is that of premature biological ageing which may explain the increased progression in the alteration of vascular structure and function.

The accelerated ageing may instigate the development of CVD in patients with COPD who were associated with structural and functional changes of the cardiovascular system (Sharma et al. 2009). Besides, several common known factors including smoking, sedentary lifestyle and low socioeconomic class are shared risk factors for the development of COPD and CVD, making it difficult to distinguish the mechanism linking the two conditions (Maclay and MacNee 2013).

1.19 Statement of the problem, study aims and objectives

1.19.1   Statement of the problem

The review of evidence demonstrates that COPD is associated with higher risk of CVD including risk of stroke. Increased arterial stiffness is a predictor of CVD and was shown to be elevated in COPD. Increased arterial stiffness is associated with CVD and stroke in other populations. There is also evidence connecting COPD and increased risk of stroke, albeit confined by several methodological limitations. Many of the studies were cross-sectional hence this association does not confirm causality. The progression of cardiovascular risk factors is unclear, the investigation of which may provide more insight into the mechanisms hence studies with longitudinal follow-up are required. There is a general lack of consensus across studies as to what determines the association between COPD and increased risk of stroke. There is also a lack of clarity as to the exact role of arterial stiffness in increasing stroke risk in this group, and it is not understood if this association is direct or indirectly due to alteration in the cerebral blood flow pattern. The study of pulsatility in the MCA and how it relates to arterial stiffness and COPD characteristics may explain the increased risk of stroke in COPD.

1.19.2   Aims and objectives of the thesis

The thesis aimed to evaluate central and cerebral haemodynamics and measures of health status in patients with COPD, compared to age-matched controls to study the link between central and cerebral circulation.

This overall aim was integrated into three main studies undertaken in this thesis, each with specific aims and objectives. The following details each study aims and objectives and their corresponding chapters numbers.

Chapter 3; Longitudinal changes in aPWV and measures of health status in patients with COPD and controls

The study aim was to measure central haemodynamic and measures of health status longitudinally at three-time points; baseline, two years, and six years in patients with COPD and aged-matched controls.

The objectives of the study were:

1. To evaluate change in aPWV at three time points in patients with COPD and controls.

2. To measures health status including physical function and patients reported measures at three time points in patients with COPD versus controls

3. To establish the relationship between aPWV and vascular measurements in patients with COPD and controls.

4. To establish the relationship between aPWV and measures of health status including objective and subjective measures.

5. To establish the relationship between aPWV and COPD characteristics as measured by FEV_1 in patients with COPD.

Chapter 4; Investigation of the relationship between MCAPI and central haemodynamics

The study aimed to investigate the relationship between central arterial stiffness measured by aPWV and the downstream cerebral flow pulsatility measured by MCAPI in patients with COPD and aged-matched controls.

The objectives of the study were:

1. To evaluate the difference in aPWV and MCAPI in patients with COPD and controls.

2. To establish the relationship between aPWV and MCAPI in patients with COPD and controls.

3. To establish the relationship between MCAPI and COPD characteristics as measured by FEV_1, FEV_1/FVC, CAT and SGRQ in patients with COPD.

4. To establish the relationship between MCAPI and health status measured subjectively by the Comprehensive Geriatric Assessment (CGA) and objectively by the 6 Minute Walk Test (6MWT) and Time up and Go test (TUG) in patients with COPD and controls.

5. To identify the predictors of MCAPI in patients with COPD and controls.

Chapter 5; An exploratory study investigating the relationship between vascular haemodynamics and cerebral volumes

The aim of this pilot study was to measure the global cerebral volumes, including the global WM, GM and CSF volumes in patients with COPD and age and gender matched controls using magnetic resonance imaging MRI technique.

The objectives of the study were:

1. To compare the cerebral volumes including the global WM, GM and CSF volumes in patients with COPD versus healthy controls.

2. To establish the relationship between measures of vascular hemodynamic including aPWV, Central Pulse Pressure (CPP) and Middle Cerebral Artery Pulsatility Index (MCAPI) and the cerebral volumes as measured by White Matter (WM), Grey Matter (GM) and Cerebrospinal Fluid (CSF) volumes.

3. To establish the relationship between cerebral volumes including the WM, GM and CSF volumes and airflow obstruction as measured by Forced Expiratory Volume in one second (FEV_1) and the ratio of Forced Expiratory Volume in one second /Forced Vital Capacity (FEV_1/FVC).

Chapter 2

Methods and methodological considerations

2 : Methods and methodological considerations

This chapter is divided into two sections: Research methods and methodological considerations as follows:

Research methods:

This section provides a summary of the research design, inclusion and exclusion criteria for participants, recruitment of participants, ethical considerations, data collection tools.

Methodological consideration:

This section provides details of some of key methodological aspects for the primary outcomes which were the aPWV measured noninvasively using a sphygomocor device and MCAPI measured using Trans Cranial Doppler (TCD). It includes discussion of the evidence concerning the measurement tools used to obtain these outcomes. It highlights the reproducibility and factors influencing the accuracy of the obtained measurements. These methodological aspects were briefly discussed to justify the methodological studies conducted in this thesis.

Methodological studies conducted and presented in this section includes the:

1. Inter-rater reliability of aPWV.

2. Inter-rater reliability for measuring MCAPI by TCD.

3. Intra-rater reliability for measuring MCAPI by TCD.

4. Validation of measurement positions of MCAPI

2.1 Design Setting and Recruitment

The research designs included in this thesis include longitudinal and cross-sectional studies. ARCADE study is a longitudinal study that commenced in 2011 which aimed to complete observational follow-up at three-time points; baseline, two years, and after six years. Data for the baseline and the two years were previously collected and the current study formed the six-year follow-up (Gale et al. 2014; Gale et al. 2019).

The cross-sectional studies aimed to examine the relationship between central arterial stiffness and MCAPI, in addition to assessing the relationship between arterial stiffness and cerebral volumes in patients with COPD. Both studies were assessed during the ARCADE study six-year follow-up.

Participants were recruited from the ARCADE study (n=600 COPD and 150 controls) NCT 01656421 (Gale et al. 2014) through postal invitation. All assessments related to this PhD were conducted at the Health Assessment Suite, School of Health Sciences, Cardiff Metropolitan University. The inclusion and exclusion criteria for patients with COPD and controls are detailed in Table 2.1. The aim was to recruit as many as possible for follow up, hence no power calculation was done for this particular follow-up (Fitzmaurice 2011).

Table 2.1: The inclusion and exclusion criteria for patients with COPD and the control group

	COPD Group	Control Group
Inclusion criteria	• Age between 35-80 years old • Able to give informed consent and willing to participate. • Diagnosed with COPD and their FEV_1: FVC <0.70	• Age between 35-80 years old • Able to give informed consent and willing to participate. • Smokers or ex-smokers
Exclusion criteria	• Unable to give informed consent • Dementia • Pregnancy • History of malignancy in the last 5 years. • Participants with any other diseases identified as having an inflammatory or metabolic component e.g. rheumatoid diseases, or active endocrine disorders • Disorders affecting mobility e.g. Parkinson disease or stroke.	• Unable to give informed consent • Chronic respiratory disease eg COPD or asthma • Dementia • Pregnancy • History of malignancy in the last 5 years. • Participants with any other diseases identified as having an inflammatory or metabolic component e.g. rheumatoid diseases, or active endocrine disorders • Disorders affecting mobility e.g. Parkinson disease or stroke.

2.2 **Ethical consideration and data management**

The current study was part of the ARCADE study for which NRES approval was gained (Ethics-11WSE027). This thesis includes the data for the assessment at the six-year visit with additional novel assessments that were added as an amendment and approved (IRAS project ID 58909, amendment date 16/6/2016) (Appendix 1).

Participation in the study was voluntary, and subjects had the right to withdraw at any time. An information sheet for participants in the COPD group and the control group (Appendix 2 and 3) was provided at least 24hrs before the start of the study at each time point to allow participants the opportunity to ask questions before written informed consent was provided (Appendix 4 and 5).

Data were recorded on a Data Collection Form. Participants' names were coded (using the original coding from the ARCADE database) to maintain anonymity and the documents that included any personal data were stored in a locked cupboard. All gathered data remained confidential and was only used for the study purpose.

All documents, including raw data, were stored with the ARCADE data at Cardiff Metropolitan University in line with local governance arrangements. Electronic data were stored on a password protected computers accessible to the researcher alone for five years. Any data presented at conferences or in publications were in aggregate form with no reference to identifiable or individual data.

With participants consent, clinically relevant data and any assessments indicating significant risk were shared with the general practitioner for consideration and further investigations.

2.3 **Data collection tools**

Participant's demographic data including age, gender, self-reported comorbidities, and list of prescribed medications were collected. Assessment included:

2.3.1 Anthropometric measurements

2.3.1.1 Height measurement

The height of participants was measured using a stadiometer (Seca CE 1023, UK). Participants were asked to stand barefoot with heels together against the height rod. The headboard was lowered until it is in contact with the participant's head, and measurement was recorded in centimetres.

2.3.1.2 Waist/Hip measurement

Waist and hip circumference were measured using a standard stretch-resistant measuring tape. Participants were asked to stand with their arms away from their body. Waist and hip circumference measurements were done on the skin or over a light layer of clothing.

2.3.1.3 Body composition

A detailed regional body composition was measured using multi-frequency bioelectrical impedance using Tanita BC-418 MA Segmental Body Composition Analyzer (Vlachopoulos et al. 2015) (Figure 2.1). The device runs minor un-noticeable electrical currents through the body in which a detailed analysis of body composition can be established. It operates based on the principle that tissues with high water content such as muscles transmit currents well, whereas fat tissues are a poor conductor to currents (Kyle et al. 2004). The device has a built-in printer which provides an instant printout of a complete body composition profile of

different body segments. These include total body weight, the total fat % and Body Mass Index (BMI).

Procedure:

Details of age, height, gender and the athletic/non-athletic status were entered in the system. Participants were asked to stand bare feet on electrodes platform with both feet positioned within the plates. They were asked to hold the handgrip in each hand with a slightly abducted elbows and to keep still until the measurement was completed. As the machine sends very minimum electrical currents, this test was not done for participants with a fitted pacemaker (Astorino et al. 2012).

Figure 2.1: Tanita BC-418 MA Segmental Body Analyser

2.3.2 Vascular measurements

2.3.2.1 Blood Pressure (BP)

Blood Pressure was measured using a semi–automated oscillometric sphygmomanometer (Omron M2 HEM-7121-E, OMRON Healthcare UK Ltd) (Figure 2.2).

Figure 2.2: The Omron Blood Pressure Monitor

Procedure:

After ten minutes of rest, the BP cuff was positioned on the right arm of the participant. Measurement of BP was taken twice in the sitting position, and the average of the two readings was recorded. Then, participants were rested in the supine position, and BP was also measured twice. The average of the two readings was recorded. In the case of variation by >5mmHg between the two readings, a third reading was taken. The systolic and the diastolic blood pressure were recorded in mmHg (Topouchian et al. 2011).

2.3.2.2 Measurement of aortic Pulse Wave Velocity (aPWV)

Arterial stiffness was measured by calculating aPWV using the SphygmoCor system (AtCor Medical, Sydney, Australia) (Figure 2.3). Mathematically, velocity is measured by dividing the distance travelled by time taken. Hence, the distance travelled by the pulse wave divided by the time taken to travel the distance was the basis of aPWV calculations (Townsend et al. 2015; Vlachopoulos et al. 2015).

Figure 2.3: The SphygmoCor System
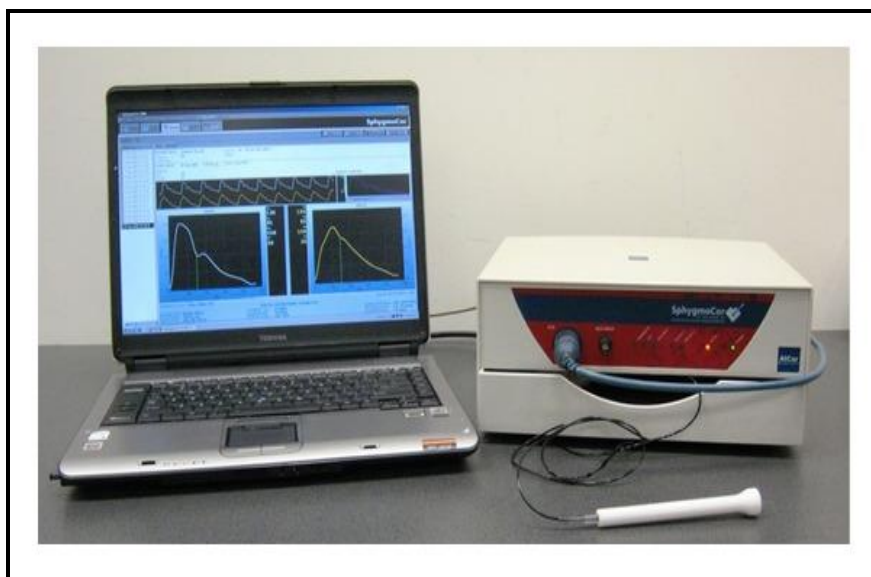
The measurement of aPWV was performed after BP measurement with participants in the supine position. Age, height, and the average of their supine BP were input in the SphygmoCor system for the PWV measurement. A three lead Electrocardiogram (ECG) system was attached to electrodes placed under the right and left clavicles and at the lower edge of the left rib cage.

Then, the best pulse points for the right carotid and femoral arteries were identified by palpation and marked. A non-stretch standard measuring tape was used to measure the distance between the marked carotid pulse to the suprasternal notch and also between the

marked femoral pulse to the suprasternal notch. The measured distances recorded in millimetres were input in the SphygmoCor system. Then, a single high-fidelity tonometer was gently pressed over the marked carotid and femoral pulses in turns to capture the pulse waveforms. The measurement was repeated twice, and the average was taken provided the measurements meeting specific quality control criteria. They were accepted if the difference between the two recordings were within 0.5 m/s with a lowest Standard Deviation (SD).

2.3.2.3 Measurement of Central Pulse Pressure (CPP)

The measurement of CPP was derived using Pulse Wave Analysis (PWA). The transfer function enables the SphygmoCor system to accurately recreate a central pressure waveform from a radial assessment of pulse waveforms. Analysis of the pulse wave then allows for recording of AIx, Central systolic/diastolic BP, CPP and Mean Arterial Pressure (MAP).

Age, height and the average of participants seated BP were input into the SphygmoCor system for the measurement of PWA. Participants were made to sit comfortably with the right arm rested on a pillow. The best pulse point for the radial artery was identified. A single high-fidelity tonometer was gently pressed over the radial pulse. Once the pulse waveform met the required quality criteria, two measurements were captured. Quality control criteria for an acceptable PWA recording includes an operator index of above 75%, and readings were all in the green zones (indicating acceptable quality), with 2-3 screens of good and consistent waveforms and signal strength between 100-250. The reading was accepted if the difference of the AIx between the two measurements was within 5%.

2.3.2.4 Measurement of MCAPI by Transcranial Doppler

The Pulsatility Index PI of the middle cerebral artery MCA was assessed using Transcranial Doppler (DWL Doppler-Box[TM] X, Compumedics, Germany) (Figure 2.4).

Figure 2.4 : The Transcranial Doppler Box X

Transcranial Doppler is an ultrasound examination method used in the assessment of intracranial cerebral circulation that uses the process of insonation, which involves an exposure to ultrasound. It does not provide a direct visualisation of insonated vessels compared to other ultrasound systems however, vessels are evaluated indirectly by a 2 MHz ultrasound beam produced from piezoelectric crystals. Within the insonated artery, the beam bounces off the erythrocytes which is received by the transducer and converted to an electrical signal (Kassab et al. 2007).

Transcranial Doppler does not allow a visualisation of the arteries hence, knowledge of anatomical landmarks is crucial to measure the required artery. In order to ensure the insonation of the correct artery, certain criteria were followed throughout the study. These

are the insonation depth, the direction of the blood flow at that depth, the site of the probe position and the direction of the ultrasound beam (Ringelstein et al. 1990).

The main three components extracted from the recorded spectral waveform were the Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV), Pulsatility Index (PI) and Resistive Index (RI). These values are indicators for evaluating intracranial arterial elasticity, vascular resistance, and cerebral blood perfusion status. The most frequently used TCD parameter to determine the downstream flow resistance and recorded in the current study is the PI which is also known as the Gosling pulsatility index. It is calculated by subtracting end diastolic velocity from peak systolic velocity and dividing the value by Mean Flow Velocity. It is independent of the angle of insonation, has no unit, and has a value that ranges from 0.65 to 1.10 with higher values representing high pulsatility in blood flow that results from a larger difference in contraction and diastolic blood velocity (Ringelstein et al. 1990; Kassab et al. 2007; Bathala et al. 2013; Robba et al. 2018).

Procedure:

Participants were in the supine position while the assessor sat at the side of the bed or the beds head. Enough aquatic coupling gel was applied to the tip of the TCD probe. Then, the TCD transducer was placed over the temporal area above the zygomatic arch, and in front of ear tragus (Figure 2.5). The ultrasonic probe was oriented slightly upward and anteriorly, and adjustment to its angel was made until waveforms of the MCA appeared on the screen. Because other cerebral arteries such as Anterior Cerebral Artery and Posterior Cerebral Artery can also be insonated within the same window, an established reference for depth and flow direction for each cerebral vessel was used. (Table 2.2) (Kassab et al. 2007). The signal was recorded once the MCA was correctly identified, and a clear signal was achieved (Figure 2.6).

Table 2.2: Guidance parameters for the measurements of cerebral arteries by TCD[5]

Artery	Window	Depth (mm)	Direction from the probe	Mean Flow Velocity
Middle Cerebral Artery	Temporal	30 to 60	Toward	55 ± 12 cm/s
Anterior Cerebral Artery	Temporal	60 to 85	Away	50 ± 11 cm/s
Posterior Cerebral Artery	Temporal	60 to 70	Bidirectional	40 ± 10 cm/s

Figure 2.5: Transducer Placement for the Measurement of MCAPI
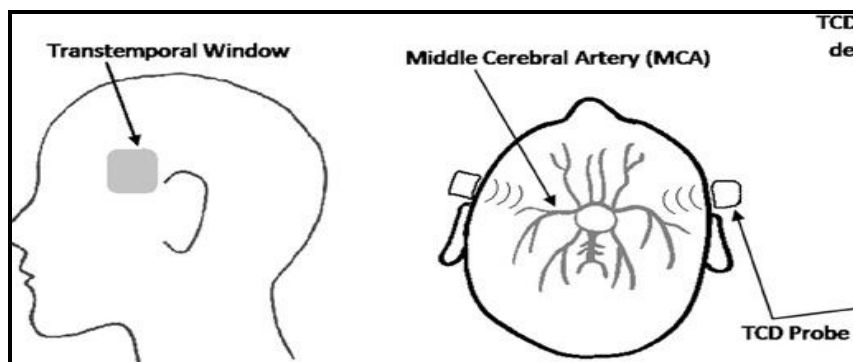
Figure 2.6: Blood Flow Waveform for MCA as recorded by TCD

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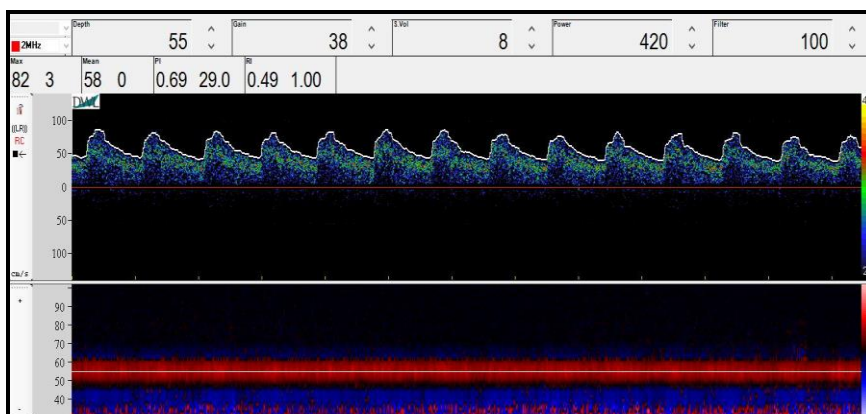
[5] Kassab et al (2007)

2.3.3 Lung function measurement

Lung function was measured using the Vitalograph ALPHA 6000 (Vitalograph Ltd. UK) (Figure 2.7). The results were recorded as measured volumes and % predicted. This includes the following:

- FVC: Forced Vital Capacity
- FEV_1: Forced Expiratory Volume in 1 second
- FEV_1/FVC ratio: The ratio of the forced expiratory volume in the first one second to the forced vital capacity of the lungs.

Figure 2.7: Vitalograph Alpha

Safety considerations

All participants underwent a lung function test as per the ATS/ERS guidelines (ATS-Statement 2002). Participants with any of the following contraindications were excluded from the test: haemoptysis, pneumothorax, high BP (SBP >200 or DBP >100), thoracic, abdominal or cerebral aneurysms, recent eye surgery, recent thoracic or abdominal surgery (Cooper 2011).

To prevent contamination, a disposable mouthpiece was used for each participant. The test was done in sitting position to prevent dizziness when standing may be risky for some participants including individuals with poor balance or those who use walking aids. An appropriate resting period between each manoeuvre was given to avoid the risk of over ventilation and exhaustion.

Procedure:

The Vitalograph was calibrated before each assessment with a 3-L syringe to confirm the device is within the accepted calibration limits. The test was fully explained and demonstrated to participants. Participants' details including age, height, gender, smoking history, and ethnicity were input followed by selecting the FVC test. Ideally, the test should be done in the standing position, but for safety and standardisation, it was done in the sitting position (Miller et al. 2005).

The procedure for measuring lung function was as follow:

- A nasal clip was used to prevent air leaking out of the nose while performing the test where possible.
- Participants were asked to breathe in deeply, filling their lungs as much as possible.
- Then, place the mouthpiece in the mouth and seal the lips around the mouthpiece well to prevent the escape of air.
- Next, they exhaled as quickly and forcefully as possible and to keep going until they feel no more air can be exhaled.
- Three tests were done but, in some cases, participants were requested to perform more than three tests if their attempt did not meet the requirement of a good manoeuvre. Manoeuvres were accepted if the difference in FVC were ± 0.150 L and in FEV_1 was ± 0.150 L (ATS-Statement 2002).
- To avoid fatigue, the upper limits of the repeated attempts were eight.
- Once the test was completed, the result was printed, and the highest value was recorded.

For participants with COPD:

Participants with COPD were advised to refrain from using their inhalers on the morning of the test, unless they needed to. A reversibility test was done by giving participants a 400 μg of salbutamol 10-15 minutes before conducting the test. The medication was administered through a volumatic spacer device (Allen and Hanbury) valved spacer where four separate doses of 100 μg were given with an interval of 30 seconds. When each dose was actuated, participants were asked to breathe from the spacer to their total lung capacity and then hold the breath for 5-10 seconds before exhaling. The spacer device was thoroughly washed between each use.

Interpretation of the Outcomes:

Spirometry is used in the diagnosis of COPD and can be used to classify the severity of airflow obstruction and the severity of COPD (Table 2.3).

Table 2.3:The classification of COPD severity based on spirometry[6]

COPD stages	FEV_1%	FEV_1/FVC %
Stage I: Mild COPD	FEV_1 > 80% predicted	FEV_1/FVC < 70%
Stage II: Moderate COPD	50% < FEV_1 < 80% predicted	FEV_1/FVC < 70%;
Stage III: Severe COPD	30% < FEV_1 < 50% predicted	FEV_1/FVC < 70%
Stage IV: Very Severe COPD	FEV_1 < 30% predicted or FEV_1 < 50% predicted plus chronic respiratory failure	FEV_1/FVC < 70%;

[6] Vestbo et al 2013

2.3.4   Subjective measures of health status

Generic or diseases-specific measure of physical function are widely used in research or clinical practice. These are used to identify problems, effects of the disease on someone's life or to assess the effectiveness of disease management (Higginson and Carr 2001).   An example of a generic instrument is the Comprehensive Geriatric Assessment (CGA), and two disease specific tools are the COPD Assessment Test (CAT) and the St George's Respiratory Questionnaire (SGRQ).

2.3.4.1 Comprehensive Geriatric Assessment (CGA)

The Comprehensive Geriatric Assessment (CGA) is a multidimensional assessment used in the evaluation and in planning the appropriate management of frailty in older adults (Appendix 6). It focuses on multiple domains including physical symptoms and illness, mental health symptoms, level of function in daily activity for personal care and for life functions, social support networks, living environment, level of participation and individual concerns and the compensatory mechanisms and resilience (Rockwood and Mitnitski 2011).

The researcher completed the questionnaire on the assessment day with all participants before commencing the measurements to ensure the suitability of the participants for the study and to identify potential contraindications for any of the measurements. The CGA was adapted for use with community dwelling individuals and included 17 items. Each item was scored and summed with high scores indicating greater impairment.

2.3.4.2 COPD Assessment Test (CAT)

The COPD Assessment Test (CAT) is a disease specific eight item questionnaire that identifies the impact of COPD on the health status of patients (Appendix 7). It describes symptoms affecting the lungs including cough, sputum and symptoms of chest tightness affecting everyday function, confidence leaving their home, sleep and energy (Jones et al. 2009). The test was self-completed by patients with COPD on the day of the assessment.

Each statement has a Likert scale from 0 to 5 where 0 indicates no symptoms or not being affected by the diseases whereas a score of 5 indicates symptoms being worse and or the disease is negatively impacting the health of the patient. The participants were asked to mark the number that best represents their symptoms. The scores were added to give a total score which ranges from 0-40, with highest scores indicating a greater impact of COPD. The CAT Development Steering Group had summarised the CAT scoring and its impact level (Jones et al. 2009) as follows:

- Score >30 (very high impact)
- Score >20 (high impact)
- Score 10-20 (medium impact)
- Score <10 (low impact)
- Score 5 (Upper limit of normal in healthy non-smokers)

2.3.4.3 St George's Respiratory Questionnaire (SGRQ)

St George's Respiratory Questionnaire (SGRQ) is a disease specific supervised self-administered questionnaire aims to identify aspects of COPD symptoms that have a significant impact on the life and health status of patients with COPD (Appendix 8).

The questions are structured to give results in domains including symptoms, activity, and impacts (psycho-social) as well as a total score. Patients with COPD self-completed the questionnaire independently with help from the researcher whenever necessary. An Excel-based scoring calculator was used to calculate the scores of the questionnaire and was summarised in three main score components:

- Symptoms: which relates to the effect of respiratory symptoms, their frequency and severity.
- Activity: which relates to activities that cause or are limited by breathlessness

- Impacts: which relates to the effect of the airway disease on the social, functional, and psychological status of the patients.
- Total score: calculated by summing the weights to all the positive responses in each of the three components.

Each component is scored separately by providing each response with a unique empirically derived weight range from 0 as lowest score indicating the best possible health status to 100 as the highest score indicating the worst possible health status (Jones and Forde 2009).

2.3.5 Objective measures of physical function

2.3.5.1 Handgrip strength

Handgrip strength was measured using the Grip Strength Dynamometer GRIP-D/ T.K.K.5401 (Takei Scientific Instruments Co Ltd, Tokyo Japan) (Figure 2.8).

Figure 2.8: Grip Strength Dynamometer

Procedure:

Participant's dominant hand was noted. The height of the handle was adjusted to suit the participant's grip by ensuring that the middle phalangeal joint of the index finger is at 90˚ on the handle. The test was done while the participant was in standing position with arms at his/her side, not touching the body and slightly bent elbows. He/she was asked to squeeze the handle of the dynamometer with as much force as possible. Two measurements were taken for each hand with a pause of 10-20 seconds to avoid fatigue. The average of the two measurements for each hand was recorded (Watanabe et al. 2005).

2.3.5.2 Timed Up and Go test (TUG test)

The TUG test is a simple, quick test used to determine fall risk in older populations. It assesses the progress of balance, sits to stand, and walking in those individuals (Barry et al. 2014).

Equipment:

- 46 cm height chair without armrests
- 3 meters measured distance
- 1 marking cone
- Stopwatch

Procedure:

The test was fully explained and demonstrated to the participants. Participants were asked to get up from a chair, walk at a comfortable pace at a marked distance of 3 metres, turn around, walk back to their chair, and take their seat (Figure 2.9). They did one trial of practice prior to the actual recording. They were asked to use their usual walking aid if they use any. The time spent in completing the test was recorded in seconds.

Figure 2.9: Time Up and Go Test
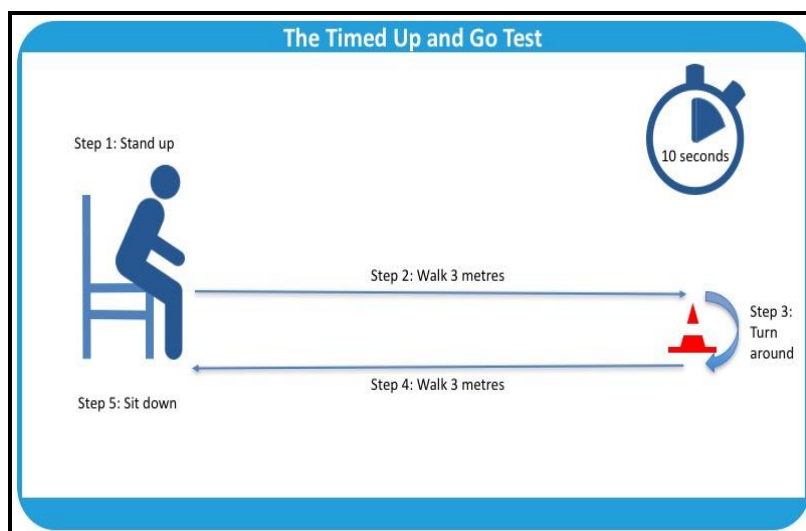
2.3.5.3 Six Minute Walk Test (6MWT)

The 6MWT is one of the most used walking tests in a variety of patients' populations. It evaluates the global and integrated responses of all systems involved during exercises. These systems include the cardiovascular, pulmonary, neuromuscular and peripheral circulations (ATS-Statement 2002).

Equipment:

- 10-metre straight flat surface corridor (due to limited space, a 10-metre corridor was used instead of the recommended 30 metre) (Figure 2.10).
- 2 marking cones
- Stopwatch
- Fingertip pulse oximeter for measuring Heart Rate (HR) and fraction of oxygen-saturated haemoglobin (SpO_2).
- The modified Borg Rating of Perceived Exertion Scale
- Recording sheet
- Two chairs at the two ends of the corridor
- The modified Borg Rating of Perceived Exertion Scale (Appendix 9).

Figure 2.10: The 6 Minutes Walking Test
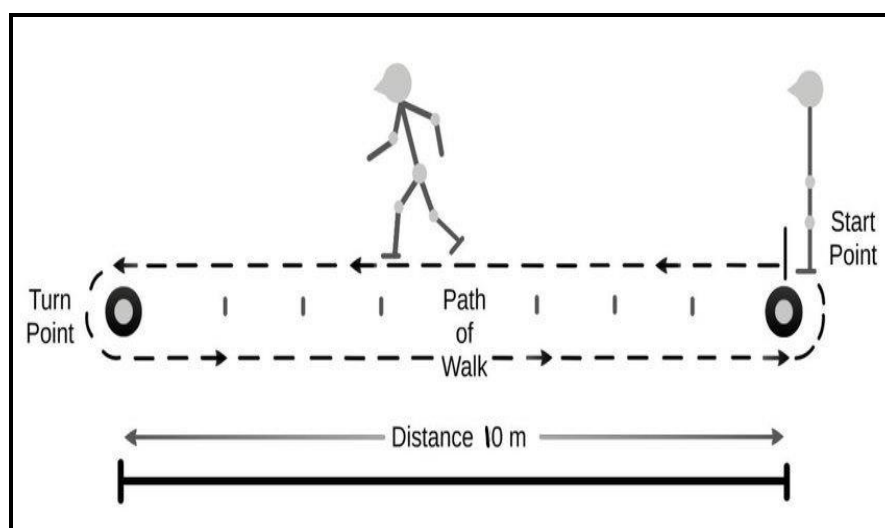
Procedure:

The test was conducted based on the recommended guideline by the American Thoracic Society (ATS) (ATS-Statement 2002). Participants were requested to wear comfortable clothing and a flat pair of shoes. Their HR and SpO_2 were measured and recorded at rest. Participants also indicated their breathlessness level from the Borg scale at rest, which is used to measure an individual's effort and exertion, breathlessness and fatigue during physical work (Borg 1982). Then, they were asked to walk at their own pace for 6 minutes, with their regular walking aid if necessary. The assessor walked behind the participants to avoid the influence of participants changing their pace of walking. Participants could stop walking if they needed, and they were encouraged to resume immediately once able. The number and the time of the rests were recorded. Standardised encouragement phrases were used after each minute. HR, SpO_2 and Borg scale were recorded in the sitting position immediately after the test completion. The distance covered by the end of the 6 minutes were measured and recorded. Participants remained seated for 5 minutes and were monitored for any symptoms before leaving the assessment area.

Safety Considerations:

Participants with conditions such as unstable angina, high blood pressure (SBP >200 or DBP >100) or low resting SpO_2 (90%) were excluded from the test. They were advised to stop the test if they had any symptoms of chest pain, intolerable dyspnoea, leg cramp, staggering, diaphoresis, and pale or ashen appearance. Participants using ambulatory oxygen were advised to continue using it while doing the test. The test was done in an area with accessible telephone, first aider and a defibrillator.

2.4 Data analysis

The details of specific data analysis for each study are included with the specific chapter.

2.5 **Methodological considerations**

2.5.1 Reliability in research

One of the fundamental aspects for utilising research findings is that data must be derived from a well-conducted quality rigour research. In quantitative research, this can be achieved by implementing critical statistical properties of measurement by ensuring that the used tools are reliable (Lachin 2004; Heale and Twycross 2015).

Reliability refers to the extent to which the measurement is inherently reproducible or the degree to which the measurement is influenced by measurement errors (Lachin 2004). There are two main types of reliability testing in quantitative research which were the focus for this methodological study: the inter-rater and intra-rater reliability. Inter-rater reliability concerns the variation between two or more raters who measure the same group of subjects under the same conditions. Good inter-rater reliability implies that the measurements obtained by one rater are likely to be representative of subject's true score and therefore, the result can be interpreted and applied with greater confidence. On the other hand, the intra-rater reliability indicates the degree of agreement between measurements taken by a single rater and assesses consistency over time (also called test-retest reliability) (Portney and Watkins 2013).

The degree of concordance in the results between two or more measurements of the variable of interest can be measured using several statistical tests, for example, the Interclass Correlation Coefficient (ICC) and the Bland–Altman limits of agreement. The ICC is a statistical measurement that considers the difference in the means of the measures where interval/ratio data have been collected (Liu et al. 2016). It is used to measures the extent to which there is a perfect agreement by examining how the measured points vary around a line of perfect unity (Peacock and Peacock 2011). It reflects both degrees of correlation and agreement between measurements (Koo and Li 2016). Generally, coefficients below 0.50 indicate poor reliability, coefficients from 0.50 to 0.75 suggest moderate reliability, and

values above 0.75 indicate good reliability (Portney 2014). However, one of its fundamental limitations that may challenge researcher includes selecting an appropriate ICC model, its sensitivity to between-subject variability and its inability to identify systemic errors (Lee et al. 2012).

The Bland-Altman limits of agreement provide a measure of the agreement by estimating how the two measurements are spaced out (Peacock and Peacock 2011). It is a widely used method that assesses the agreement between two methods of measurement. It examines the average difference between the methods and estimates the 95% limits of agreement between them (Bland and Altman 2007). For a good agreement, about 95% of the data points should lie within ± 2 standard deviations of the mean difference (Giavarina 2015).

In many cases, no single reliability test is sufficient on its own because using ICC alone can be indefinite as it provides no measure of the closeness of the measurements. The use of Bland-Altman to compliment the result of the ICC is highly recommended in studies concerning reliability. By combining the two tests, the appropriate ICC is calculated, and also descriptive statistics are graphically presented to check for reliability (Hicks 2009).

2.5.2 Background and statement of the problem

The measurement of aPWV is considered the gold standard method for assessing aortic stiffness noninvasively and had been recommended by the European Society of Cardiology guidelines as a tool to assess the arterial system damage and therapeutic efficacy (Mancia et al. 2013). As the measurement is operator dependent, ensuring measurement accuracy is key in establishing the true risk. Despite some element of operator influence in the acquisition of aPWV measurement, appropriate training, and the use of quality control criteria to assess the quality of measurement is vital in reducing operators related variations that provides assurance when using this measure in clinical decisions (Sigrist et al. 2010; Elliot et al. 2020).

There are two main types of TCD equipment that use the same operating principles and produce a similar measurement but differ in their ability to display the cranial vessels. These systems are referred to as imaging or non-imaging TCD. The imaging TCD provides an overview of the anatomical landmarks that allows the visualisation of the Circle of Willis and guide the identification of cerebral arteries. On the other hand, the non-imaging, also known as the blind technique, is a widely available system which relies on the operator experience and knowledge as there is no provision for the anatomical visualisation (Inusa et al. 2019). There have been some reported discrepancies when using the two systems interchangeably; however, discussing these differences is beyond the scope of this study as the most commonly used and accepted method of the non-imaging system was only used in this study.

The complexity of the cerebral arteries and their anatomical position within the cranium can lead to challenges in identifying and measuring the corresponding artery. There are specific criteria which should be followed to help guide the measurement of a specific artery based on their anatomical position and characteristics. These include the identification of the appropriate acoustic window and the insonation depth for each artery. Besides, ensuring the correct direction of the blood flow at that depth, the position of the probe and the direction of the ultrasound beam (Ringelstein et al. 1990). These challenges may lead to variations in the obtained measurement and hence, this section aimed to highlight some of these methodological issues including inter and intra-rater reliability.

A total of four test-retest studies were conducted in this research to ensure that data collected for the primary research was reliable. These were concerned with two main measurement tools for the main research outcomes, which are the measure of central arterial stiffness by aPWV and measure of cerebral pulsatility by the MCAPI. The study had 4 main aims which were:

1. Establish the inter-rater reliability of measuring aPWV using the SphygmoCor system.

2. Establish the inter-rater reliability of measuring MCAPI using TCD.

3. Establish the intra-rater reliability of measuring MCAPI using TCD.

4. Establish the validity of measurement positions of MCAPI using TCD.

The next section details the methods of these studies and provides an interpretation of their result with a comparison to data from relevant research.

2.5.3 Study design

This reliability study aimed to establish the inter and intra-rater reliability of the measurement of aPWV and MCAPI in addition to the validation of test-retest of measurement positions of MCAPI in healthy individuals. It concerned measuring these outcomes by the researcher against a proficient assessor in a standardised manner.

2.5.4 Study participants

Participants from the ARCADE database and staff and students from the School of Health Sciences and Sport at Cardiff Metropolitan University were invited through post or emails to take part in this study. Ethical approval for these studies was granted from the Cardiff School of Sport and Health Sciences at Cardiff Metropolitan University (Appendix 10). Participants were included if they were able to give consent and aged between 20 to 65 years.

2.5.5 aPWV measurement

Participants from the ARCADE database were invited to complete repeated measurements of aPWV obtained by SphygmoCor system (AtCor Medical, Sydney, Australia) to establish the inter-rater reliability. The measurement was carried by the researcher and an experienced assessor in a random order on the same day. Measurements were completed by the two assessors in succession to minimise the impact of any physiological changes in HR and BP. The detailed method for the measurement of aPWV was fully described in the previous section of this chapter (2.3.2.2) and in accordance with recommendations.

2.5.6 MCAPI measurement

Participants were invited to complete repeated measurements of MCAPI obtained using the DWL Transcranial Doppler Sonography Box X (Chapter 2.3.2.4). To establish inter-rater reliability, the measurement of MCAPI was obtained from the left side by two assessors successively in a randomised order using simple randomisation on the same day. Each assessor independently recorded MCAPI without the second assessor being in the room. Any gel used during the measurement was also wiped off to prevent clues of the measurement location.

Then, participants completed repeated measurements of MCAPI by a single operator to establish the intra-rater reliability. Two measurements were obtained with a gap of 2-3 minutes. In most participants, measurements were obtained from the left side. However, in some participants, it was taken from the right side due to poor acoustic window at the left side.

Last, a single assessor recorded MCAPI measurement from two pre-defined anatomical points as shown in (Figure 2.11) to establish measurement consistency between the two measurement positions.

Figure 2.11: measurement positions for MCAPI by TCD
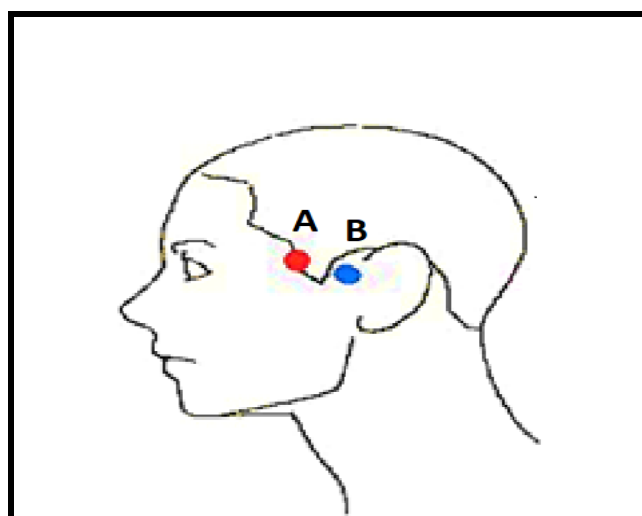
2.5.7 Data analysis

Results were checked for normality and displayed as mean and standard deviation. The intraclass correlation coefficient was used to assess the statistical agreement between the observers. The limits of agreement between the two measurements were analysed using scatter plots and the Bland-Altman's plots. In these plots, the differences between the studied parameters were plotted against their mean values. Limits of agreement were considered as being within 2 SDs of the mean differences, and variation in 95% of the cases (Bland and Altman 2007).

2.6 **Results**

The number of participants and their characteristics for each of the sub-studies are detailed below.

2.6.1 Study 1: Inter-rater reliability of aPWV measurement

The characteristics of the 19 participants and aPWV measurements by both assessors are summarised in Table 2.4.

Table 2.4: Participant's characteristics and aPWV measurements for the Inter-rater reliability

n=19	Mean ± SD
Gender Female: Male	12:7
Age (years)	68±9.8
aPWV (m/s) assessor 1	10.7±3.2
aPWV (m/s) assessor 2 (Proficient user)	10.8±3.2

The result of the Intraclass Correlation Coefficient (ICC) showed a high degree of reliability between aPWV measurements by the two assessors. The average measure ICC was 0.996 with a 95% confidence interval from 0.991 to 0.999 (F (18, 18) = 273.41, p<.001). In addition, scatter plot represent data very close to the line of best fit (Figure 2.12 A). Also, Bland-Altman plots showing acceptable mean measurement differences and limits of agreement between series of MCAPI measurements without outliers (Figure 2.12 B).

Figure 2.12: Scatter plot showing the line of perfect agreement (a) and the Bland-Altman plots (b) of level of agreement between aPWV measurements by the two assessors

A
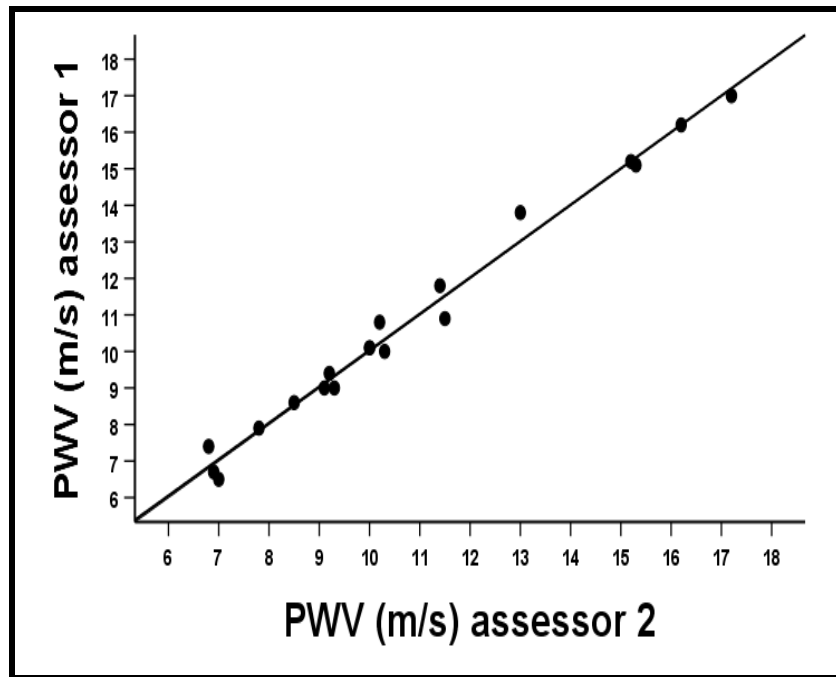
B
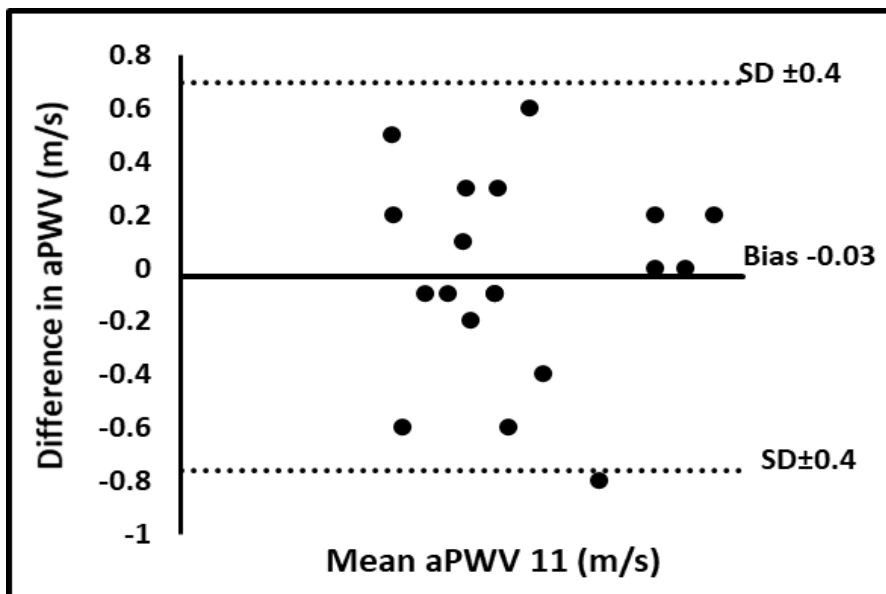
2.6.2 Study 2: Inter-rater reliability of MCAPI measurement

Twenty participants (mean age 37±15 years) took part in the study. The characteristics of the participants and the MCAPI measurements by both assessors are summarised in Table 2.5.

Table 2.5: Participant's characteristics and MCAPI measurements for the Inter-rater reliability

n=20	Mean ± SD
Gender Female: Male	8:12
Age (years)	37±15
MCAPI assessor 1 (researcher)	0.75±0.12
MCAPI assessor 2 (proficient user)	0.72±0.10

There was an excellent degree of reliability between MCAPI measurements by the two assessors as shown by the ICC and the Bland-Altman (Figure 2.13). The average measure ICC was 0.956 with a 95% confidence interval from 0.890 to 0.983 (F (19, 19) = 22.979, p<.001). The two measurements are presented by the scatter plots showing data closely aligned around the line of best fit (Figure 2.13.A) and the Bland-Altman showing acceptable mean measurement differences and limits of agreement between series of MCAPI measurements with one outlier beyond 2 SD's (Figure 2.13 B).

Figure 2.13: Scatter plot showing the line of perfect agreement (a) and the Bland-Altman plots (b) of level of agreement between MCAPI measurements by the two assessors

A
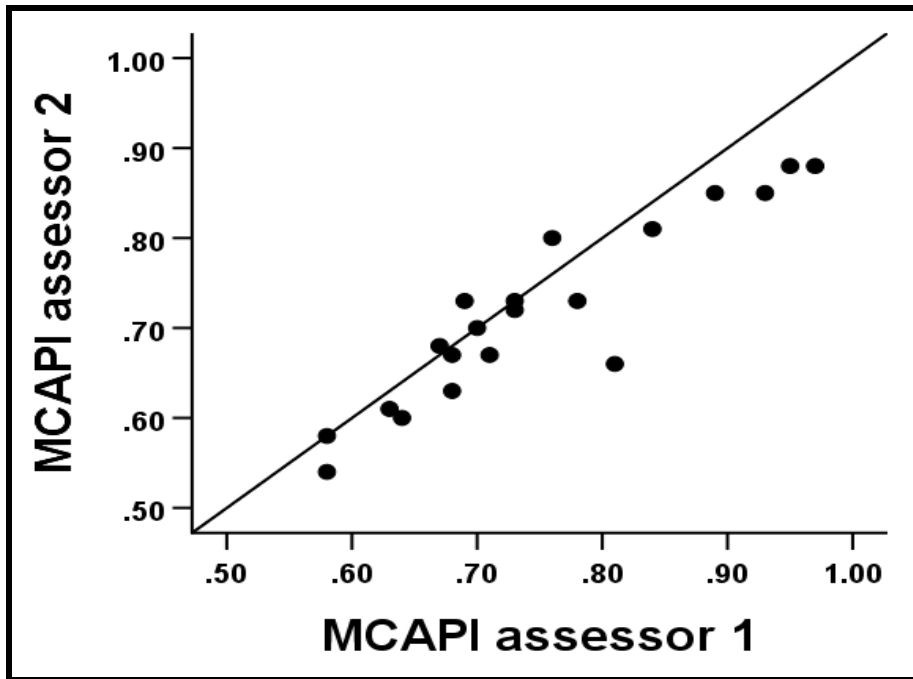
B
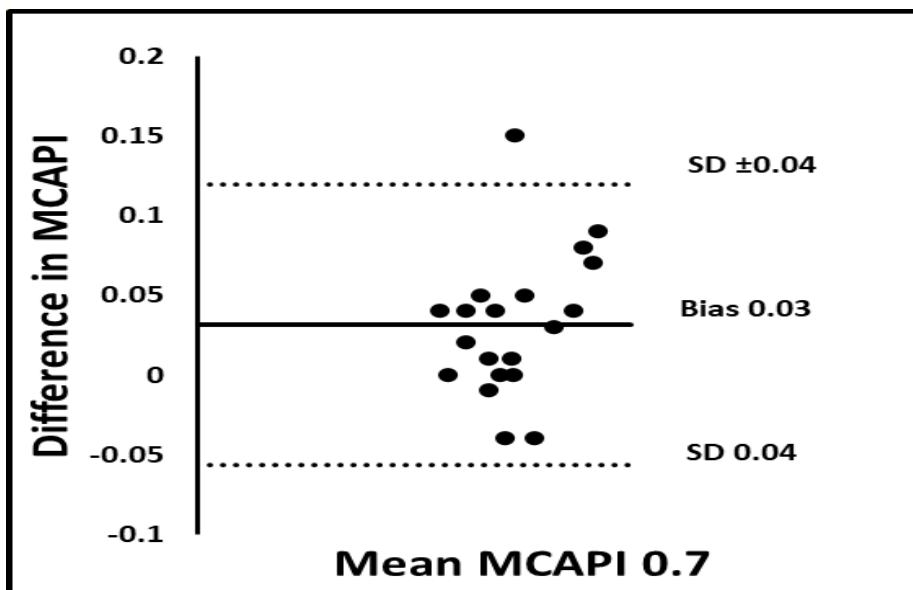
2.6.3 Study 3: Intra-rater reliability of MCAPI measurement

A total of 25 participants completed repeated measurements of MCAPI taken by a single assessor. The characteristics of participants including age and gender, in addition to the two MCAPI measurements, are summarised in Table 2.6.

A high degree of reliability was found between the two MCAPI measurements by single assessor. The average measure ICC was 0.980 with a 95% confidence interval from 0.954 to 0.991 ($F_{24,24}$ = 48.92, $p < 0.001$). A visual presentation of the level of agreement between the two measurements using scatter plots showed that the two measurements were closely related (Figure 2.14.A). Furthermore, the Bland-Altman showing acceptable mean measurement differences and limits of agreement between series of MCAPI measurements with one outlier (outside 2 SD's) that relate to a participant with extreme value of MCAPI (Figure 2.14.B).

Table 2.6: Characteristics of the participants and MCAPI repeated Inter-rater reliability

n=25	Mean ± SD
Gender Female: Male	16: 9
Age (years)	65±7
MCAPI 1	0.83±0.17
MCAPI 2	0.84±0.18

Figure 2.14: Scatter plot showing the line of perfect agreement (a) and the Bland-Altman plots (b) of level of agreement between MCAPI measurements by single assessor

A
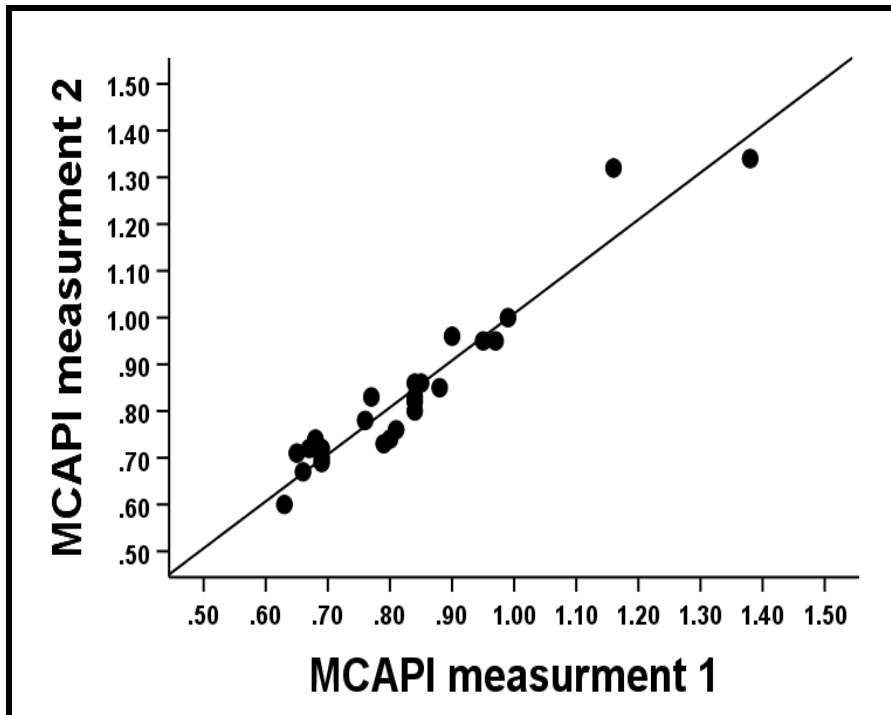
B
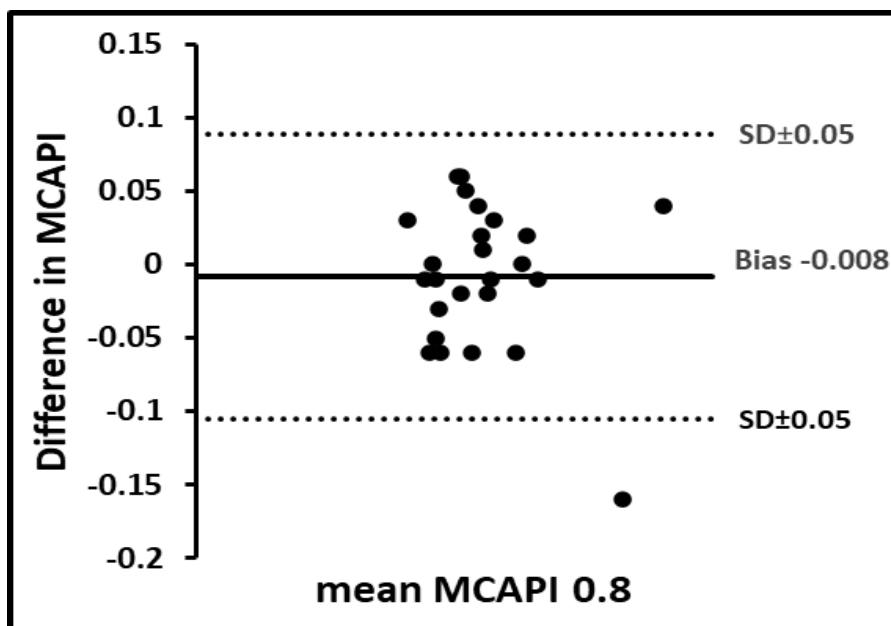
2.6.4 Study 4: Validation of measurement positions of MCAPI

A total of 21 participants completed the measurement of MCAPI at the two measurements positions in one session by a single assessor. The characteristics of the participants, including age, gender and the measurements of MCAPI are summarised in Table 2.7.

Table 2.7 : Participant characteristics for the test-retest validity of MCAPI measurement points

n=21	Mean ± SD
Gender Female: Male	11:10
Age (years)	67±6.9
MCAPI Position A	0.77±0.11
MCAPI Position B	0.76±0.12

An excellent degree of reliability was found between the two MCAPI measurement positions. The average measure ICC was 0.905 with a 95% confidence interval from 0.768 to 0.961 ($F_{(20, 20)} = 10.311$, $p<.001$). The ICC represents a moderate corelation between measurements obtained at the two positions (Figure 2.15.A) and Bland-Altman graph presents this strong agreement with two outliers (outside 2 SD's) (Figure 2.15.B).

Figure 2.15: Scatter plot showing the line of perfect agreement(a) and the Bland-Altman plots (b) of level of agreement between the two measurement positions of MCAPI

A
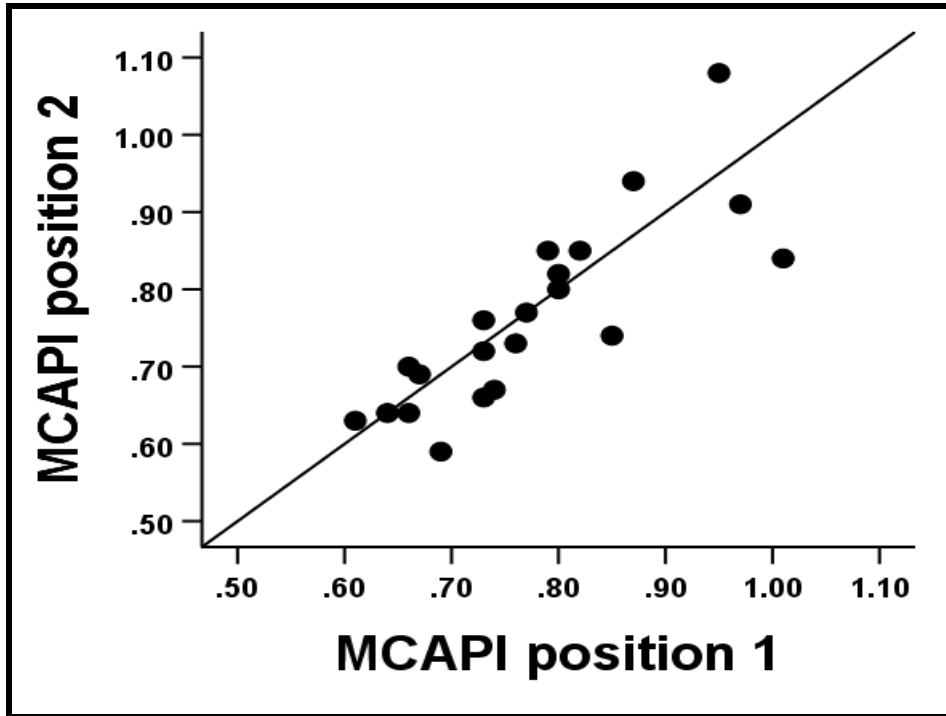
B
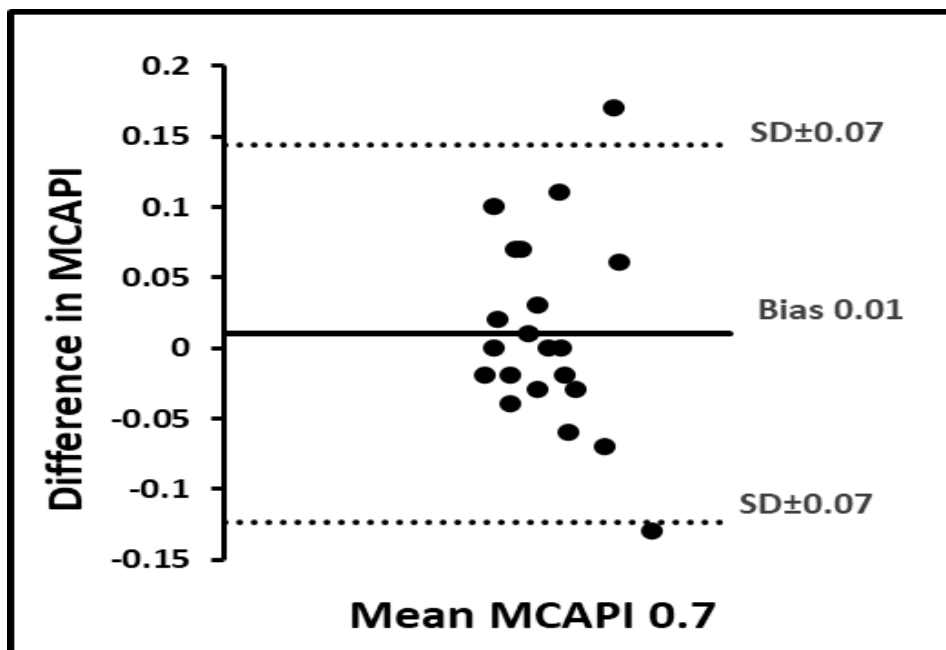
2.7 **Discussion**

The methodological studies were conducted to establish the test-retest reliability of key outcomes for the main study which were the measurement of aPWV and MCAPI.

2.7.1 Inter-rater reliability of aPWV measurement

The aim of this study was to establish the inter rater reliability of aPWV measurement in healthy individuals. The result indicated an excellent inter-rater reliability, as confirmed by the high reliability with all ICC >0.09 (Portney and Watkins 2013).

The results from the current study are in keeping with the results from other studies assessing reproducibility of aPWV in patients with COPD and other populations. A group of researchers reported a good agreement when aPWV measurement was repeated in 15 moderate to severe COPD patients (mean age 64 years). A single assessor measured aPWV five times in three separate days within a 10-day gap. There was good reliability of aPWV measurements as represented by ICC = 0.75 (95% CI, 0.53–0.89). There was less than 10% variation in the measurements between the different assessments' days which is an acceptable threshold for aPWV measurement (Sima et al. 2016).

In addition, a study examined the within-day and between-day reproducibility of aPWV in 17 patients with COPD (mean age 56 ± 2years; FEV1 51.5% predicted). The within-day measurement of aPWV where measurements were taken 3 hours apart, showed significant ICC and Pearson correlation coefficients (ICC. 0.988, P≤0.01) with one point that falls outside of the 95% confidence interval on the Bland–Altman plot. In addition, the between-day reproducibility of aPWV where the measurement was repeated with seven days showed a significant ICC and Pearson correlation coefficients (ICC 0.963, P≤0.01), and all points fall within the 95% CI on the Bland–Altman plot (Rodriguez-Miguelez et al. 2015).

In addition, a study of aPWV in 20 individuals (13 with Chronic Kidney Disease and 7 without) by four observers who underwent a training course of 3-hour sessions twice weekly, for 6 weeks. The interclass correlation coefficient for all four observers assessing was 0.95, indicating a high concordance measurement (Sigrist et al. 2010). Although having a small sample size, the study assessed level of agreement of aPWV by four observers, which has not been assessed previously by this many observers.

Furthermore, a group of researchers examined the inter-rater reliability of measuring aPWV in 20 healthy participants (mean age 45±12 years) and 16 patients with end-stage renal disease (mean age 63±16 years). Two research nurses independently took measurement of aPWV at two study visits within a week. The result showed that the level of concordance between measurements taken by the two operators was higher in the patient group (ICC 0.87) compared to the healthy group (0.461) (Rodriguez et al. 2016).

The current study and previous evidence demonstrate that trained assessor with limited previous experience of aPWV measurement can produce reliable data that can be utilised in research.

2.7.2 Inter-rater reliability of MCAPI measurement

Since measuring MCA velocities by TCD is partly operator dependent, the operator knowledge and experience can influence the accuracy of measurement to a greater extent (Inusa et al. 2019). The current study was aimed to establish the inter and intra-rater reliability of MCAPI. The results showed an excellent degree of reliability with an ICC >0.9 between MCAPI measurements by the two assessors (an ICC of above 0.90 is considered an excellent reliability) (Portney and Watkins 2013).

The consistency can be linked to training that provided an understanding of the principles of measurement in addition to appreciating variations in different age groups and genders.

Previous researchers investigated the impact of the level of experience on the level of agreement of measurements obtained by TCD. A total of 216 measurements of MCA velocity were completed in 36 healthy young individuals by four assessors, two experienced and two inexperienced, with experience level quantified based on the duration and the frequency of performing TCD measurements over the year prior commencing the study. For inter-operator reproducibility, there was a higher consistency with more experienced assessors than that of the mixed and inexperienced groups (±22.1, ±39.3 and ±40.1 cm/s, respectively). However, irrespective of the level of experience, the intra-operator reproducibility was strong (The 95% limits of agreement for the whole group are ±16.9 cm/s) (McMahon et al. 2007).

In addition, a modular training program in measuring MCA velocity using TCD in patients with sickle cell disease has shown its effectiveness in standardising TCD measurement. Operators from three centres who completed the training were able to produce consistent measurements that were comparable to an acknowledged sickle cell classification metric (ANOVA F = 1.9, p = 0.15). The consistency in the measurement was irrespective of the mode of the TCD used, however, the imaging TCD was preferred as it offered a shorter learning curve since the vessels visualisation and localisation was possible compared to the non-imaging TCD (Inusa et al. 2019).

2.7.3 Intra-rater reliability of MCAPI measurement

As for the intra-rater reliability of MCAPI measurement, the results showed an excellent short-term reproducibility of MCAPI (ICC above 0.90 represent an excellent reliability) (Portney and Watkins 2013), which is in line to what had been reported by several studies in diverse populations. A group of researchers measured blood flow velocity twice with an hour gap and repeated the measurement within 14 days in 20 healthy young individuals (mean age 31±9 years). The ICC for both repeatability and reproducibility indicated good reliability (ICC 0.75–0.90). The same protocol was also repeated in 20 patients with symptomatic carotid artery stenosis (mean ago 72±11 years) and the result was comparable to the one obtained from the younger healthy participants (ICC > 0.90) (Kaczynski et al. 2018). A similar study in healthy adults where MCAPI was measured by seven blinded assessors also reported a good intra and inter-rater reproducibility. The MCAPI intra-rater ICC was 96% (95% CI 94.3-98.6%) and the MCAPI inter-rater ICC was 75.0% (95%CI 70.2-79.7) (Venturelli et al. 2015).

In addition, studies also showed that MCAPI was reproducible over the longer term. Ortega-Gutierrez et al demonstrated a fair to good reliability (ICC=0.632, L ICC=0.576; p<0.001) for repeated measurements at a median time of 17 days in 19 healthy middle-aged subjects (Ortega-Gutierrez et al. 2014). The measurement interval was not consistent in all study participants, which may add additional variability and therefore causing a potential error. However, this variability is prevalent in clinical settings as measurements may not be performed at an exact interval.

To conclude, MCAPI measurement is reproducible and appropriate training and practice can help achieve MCAPI measurement proficiency.

2.7.4 Validation of measurement positions of MCAPI

Although TCD is a widely accepted non-invasive estimate of cerebral flow velocity, acquiring the measurement in some individuals may be challenging (Nader et al. 2015). It is acknowledged that one of the leading technical challenges in TCD measurement is the availability of the optimal temporal window to insonate the MCA, generally known as an absent window or Temporal Acoustic Window Failure (TAWF) (Kwon et al. 2006). A large body of evidence suggests that TWAF is a prevalent problem and was reported in 10% to 54% of studied populations. Its prevalence increases especially in old age, female and in individuals from specific ethnicity background. The variation of failure rate between the studies may be caused by the use of different definitions for TAWF, variation in populations age, gender or ethnicity, operator experience and different methodologies. (Bos Michiel et al. 2007; Wijnhoud et al. 2011; Brunser et al. 2012; Lin et al. 2015b; Nader et al. 2015; Del Brutto et al. 2016; Kang et al. 2019).

There are some established classifications with regards to the acoustic window when using TCD. The use of names including an optimal, suboptimal, or absent window is widely used (Del Brutto et al. 2016), but it is also classified numerically into classes one, two or three (Jarquin-Valdivia et al. 2004). Typically, an absent window occurs when no flow signals are detected after ensuring that the right window was adequately mapped to insonate the vessel and hence, no velocity parameters are produced. However, a suboptimal window is present in some cases where flow signals can be detected, but the decreased signal energy does not allow for an accurate calculation of velocity parameters. The reduced signal energy causes the algorithm to fail to differentiate between the Doppler signal from the background noise leading to the presence of artefacts. Whereas an optimal window implies the existence of flow signals and velocities parameters. (Lorenz et al. 2007; Nader et al. 2015; Del Brutto et al. 2016).

To insonate the MCA, the probe is placed over the temporal area above the zygomatic arch, and in front of the ear tragus (point A) (Figure 2.11) (Purkayastha and Sorond 2012). However, the success rate of insonating the MCA to achieve suitable quality waveforms was

low in the initial days of data collection. In the present study, the success rate improved dramatically when the probe was moved closer to the tragus of the ear (point B) (Figure 2.11). However, the change in probe placement could have affected the accuracy and consistency of the measured MCAPI. This methodological modification through the data collection phase necessitated validation. Hence, a validation study was conducted to establish the consistency of MCAPI measurement taken at the two different measurement locations in healthy young participants and in a smaller sample of elderly participants.

The moderate level of agreement between the MCAPI measurements taken at the two positions in this study suggest that either position can be used to maximise the attainment of this measurement in individuals with challenging acoustic window.

It is vital to acknowledge that changing the probe position can alter the angle of insonation to a great extent. The angle of insonation is the angle between the ultrasound beam and the direction of the MCA on a horizontal plane, known mathematically as the cosine function of the angle of insonation. (Aaslid et al. 1982). The cosine angle is one of the principal elements in the Doppler formula that is used to derive blood flow velocity. It is assumed to be as close to zero as possible and < 30 degrees (Jarquin-Valdivia et al. 2004). Besides, insonating MCA through the anterior window provides a near-zero insonation angle, making absolute velocity measurements possible unlike using the posterior window (Willie et al. 2011).

In some ultrasound modalities, this angle can be corrected. However, in TCD, blood flow velocities are measured regardless (Purkayastha and Sorond 2012). This angle was measured by CT scan in 100 healthy adults and was estimated to be around 33.9 degrees suggesting the appropriateness of correcting the angle to 30 degrees rather than 0 degrees. Because the CT scans were done as part of routine care, the head position that allows the MCA to lie on the same CT slab was not achieved in all participants. However, the impact of position differences was very minimal (Jarquin-Valdivia et al. 2004). Nonetheless, even when the angle is assumed to be sharp (between 0-30°), the maximum errors are estimated to be less than 15% (Aaslid et al. 1982) and between 0-13% (Ringelstein et al. 1990) suggesting that vessels diameter plays a more significant role in the blood flow velocity than the angle of insonation.

However, an in-vitro experiment showed that the angle of insonation contributed the most to the measured flow velocity. However, an in-vivo experiment by the same team showed that insonation places highly contributed to the measured velocity. Besides, signal quality was not affected by the changes in the insonation angle but was indeed affected by changing insonation place. The changes in the insonation place meant that bone thickness was not consistent, and this had caused a variation from the initially measured mean flow velocity by about 65% to 80% (De jong et al. 2014).

Considering the above-reported variations caused by changing the probe position and angle of insonation, standardising the probe orientation can be challenging, and there can be inherent variability within individuals when doing repeated measures. However, data from this study indicated a good consistency between the two measurement positions in healthy individuals supporting the limited effect of the angle in the measured velocity and variations, if any, might be linked to changes in vessels diameter.

Age and Gender

A large body of evidence supports the effect of age and female gender in increasing TWAF. It was reported that the mean age of patients with TAWF was significantly higher than those with a good acoustic window (Farhoudi et al. 2011; Lee et al. 2020) hence the current study included participants of older age.

In a study including patients with acute ischemic stroke, 75% of patients with TAWF were female who had a significant increase in the temporal bone thickness (3.04 ± 0.77 mm) compared to men (2.41 ± 0.58 mm). In addition, the inhomogeneous temporal bone was correlated with a lower bone mineral density which explains why TAWF is more common in females (Kwon et al. 2006).

Increased TWAF in older individuals and females may relate to structural changes with advancing age and hormonal changes in women after menopause. (Lin et al. 2015b). Age increases temporal thickness and inhomogeneity that can lead to attenuation of ultrasound

waves. When bone loses its density, air pockets are formed and multiple layers of different media overlapping within the bone, making it inhomogeneous. As ultrasound waves pass through, they get absorbed, reflected and scattered, resulting in ultrasound energy loss (Lee et al. 2020). The inhomogeneous temporal bone was correlated with lower bone mineral density, commonly seen in females who are more prone to have bone density reduction after menopause which explain why TAWF is more common in females (Farhoudi et al. 2011).

One of the main factors that has been shown to influence TAWF independent of age and gender are increased temporal bone thickness, low temporal bone density and soft tissue thickness (Lorenz et al. 2009; Wijnhoud et al. 2011; Del Brutto et al. 2016; Lee et al. 2020).

A study by Del Brutto and colleagues showed that the mean thickness of the temporal bone was significantly higher in individuals with suboptimal or absent window compared to individuals with optimal temporal window. For every millimetre increase in thickness, the chance of having a suboptimal/absent window was higher by 2.9 times (Del Brutto et al. 2016). Their result was further supported by a similar study which assessed skull thickness and bone density influence on TWAF by evaluating CT scans in 182 patients with a transient ischemic attack or minor ischemic stroke. They reported that skull thickness at the temporal bone window was an independent prognostic factor of TAWF after adjusting for age and gender (Wijnhoud et al. 2011).

Lorenz and colleagues developed a model that simulate poor bone windows where the spectral power of the received ultrasound signal was reduced by artificially increasing the thickness. They studied its impact on the calculated blood flow velocity of the MCA while measuring cerebral autoregulation in a group of healthy young individuals. The model resulted in significant bias in TCD autoregulation parameters which was directly linked to the reduction in signal quality. There was a significant reduction in all blood flow velocities, including the diastolic blood flow velocities and a higher MCAPI (Lorenz et al. 2009).

The structure and thickness of the skull play a vital role in the transmission of the ultrasound beam. Homogenous bone allows smooth infiltration of ultrasound wave; however, when the

bone is inhomogeneous, attenuation and scattering of ultrasound signals can occur (Kwon et al. 2006). When the signal loses its strength, the distance between the signal and the background noise shrinks leading to failure in the envelope curve algorithm and as a result, producing artefacts.

It is practically difficult to determine if a lack of signals was a result of TAWF or duo to occlusion in the MCA. The lack of certainty of the source of TWAF can impose a risk of misdiagnosis, especially when using TCD to guide the thrombolysis use for manageing patients with stroke (Kwon et al. 2006).

Kwon and colleagues suggested that initial brain CT taken at the time of stroke could predict TAWF. For example, the possibility of TAWF was 93.5% if the brain CT scan showed inhomogeneous and thickness in the temporal bone ($\geq$ 2.7 mm) whereas if the temporal bone was homogenous and thin , TAWF was only 5.4% (Kwon et al. 2006). This suggests that when a CT scan is available, confirmation of TAWF is possible to a greater extent and can potentially produce a better management outcome.

Nonetheless, colour-coded Doppler velocity imaging coupled with B-mode imaging, is now widely used for TCD examination and may help assess the temporal acoustic window and find anatomic landmarks, thus potentially reducing insonation failure rates (Del Brutto et al. 2016).

These findings have significance when interpreting parameters obtained from a suboptimal window as systematic error can occur. To avoid this error, data was included to represent parameters derived from an optimal window to avoid any bias caused by signal artefacts and consequently, the sample size decreased.

2.8 **Strength and limitations**

The strength of this chapter was the steps undertaken to ensure research rigour by conducting several methodological studies. The outcome of these studies provided confidence that measurements taken for the three primary studies within this thesis were not affected by measurement variability. This was achieved by considering several factors, including recruiting an appropriate sample, controlling for caffeine intake and exercise before the assessments, following established protocols, randomising measurement order, using validated measurement tools, comparing results to proficient assessors, and using well-established analysis tests for reliability studies.

2.9 **Conclusion**

In this group of participants, using ICCs and Bland-Altman plots, the results have shown that the measurements of aPWV using the SphygmoCor system are repeatable when measured by different assessors following the recommended guidelines. It also showed that the measurement of MCAPI using TCD is reliable when repeated by one assessor or by several assessors. It also showed that using several measurement locations within the recommended measurement zone was accepted and reduced the chance of measurement failure. Therefore, these measurements are suitable for use in longitudinal and observational studies.

Chapter 3

Longitudinal changes in aPWV and measures of health status in patients with COPD and controls

3 Longitudinal study; changes in aPWV and measures of health status in patients with COPD and controls

3.1 Introduction

Chronic Obstructive Pulmonary Diseases (COPD) is a chronic inflammatory disease that primarily affects the lung and results in progressive airflow obstruction that is not fully reversible (Rabe et al. 2007; Burkes and Donohue 2018). However, surmounting evidence support that COPD is frequently associated with other comorbidities that have a significant impact on COPD management and prognosis (Wang et al. 2017b), high excess costs (Huber et al. 2015) in addition to increasing the mortality risk (Cavailles et al. 2013).

Cardiovascular disease (CVD) is among the comorbidities highly prevalent in patients with COPD who present with 2-5 times greater risk of developing different forms of CVD (Chen et al. 2015). In addition, the risk of mortality from CVD was higher than deaths caused by pulmonary complications (McAllister et al. 2007; Lahousse et al. 2016).

The relationship between COPD and CVD is complex, but a plausible mechanistic connection explaining the coexistence of both conditions is the presence of shared risk factors including old age, smoking, exposure to environmental pollution and systemic inflammation (Mills et al. 2008; Mullerova et al. 2013; Fisk et al. 2018).

In addition to traditional risk factors, aortic PWV (aPWV) estimated by c-f PWV is considered the gold standard surrogate marker for arterial stiffness and can be measured noninvasively (Laurent et al. 2006). Importantly, increased aPWV may reflect subclinical CVD (Sabit et al. 2007), with a large body of evidence supporting its predictive role for CVD events irrespective of other traditional risk factors (Vlachopoulos et al. 2010; Mullerova et al. 2013; Ben-Shlomo et al. 2014; Chen et al. 2015; Zhong et al. 2018).

Increased aPWV in patients with COPD is well documented in the literature and was associated with age, higher peripheral systolic and diastolic blood pressure (Mills et al. 2008; Fisk et al. 2018). However, the exact mechanisms linking it to COPD specific characteristics are not fully understood. As cross-sectional studies have their limitations of establishing causality, longitudinal studies examining such interaction over time are much needed. A considerable amount of evidence reported the progression of aPWV in several populations (Benetos et al. 2002; Oliveras et al. 2016; Cecelja et al. 2018; Maloberti et al. 2019), however, studies measuring rates of progression of aPWV in COPD are limited.

3.1.1 Study aim, objectives, and hypothesis

Therefore, the study aim was to measure central haemodynamics and measures of health status longitudinally at three-time points; baseline, two years, and six years in patients with COPD and aged-matched controls.

3.1.1.1 The objectives of the study

1. To evaluate change in aPWV at three time points in patients with COPD and controls.

2. To measure health status including physical function and patients reported measures at three time points in patients with COPD versus controls.

3. To establish the relationship between aPWV and vascular measurements in patients with COPD and controls.

4. To establish the relationship between aPWV and measures of health status including objective and subjective measures.

5. To establish the relationship between aPWV and COPD characteristics as measured by FEV_1 in patients with COPD.

3.1.1.2 The hypothesis of the study

Null hypothesis:

1. There is no difference in aPWV and its progression in patients with COPD versus controls.

2. There is no difference in health status between patients with COPD and controls at three time points.

3. There is no relationship between aPWV and vascular measurements in patients with COPD and controls.

4. There is no relationship between aPWV and measures of health status in patients with COPD and controls.

5. There is no relationship between aPWV and COPD characteristics in patients with COPD.

Alternative hypothesis:

1. There is a difference in baseline aPWV and in it's progression in patients with COPD versus controls.

2. There is a difference in health status between patients with COPD and controls.

3. There is a relationship between aPWV and vascular measurements in patients with COPD versus controls.

4. There is a relationship between aPWV and measures of health status in patients with COPD and controls

5. There is a relationship between aPWV and COPD characteristics in patients with COPD versus control.

3.2 Method

3.2.1 Design setting and recruitment

A longitudinal study measuring aPWV and its determinates over a period of time in a group of patients with COPD and age matched control group from the ARCADE study NCT 01656421 (Gale et al. 2014). Data for the baseline and the two year follow up were previously collected, with the current study forming the six-year follow-up (Gale et al. 2019). The inclusion and exclusion criteria are detailed in methods chapter (Table 2.1). Participants had a postal invitation, and the study was fully explained before considering participation and consenting to take part in the study.

3.2.2 Participant characteristics

Height, weight, waist circumference and total fat % were measured in all participants. History of CVD and use of CVD medications were also recorded.

All study participants underwent pulmonary function testing according to ATS/ERS guidelines (Miller et al. 2005) using the Vitalograph ALPHA 6000 (Vitalograph Ltd. UK) as detailed in (Chapter 2.3.3).

3.2.3 Assessments

3.2.3.1 Vascular measurements

Blood pressure was measured using a semi–automated oscillometric sphygmomanometer (Omron M2 HEM-7121-E, OMRON Healthcare UK Ltd). Measurements were taken twice in seated position and supine position. The average of the two measurements were recorded for each position (Chapter 2.3.2.1).

Arterial stiffness was measured using the SphygmoCor system (AtCor Medical, Sydney, Australia). Measurements of aPWV was obtained by measuring the c-f PWV as per the protocol endorsed by Expert consensus document on arterial stiffness (Laurent et al 2006) and fully explained previously (2.3.2.3). The recorded measurements were AIx, central systolic/diastolic BP, heart Rate, and mean arterial pressure (MAP), central pulse pressure (PP) and aPWV.

3.2.3.2 Measures of health status

General subjective and objective measures of health status were evaluated in all participants, with some measures specific to the COPD group. The subjective measures included the Comprehensive Geriatric Assessment (CGA), a multidimensional questionnaire used in the assessment of frailty in older adults. In addition, the COPD Assessment Test (CAT), a short questionnaire that measures the impact of COPD on the life of patients with COPD was completed by participants with COPD. Participants in the COPD group also completed the St George's Respiratory Questionnaire (SGRQ). It is a detailed assessment structured to describe three main elements related to COPD which are symptoms, activity, and impact.

The objective measures included handgrip strength using the Grip Strength Dynamometer GRIP-D/ T.K.K.5401 (Takei Scientific Instruments Co Ltd, Tokyo Japan) with an average of two measurements for each hand was recorded in kg. Participants also completed the Timed Up and Go test (TUG test), a simple test assessing the progress of balance, sit to stand, and walking at a comfortable pace at marked distance of three meters. The time spent in completing the test and was recorded in seconds. In addition, participants completed the Six-Minute Walk Test (6MWT) walking at their own pace for six minutes in a 10-meter straight corridor using their walking aid if used. HR, SpO_2 and Borg Rating of Perceived Exertion Scale were recorded prior and after test completion. The distance (m) covered by the end of the six minutes were measured and recorded.

3.3 **Data analysis**

Data were analysed using SPSS Statistics 25. Data included continuous (interval ratio) and categorical data. Interval ratio data were checked for normality. Descriptive data included mean and standard deviation for parametric data and median IQR frequencies and percentages for nonparametric data.

The longitudinal changes in aPWV were assessed using the ANOVA test. The Pearson's correlation test was used to assess the relationship between aPWV and vascular measurement and COPD characteristics. The non-parametric equivalent was used to assess the relationship between aPWV and comorbidities. In addition, Friedman's test was used to estimate the mean rank and the difference in the mean rank in the number of cardiovascular acting medications between baseline and follow-up in both groups.

3.4 **Results**

3.4.1 Participant's characteristics

Total of 85 Patients with COPD and 73 matched controls attended all three visits (baseline, 2 and 6 years), but some were excluded from this analysis. The exclusion was due to incomplete measurements related to difficulties in getting the measurements or contraindications on the day of the assessment including high BP, breathlessness, or pain. Data for a total of 74 patients with COPD and 71 healthy controls who completed measurements at the three visits were included in the analysis.

At baseline, the mean age of participants in both groups was similar (65 ±7 years). Both groups were similar in terms of anthropometric measurements. Lung function was significantly reduced in the COPD group as measured by $FEV_1\%$, FVC% and FEV_1/FVC ratio (p=0.001) (Table 3.1).

At the 6-year follow-up, anthropometric measurements were also similar. Lung function was significantly reduced in the COPD group as measured by $FEV_1\%$, FVC% and FEV_1/FVC ratio (p=0.001) (Table 3.1).

3.4.2 Vascular measurements in both groups

At baseline, aPWV was higher in the COPD group (9.6±2.4 m/s) compared to the controls (8.2±1.6 m/s) and remained higher during the consecutive visits (p=0.001). At 6-year follow up, there was an increase in aPWV by 1.4 m/s in patients with COPD and 1.5 m/s in the control group (both at p=0.001). Whilst absolute values of aPWV remained consistently higher in the COPD group, both groups had shown a similar trend in the increase in aPWV from visit one to three with an approximate annual increase of 0.2 m/s/year (Figure 3.1).

Other vascular parameters such as HR and BP measurements were significantly higher in the COPD group than the control at baseline, but at visit three, no difference in BP measurements was observed between the groups.

3.4.3 Measures of physical function in both groups

Patients with COPD had a significantly lower level of function at all three visits as measured subjectively and objectively when compared to participants in the control group (Table 3.1).

Patients with COPD had a higher score representing a greater limitation in the self-reported comprehensive geriatric assessment (CGA) when compared to control group (p=0.001). Both groups had shown a similar significant increase in the CGA score from baseline to follow-up indicating worse outcomes (p=0.001).

Patients with COPD had a significant shorter walking distance in the 6MWT at baseline and follow-up than the control group (p=0.001). However, the within group change from baseline to follow up was not significant in both groups.

In addition, patients with COPD needed a longer time to complete the TUG test at baseline and remained higher at follow up when compared to the control group (p=0.001). The change in TUG test between the baseline and follow-up was significant in COPD (change of 0.9 s, p=0.011) but not in controls (change of 0.3, p=1.00). However, the mean handgrip was similar in both groups at the three visits.

Table 3.1: Demographics, vascular measurements, and measures of function in patients with COPD and controls

Measurements		COPD (n=74)			Controls (n=71)			COPD vs controls	COPD vs controls
		Baseline Mean ± SD	Mean change at 6yr follow up	Within group difference P=	Baseline Mean ± SD	Mean change at 6yr follow-up	Within group difference P=	Difference at 6 yrs. follow up P=	Difference in the change over 6 yrs. P=
Demographics	Age (Years)	65 ±7	-	-	65 ±7	-	-	0.858	-
	Gender (male, female)	M39, F36	-	-	M36, F35	-	-	0.877	-
	Body Mass Index (Kg/m2)	27.7 ±4.3	-0.3	0.743	28.1 ±3.7	0.1	1.00	0.329	0.220
	Waist circumference (cm)	99 ±15	-1.1	0.645	95 ±10	1.6	0.180	0.577	0.028
	Total Fat %	34± 8	-2.8	0.107	33± 9	-0.7	1.00	0.311	0.220
	FEV_1 % of predicted	68 ± 19	-6.6	0.001	108 ± 13	-6.5	0.003	**0.001**	0.960
	FEV_1/FVC (L)	0.6± 0.1	-0.02	0.067	0.8± 0.1	-0.02	0.065	**0.001**	0.652
Function Measurement	Comprehensive Geriatric Assessment Score	7.6± 4.6	2	0.001	2.6± 2.1	2	0.001	**0.001**	0.409
	6 Minutes Walking Test (m)	383 ± 109	7	1.000	512 ±82	-9	1.000	**0.001**	0.473
	Time Up and Go Test (S)	10± 3	0.9	0.011	8±1	0.3	1.000	**0.001**	0.169
	Mean handgrip strength (Kg)	29±10	-5	0.001	32±11	-6	0.001	0.138	0.420

FEV_1; forced expiratory volume in one second, FVC; forced vital capacity, **p≤0.05** = statistically significant

Table 3.2: Demographics, vascular measurements, and measures of function in patients with COPD and controls (continue)

Measurements	COPD (n=74)			Controls (n=71)			COPD vs controls	COPD vs controls
	Baseline Mean ± SD	Mean change at 6yr follow up	Within group difference P=	Baseline Mean ± SD	Mean change at 6yr follow-up	Within group difference P=	Difference at 6 yrs. follow up P=	Difference in the change over 6 yrs. P=
Vascular measurements								
Aortic Pulse Wave Velocity aPWV (m/s)	9.6±2.4	1.4	0.001	8.2±1.6	1.5	0.001	**0.001**	0.560
Seated Peripheral SBP (mmHg)	146 ±18	-0.9	1.000	139±17	3.2	0.210	0.240	0.093
Seated Peripheral DBP (mmHg)	82±9	0.8	1.00	80±9	2.5	0.105	0.728	0.298
Seated Central SBP (mmHg)	135±18	-1.5	1.000	128±17	12	0.168	0.500	0.123
Seated Central DBP (mmHg)	83±9	0.3	1.000	81±9	2.1	0.193	0.495	0.246
Seated MAP (mmHg)	105±11	-0.3	1.000	101±11	-27.7	0.001	0.331	**0.001**
Seated HR (bpm)	71±10	0.6	1.000	66±9	-1.3	0.757	**0.001**	0.229
Seated central Pulse Pressure (mmHg)	51±15	-2	0.548	47±13	1	1.000	0.534	0.150
Supine SBP (mmHg)	137±16	11	0.001	133±18	7	0.019	0.709	0.270
Supine DBP (mmHg)	73±8	9	0.001	72±8	6	0.001	0.631	0.157

SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; Mean arterial pressure, HR; heart rate, **p≤0.05** = statistically significant

Figure 3.1: aPWV progression at the three visits in the COPD and control groups
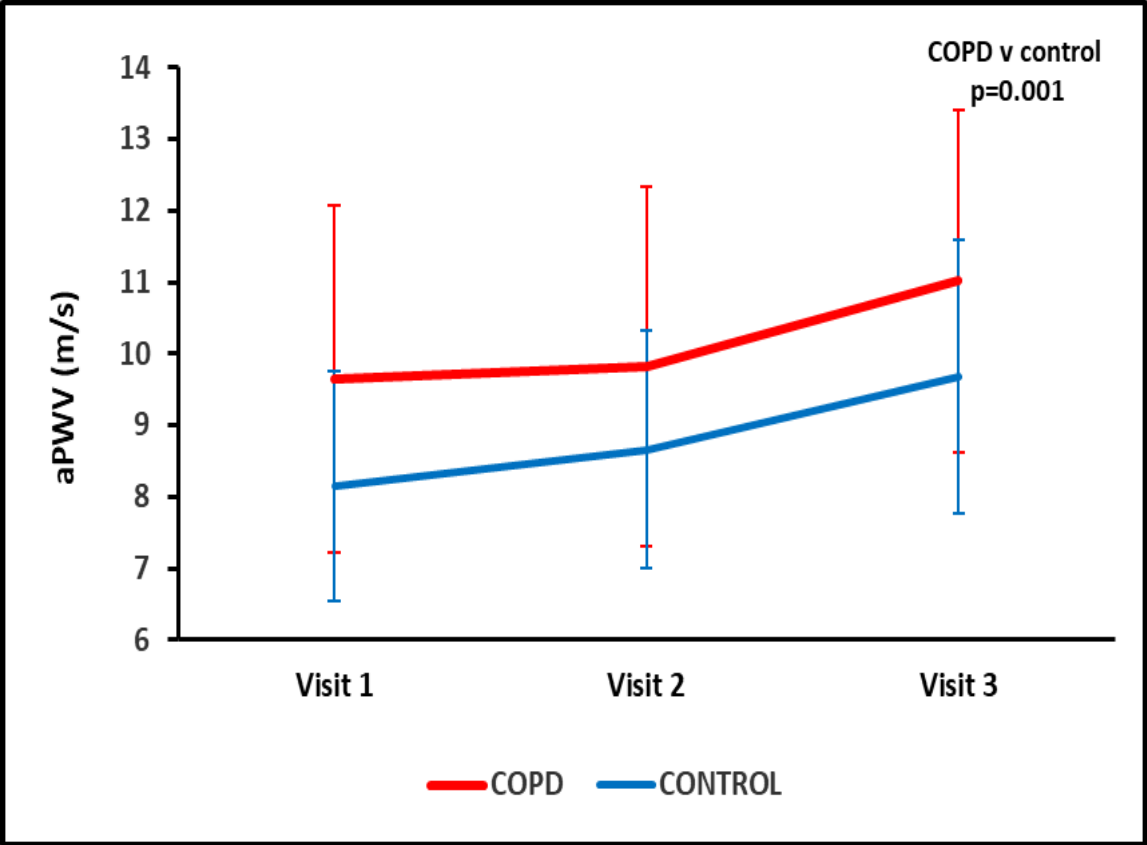
3.4.4 Cardiovascular comorbidities in both groups

Descriptive statistics showed that CVD was more prevalent in patients with COPD compared to controls during the three visits (Figures 3.2 and 3.3). At baseline, approximately 45% of patients with COPD had a diagnosis of hypertension compared to only 21% in the control group. There was a gradual reduction in the number of participants with hypertension in the COPD group over the study period, but hypertension prevalence remained unchanged in the controls.

Participants in the COPD group reported more diagnoses of Angina, Myocardial infarction, and Atrial fibrillation than controls. A small percentage in the COPD group (3%) had chronic heart failure.

In the same way, the prevalence of high cholesterol in COPD was double that of the control group during the initial two visits but significantly reduced to a level equal those in the control group who did not change over the 6 years.

There were no reports of stroke or transient ischemic attack (TIA) during the first two visits in both groups, however, at visit three, 5% of participants in COPD group and 3% in the control group reported having TIA.

Figure 3.2: Cardiovascular Disease in COPD group at all visits
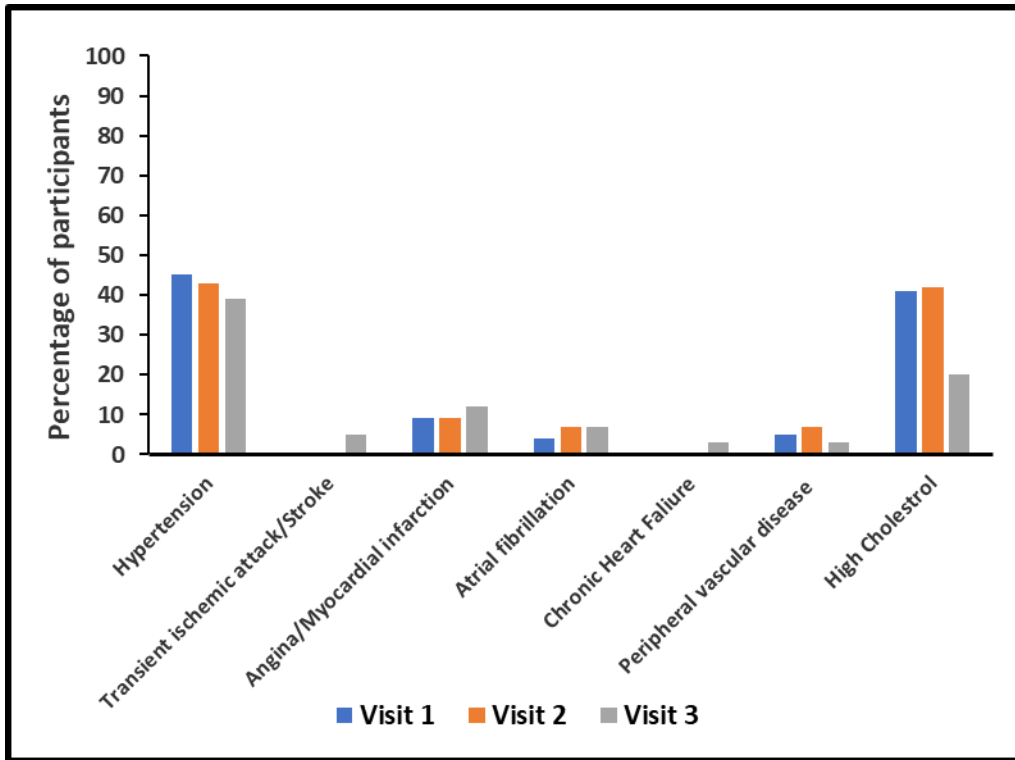
Figure 3.3: Cardiovascular Disease in control group at all visits
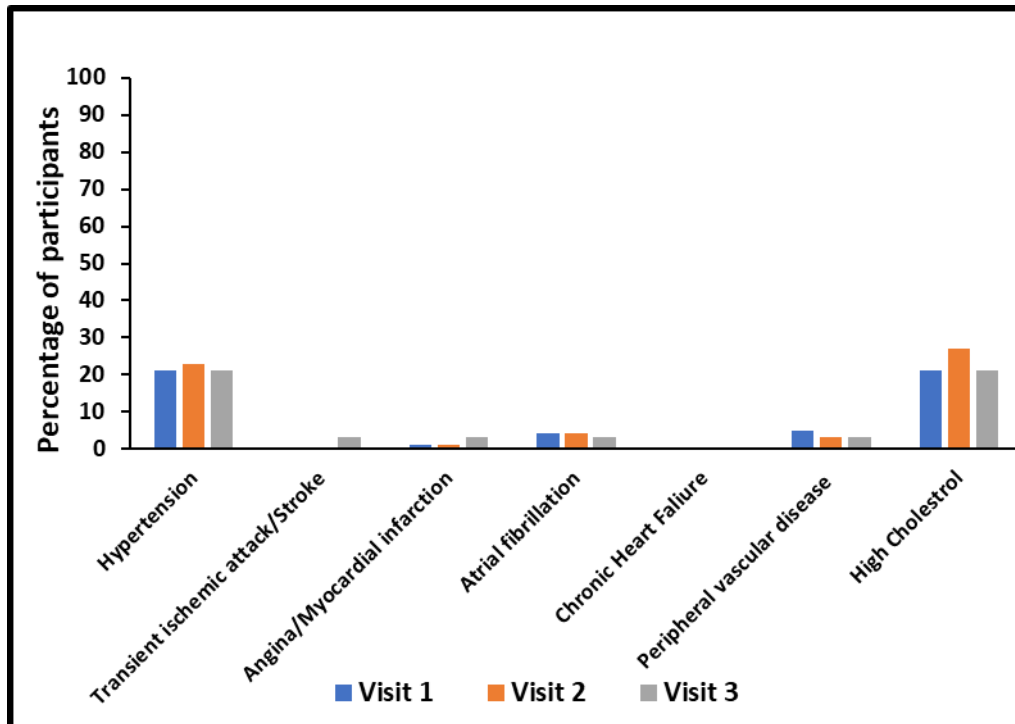
3.4.5 Cardiovascular medications use in both groups

Participants in the COPD group were prescribed more cardiovascular medications than the control group. Both groups showed an increase in the number of cardiovascular medications from baseline to the 6-year follow up. This increase was significant in the control group (Chi-square 8.0, p=0.005) and was reaching significance in the COPD group (chi-square 3.8, p=0.05) (Figure 3.4).

Figure 3.4: Number of prescribed cardiovascular acting medications at the three visits in COPD and control groups
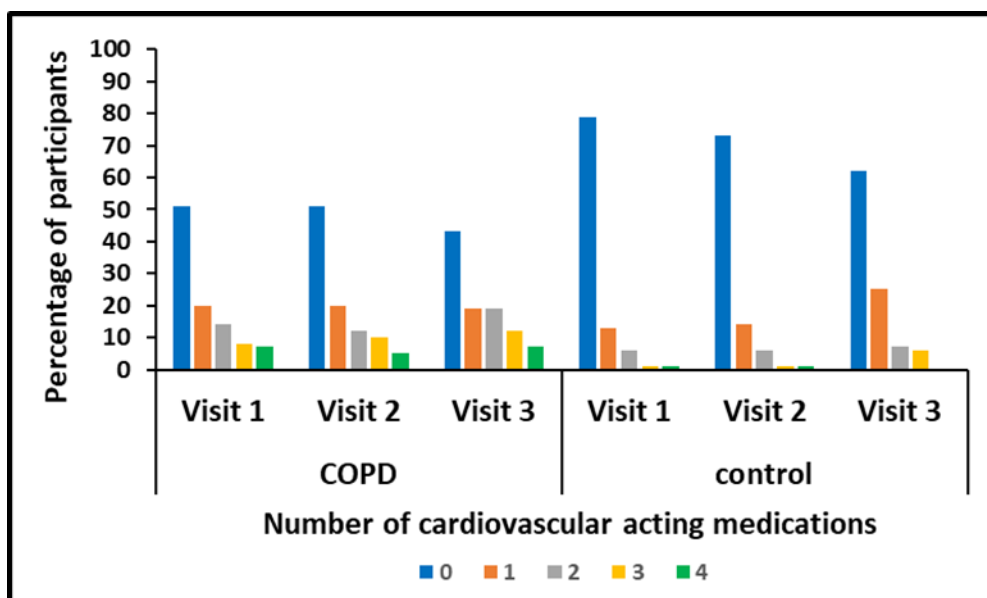
When analysing the breakdown of cardiovascular medications used among both groups, the use of different blood pressure-lowering medications by patients with COPD was also higher than the controls at all three visits. These medications include Angiotensin Converting Enzyme (ACE) inhibitors, Angiotensin Receptor Blockers (ARBs), beta-blockers BB, calcium channel blockers and diuretics. In addition, statins are the most used cardiovascular medications by participants in both groups who displayed a rise in its use at the follow-up (Figures 3.6 and 3.7).

Figure 3.5: Types of cardiovascular acting medications used in the COPD group at the three visits
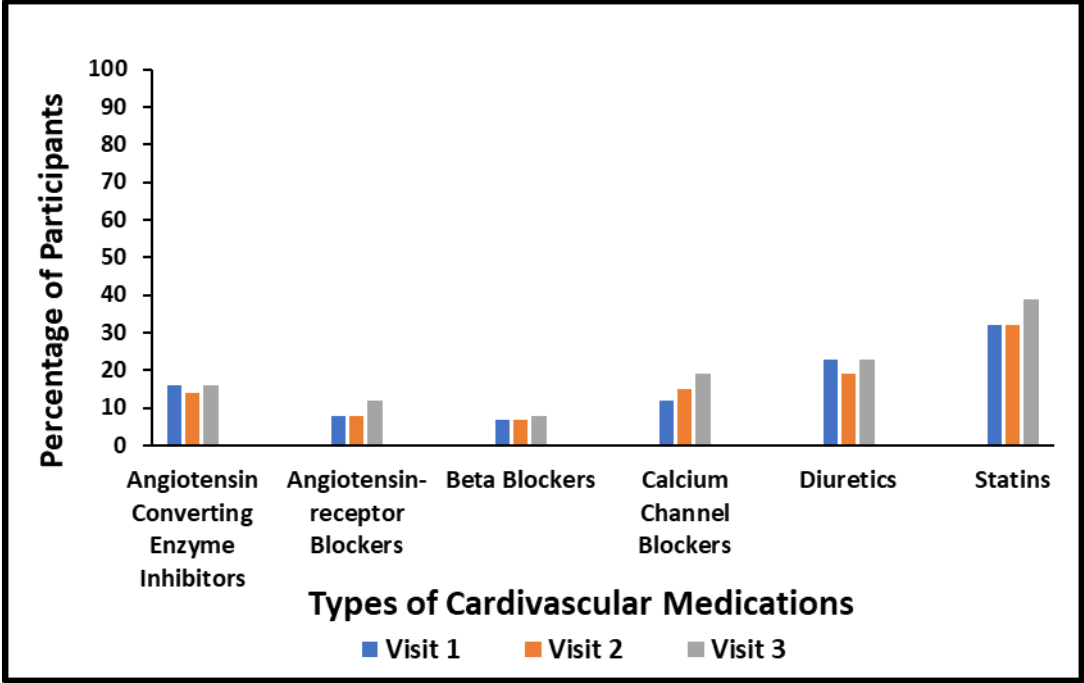
Figure 3.6: The types of cardiovascular acting medications used in the control group at the three visits
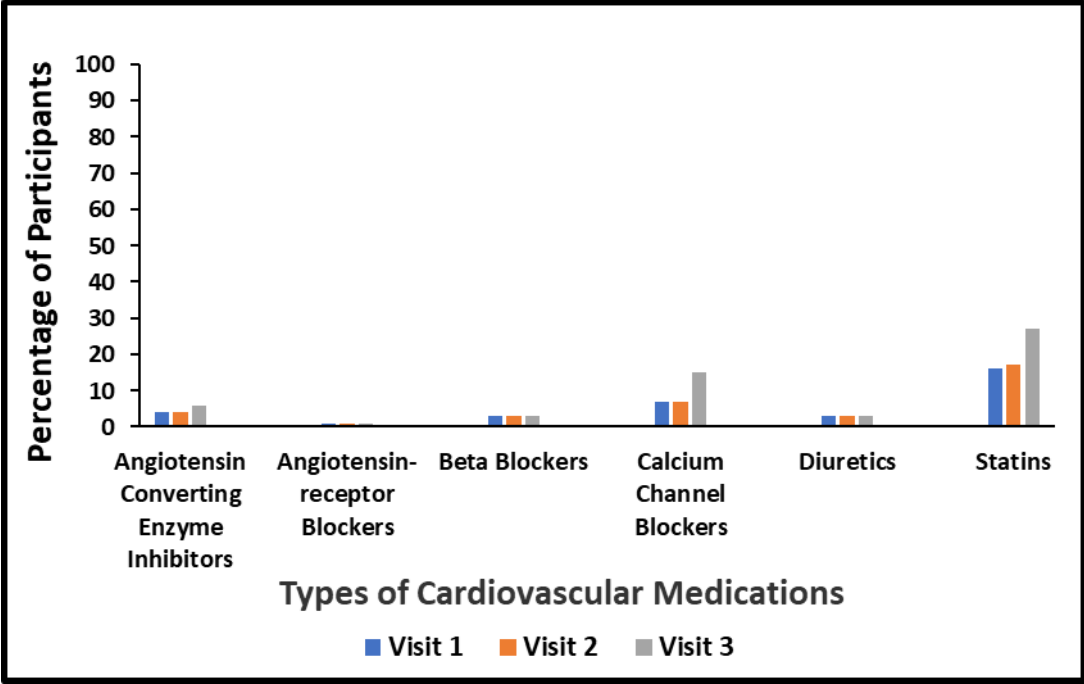
3.4.6 Relationship between aPWV and vascular, COPD related and functional measures

At 6-year follow up, bivariate correlation analysis showed that aPWV was significantly associated with seated SBP, supine central SBP, HR and AIx in the COPD group (all p<0.05). However, in the control group, aPWV was significantly associated with seated peripheral SBP, seated central SBP, seated MAP, supine SBP and CPP (all P <0.05) (Table 3.2).

On the other hand, there was no significant correlation between aPWV, and airflow obstruction as determined by FEV1 % and FEV1/FVC. Similarly, neither were related to subjective measures of COPD including the SGRQ.

As for measures of physical function, aPWV was significantly related to CGA, 6MWT and TUG, in the COPD group but only related to TUG in the control group (all at P <0.05).

In both groups, aPWV weakly correlated with the number of cardiovascular comorbidities in the COPD group (r=0.305, p=0.008) and in control group (r= 0.246, p=0.038).

Table 3.3: Relationship between aPWV and vascular measurements, COPD related measurements, measures of physical function, and cardiovascular comorbidities in both groups at the 6-year follow up

	Measurements	COPD		Controls	
		R	P	R	P
Vascular Measurements	Seated Peripheral SBP (mmHg)	0.330	**0.004**	0.539	**<0.001**
	Seated Peripheral DBP (mmHg)	0.052	0.658	0.075	0.534
	Seated Central SBP (mmHg)	0.250	0.032	0.504	**<0.001**
	Seated Central DBP (mmHg)	0.058	0.622	0.096	0.427
	Seated MAP (mmHg	0.168	0.152	0.350	**0.003**
	Seated HR (bpm)	0.253	**0.031**	0.147	0.222
	Seated Central Pulse Pressure (mmHg)	0.020	0.865	0.345	**0.003**
	Supine SBP (mmHg)	0.371	**0.001**	0.457	**<0.001**
	Supine DBP (mmHg)	0.176	0.134	0.080	0.508
COPD-related measurements	FEV_1%	-0.225	0.056	-0.064	0.599
	FEV_1/FVC	-0.055	0.642	-0.079	0.517
	St George's Respiratory Questionnaire Score	0.217	0.063	-	-
	COPD Assessment Test	0.226	0.053	-	-
Measures of physical function	Comprehensive Geriatric Assessment Score	0.360	**0.002**	0.011	0.930
	6 Minutes Walking Test (m)	-0.348	**0.002**	-0.190	0.112
	Time Up and Go Test (S)	0.428	**0.000**	0.290	**0.014**
	Mean handgrip strength (Kg)	-0.120	0.310	-0.105	0.384
Cardiovascular comorbidities	Number of cardiovascular comorbidities *	0.305	**0.008**	0.246	**0.038**

Abbreviations:

R=Pearson Corelation coefficient
 SBP-Systole Blood Pressure, DBP- Diastole Blood Pressure, FEV1-Forced expiratory volume, FVC -Forced vital capacity, FVC -Forced vital capacity
 *Spearman's **correlation** test for non-parametric data

3.5 **Discussion**

This study aimed to measure central haemodynamics longitudinally at three-time points; baseline, two years, and after six years in patients with COPD and aged-matched healthy controls. This is the first study to provide comprehensive evidence of cardiovascular risk and health status over a 6 year follow up in COPD patients. The results illustrate that patients with COPD had consistently higher aPWV than the controls at each time point; thus, the null hypothesis of no difference in aPWV between the COPD and controls was rejected. However, an important point to note, both groups showed a similar trend in the change of aPWV at each time points, despite the increased use of cardiovascular medications in the COPD group. Therefore, the null hypothesis of no difference in the change in aPWV was accepted. These novel findings are of clinical importance, as they suggest premature vascular ageing in patients with COPD and maintained increased risk when additional CV acting medications are introduced to clinical care. In addition, the level of aPWV was reaching a cut-off point proposed by established guidelines (10m/sec) that provides evidence of subclinical target organ damage of the aorta. These findings may explain the overt history of CVD and the excess use of cardiovascular acting medications in addition to limitations in health status including physical function in this COPD group. Hence, measuring cardiovascular risk over six years adds significant knowledge to the literature that lacks data on longitudinal changes in aPWV.

Furthermore, the study examined health status using a combination of objective and subjective measures in patients with COPD and controls. Our findings highlight that health status was compromised in patients with COPD compared to the controls; thus, the null hypothesis of no difference in health status measures between the groups was rejected.

Finally, a novel finding of this thesis was the observed link between aPWV and health status measures that represent physical function, including the CGA, 6MWT and the TUG test, which were better related to aPWV than lung function or COPD specific questionnaires. Therefore, the null hypothesis of no relationship between aPWV and health status was rejected, and the null hypothesis of no relationship between aPWV and COPD specific

outcomes was accepted. These results highlight the impact of reduced physical function on cardiovascular risk and that these levels of physical function may be an important determinant of vascular damage that may link to increased CVD risk.

3.5.1 Longitudinal changes in aPWV

In terms of the longitudinal progression of aPWV over six years, the annual increase in aPWV was 0.2 m/s/year in both groups who showed a similar trend in the increase in aPWV from visit one to three. The progression in aPWV that was observed in this study was somewhat higher than reports from multiple follow-up studies assessing the longitudinal changes in aPWV in the general population. In a study measuring aPWV progression in normotensive participants, the annual increase was 0.08 m/s/year (Benetos et al. 2002) whereas, in hypertensive participants, this increase was 0.14 m/s/year (Benetos et al. 2002), 0.15 m/s/year (Maloberti et al. 2019) and 0.18 m/s/year (Oliveras et al. 2016). In addition, the Twin United Kingdom cohort, a large longitudinal cohort study that includes female participants with a large proportion having hypertension, showed an annual increase of 0.14 m/s/year (Cecelja et al. 2018).

However, studies measuring longitudinal changes in aPWV in COPD are scant. The Whitehall II study, a large cohort study in the UK reported a mean change of 0.14 m/s/year in aPWV over about five years in individuals who showed a more significant decline of FEV_1, a key feature in COPD. It suggests that COPD is associated with premature vascular ageing (Okamoto et al. 2019).

Recently, a group of researchers reported vascular changes in 76 individuals with COPD followed up for six years (mean age at baseline was 62 years old) by measuring AIx, another measure of arterial stiffness. They reported a significant annual increase in AIx by 0.91%, that was higher than what was reported in other population-based studies suggesting a progressive stiffness of the arterial wall in COPD patients (Roeder et al. 2020). It is essential to consider that AIx is not directly comparable to aPWV as AIx is not a sensitive measure of

ageing in people older than 60 years, whereas aPWV is a better marker of ageing in older individuals (McEniery et al. 2005).

In the studied cohort, the overall annual progression in aPWV was found to be higher than previously reported but can be primarily explained by the older age range of the participants in this cohort. It was shown that progression pattern and age-related changes in aPWV manifest in individuals over 50 years (McEniery et al. 2005). Because participants in the present cohort were largely in their seventies, the rate of the annual progression was steeper than the younger individuals even during a similar course of time. It can be argued that the observation of a steeper rise in aPWV, especially after the 2 years follow up might relate to the overt history of CVD in the COPD group. However, an almost parallel trend was also found in the control group who have a lesser prevalence of CVD. The similarities between the groups in terms of patterns of change in aPWV progression can then be largely related to age. Nevertheless, patients with COPD had greater aPWV at each time point, suggesting their vessels may have stiffened prematurely, and they may be at heightened cardiovascular risk at each timepoint due to this increase.

A large body of evidence has confirmed a well-established association between aPWV with both age and BP as shown by a systematic review (Cecelja and Chowienczyk 2009) and by several large studies including different populations including the Anglo-Cardiff Collaborative Trial (McEniery et al. 2005), the LIFE-Adult study (Baier et al. 2018), The ELSA-Brasil study (Baldo et al. 2018), the Guimaraes/Vizela Study (Cunha et al. 2015), the Han-Chinese study (Lin et al. 2015a) and the Corinthian study (Papaioannou et al. 2016).

The increase in aPWV values observed over time in all studies can be attributed to progressive ageing. Evidence showed that aPWV progress nonlinearly with age, but it exhibits a steeper rise from midlife onwards (McEniery et al. 2005) because of elastin fatigue fracture and degradation and the increase in calcification of the aortic media with age (Kohn et al. 2015). However, it is imperative to acknowledge that chronological age is not the same as biological ageing (Boutouyrie and Bruno 2019). Evidence supports the alteration of markers related to ageing in individuals with COPD including shorter Telomere length, decreased anti-

ageing molecules such as sirtuin 1 and Sklotho and a reduced DNA repair gene, making them biologically older by about 7.5 years than individuals with similar age (Rutten et al. 2016) which may explain the premature vascular ageing found in the COPD group.

The above observation supports the vital role of ageing in increasing the risk of CVD, and as patients with COPD exhibit ageing process at a faster rate than non-COPD, they may be at higher risk of CVD. The higher aPWV at baseline in the COPD group may relate to CVD history, but causality cannot be ascertained in this case. It also suggests that COPD may contribute to the aortic stiffness to the same degree but through a different mechanism, to traditional risk factors.

A relationship between aPWV and peripheral and central systolic BP in both groups was observed. Aortic pulse wave velocity also related to CPP in the controls but not in the COPD group. Arterial stiffening can reduce the sensitivity of baroreceptor function, which may contribute to the disturbance in the regulation of BP and PP (Tarumi and Zhang 2018). Evidence supports the presence of a linear relationship between aPWV and peripheral BP in which aPWV increases by 1.5-fold in elderly with higher BP compared to younger individuals with low BP (Collaboration 2010). This relationship may support the higher annual progression in aPWV in the participants with hypertension (Benetos et al. 2002; Oliveras et al. 2016; Maloberti et al. 2019) compared to normotensive (Benetos et al. 2002). Both ageing and high BP act concurrently on the arterial wall, causing lumen diameter dilation and structural thickening of arteries, making them stiffer. In addition, any increase in BP can exacerbate these changes further (Cecelja and Chowienczyk 2009; Boutouyrie and Bruno 2019).

3.5.2 Longitudinal changes in physical function

The change in 6MWT measured in the current study from baseline to the follow-up was not significant in either COPD or control groups although it may be expected that they show some age-related decline in the walked distance. Several factors may have caused this result, such as the variation in the walked course at visit three or attrition bias as healthier

participants may have returned for follow up. Furthermore, the Hawthorne effect is also plausible, where individuals may modify their behaviour when observed (McCambridge et al. 2014).

A study determined the annual rate of decline in the 6MWT over five years in 294 patients with COPD. At baseline, the mean 6MWT distance was 380 m but declined at follow-up with the extent of decline corresponding with the severity of COPD (a decline by 19% in patients with stage III COPD compared to a decline by 26% in patients with stage IV) (Casanova et al. 2007).

Furthermore, participants in the COPD group walked a significant shorter distance in the 6MWT (mean 379 m) compared to the controls (mean 508 m) and as expected, experienced higher levels of dyspnoea than the controls.

There are several field tests used to measure function, but the 6MWT is considered the gold standard for individuals with COPD. It measures two main components which are exercise capacity by measuring the walked distance and exercise tolerance through assessing respiratory signs such as dyspnoea and desaturation (Rasekaba et al. 2009; Vaidya et al. 2017).

As discussed earlier, patients with COPD are by definition, patients with airflow obstruction, and experience symptoms including dyspnoea and fatigue that can impact their level of functions (Calverley 2006; Hanania and O'Donnell 2019) which worsens with advanced severity of COPD (Cinarka et al. 2013). The current study found a significant association between FEV_1 and measures of function such as the 6MWT and TUG tests and the quality-of-life measure by the CGA. Comparison of the findings with those of other studies confirms the role of physiologic parameters of COPD in functional abilities (Cinarka et al. 2013; Zeng et al. 2018).

A recent study including individuals with mild to moderate COPD who were younger than the present cohort by about 12 years also reported a walked distance of 425 m in the COPD

group and 530 m in their control group (Mansour et al. 2020). In another study, including individuals with COPD and controls younger than the cohort of this study, the mean distance was 360±69 m and 427±64 m, respectively (Cinarka et al. 2013). In the ECLIPSE study, a multi-countries study, a similar walked distance (mean 370 m) was also reported in the COPD group. The cohort in the ECLIPSE study was younger (mean age 63 ± 7 years), the majority had moderate to severe COPD as per GOLD classification, and about 50% had self-reported CVD. The level of airflow obstruction and severity of dyspnoea were significant clinical determinants of poor 6MWT performance in the study (Spruit et al. 2010). Although a shorter walking distance was expected in the older cohort compared to the slightly younger cohort of the ECLIPSE study. However, a possible explanation for this might be that most of the participants were having moderate COPD; hence they performed better than those with severe COPD.

In contrast, the walked distance measured in the current study was much higher than the distance walked by participants from a South Asian cohort who was comparable to the present study in term of age, disease severity but healthier as they were free from CVD (mean 287 m). The study had controls who also presented with lower 6MWT results than controls of the current study (Kumar et al. 2018). The difference in the walked distance in the current cohort compared to the studies by Kumar et al. and Mansour et al. are likely to be related to differences in reference values among different ethnicity which can be influenced by anthropometric characteristics or the speed of habitual walking. The result can also be influenced by test protocol or equations of predictive values (Casanova et al. 2011a). However, discussing these are beyond the scope of this study but warrant future investigation.

Nonetheless, a review including data of 6MWT in patients with COPD from six longitudinal observational studies concluded that walking 350 m was the threshold in which a result below this value was linked to a significant increase in the risk of mortality, hospitalisations, and exacerbations (Celli et al. 2016).

3.5.3 Mechanisms linking arterial stiffness to COPD

There are a number of potential mechanisms linking COPD to increased arterial stiffness which includes lifestyle factors, smoking and inflammation.

3.5.3.1 Lifestyle factors

It is well established that COPD is associated with reduced physical function, as shown in the current study and earlier research (Cote et al. 2007; Crisan et al. 2015) Evidence supports that lifestyle factors, including physical activity and sedentary lifestyle, were associated with vascular ageing.

Physical inactivity and reduced exercise capacity were associated with increased aPWV in community-dwelling older adults (Karimi et al. 2016; Nadruz et al. 2017; Orkaby et al. 2019; Gomez-Sanchez et al. 2020) and in COPD (Stickland et al. 2013). The current study supports previous evidence in which there was a significant relationship between aPWV and measures of physical function including the 6MWT, TUG and the CGA in the COPD group.

A possible explanation for the relationship between increased aPWV and functional limitation could be attributed to the progressive loss of pressure absorption effect in the vasculature due to increased arterial stiffness and hence affecting the microcirculation of different body organs including the skeletal muscles leading to impairments in physiologic reserve (O'Rourke and Hashimoto 2007).

In addition, there may be a vicious cycle linking aPWV to functional limitations in COPD where functional limitations may be exaggerated because of progressive airflow obstruction. Patients with COPD may avoid activity that provokes dyspnoea and, over time, become physically inactive and deconditioned, consequently increasing the risks of CVD. Nevertheless, further investigation is required to fully understand the mechanisms linking arterial stiffness to functional limitations in this group.

3.5.3.2 Smoking

One of the most accepted risk factors that is linked to both CVD and COPD is tobacco smoking (Morgan et al. 2018). Nonetheless, the notion that COPD by itself is an instigator for increased arterial stiffness is becoming questionable. Studies reporting such connection were limited methodologically by comparing COPD smokers or past smokers with smokers' controls making the exclusion of smoking effect uncertain in this population. However, the recent work by Soumagne and his team overcame this problem by measuring aPWV in two subsets of COPD individuals, smokers versus never smoked to exclude the influence of the combinations of COPD and smoking. They concluded that smoking is a risk factor for increased aPWV and that increased aPWV cannot be explained purely by COPD (Soumagne et al. 2020).

A systematic review of evidence including 39 studies linked acute, chronic and passive smoking to increased arterial stiffness, but the role of chronic smoking in increasing arterial stiffness is somewhat more controversial due to the high heterogeneity of the populations or methodologies of research examining this connection (Doonan et al. 2010).

Several mechanisms had been proposed to explain the connection between smoking and increased arterial stiffness. Smoking induces oxidative stress, which changes vascular tone that can lead to increased arterial stiffness (Cacciola et al. 2007). In addition, smoking is associated with increased inflammatory markers, leading to vascular remodelling and calcification (Boutouyrie et al. 2008; Arnson et al. 2010). Moreover, smoking alters lipid metabolism, which contributes to the increased thickness of intima-media and changes in the arterial wall structure (Chambless et al. 1997; Campbell et al. 2008).

3.5.3.3 Inflammation

A widely held theory proposed to explain the increased burden of CVD in COPD is the overspill of inflammatory mediators from the lungs leading to a low-grade systemic inflammation (Sinden and Stockley 2010; Zhong et al. 2018).

Low-grade lung and systemic inflammation can increase oxidative stress, and this has been associated with increased prevalence of CVD in COPD (Sin and Man 2003). Moreover, immune cell activation combined with the systemic inflammation adds to the arterial wall thickening and atherosclerotic plaque formation (Brassington et al. 2019) and subsequently, changes the mechanical properties of the arterial wall leading to stiffening (Zhong et al. 2018). Evidence has shown that inflammatory mediators were related to arterial stiffness in COPD. The study by Sabit and colleagues in patients with COPD reported an association between serum interleukin-6 and aPWV (Sabit et al. 2007).

On the other hand, some evidence does not support the impact of the spill over theory on the vasculature. In an earlier study by McAllister and colleagues, inflammatory biomarker including high-sensitivity CRP (Roeder et al. 2020) and leukocytes (McAllister et al. 2007) were not found to relate to aPWV in a small cohort of COPD. In addition, fibrinogen, high-sensitivity C-reactive protein and white cell count were not found to independently associate with aPWV in a large group of COPD patients in the ERICA study (Mohan et al. 2016). Similarly, Cinarka et al found no relationship between C-reactive protein and aPWV in a small group of stable COPD patients (Cinarka et al. 2013) . In addition, a lack of correlation between aPWV and high level of CRP was reported in individuals with mild COPD (Soumagne et al. 2020). Moreover, the impact of some anti-inflammatory agents commonly used to treat COPD in reducing arterial stiffness is controversial (White et al. 2013; Urban et al. 2020). This may result from the heterogeneity of studied cohorts in terms of different age and disease, the sensitivity of the measure of inflammation, the degree of inflammation or variations in measuring arterial stiffness. Hence, the exact role of inflammation in the link between COPD and CVD is indefinite and warrants further investigation.

3.5.4 Effect of heart rate (HR) on arterial stiffness

In the current study, patients with COPD had higher resting HR compared to the controls. These results are consistent with earlier studies showing higher HR in individuals with COPD (Warnier et al. 2014; Byrd et al. 2018; Kumar et al. 2018).

Mounting evidence supports the presence of a linear relationship between HR and higher risk of CVD and mortality in hypertensives (Gillman et al. 1993; Palatini 2011), smokers (Jensen et al. 2011), individuals with CVD (Diaz et al. 2005; Fox et al. 2007) and in the general population (Greenland et al. 1999; Seccareccia et al. 2001; Zhang et al. 2016).

In addition, measures of arterial stiffness have been associated with resting HR in healthy individuals (Park et al. 2010; Whelton et al. 2013; Logan and Kim 2016) and in hypertensive patients (Papaioannou et al. 2019). The Corinthia study, a large observational cross-sectional study, including 1566 subjects (70% hypertensive) showed that increased HR was significantly related to higher aPWV, and this relationship was partially mediated by MAP. A multivariate regression model also showed that an increase in HR by 20 bpm could increase aPWV by 0.5 m/s in individuals with increased aPWV (Papaioannou et al. 2019).

There is also a growing interest in investigating the relationship between HR and CVD risk and mortality in patients with COPD with several large cohort studies shedding light on this topic. However, the complexity of the relationship between COPD and CVD and the shared clinical features such as decreased stroke volume, dyspnoea and fatigue makes this connection inconclusive. Nonetheless, the implication of higher resting HR in increasing CVD mortality risk is more marked in COPD compared to individuals without COPD as shown by several studies (Mannino et al. 2008; Jensen et al. 2013; Warnier et al. 2014; Byrd et al. 2018).

Data from the SUMMIT, a randomised, double-blind outcome trial of 16,485 participants with COPD support a linear relationship between HR and CVD mortality risk (Byrd et al. 2018). In another study, lower heart rate was associated with better survival depending on the GOLD stage of the COPD. It was estimated that resting HR below 65 bpm was associated with increase survival of nearly 10 years in the stage I (mild COPD) , 7 years for stage II and 6year for stages III-IV (severe-very severe COPD) (Jensen et al. 2013). In the same way, higher HR had been shown to negatively affect life expectancy as shown by a study in which a resting HR between <65 and ≥85 bpm caused up to 10 years difference in life expectancy in individuals with moderate COPD (Mannino et al. 2008). The Copenhagen city heart study, a prospective population-based study, also reported that resting HR increased with severity of

COPD and was associated with both CVD and all-cause mortality across all stages of COPD, but more prominent in GOLD stages III and IV. Individuals with severe COPD have higher HR that puts them at 72% risk of all-cause mortality during ten years compared to only 25% risk of all-cause mortality in individuals without COPD having high HR (Jensen et al. 2013). In addition, a prospective cohort study of 405 elderly patients with COPD reported an increase in relative risk of all-cause mortality by 21% for every 10 beats/minute increase in HR. The increase in HR did not lead to an increase in pulmonary complications including exacerbations or pneumonia, suggesting that the higher mortality risk may relate to non-pulmonary causes (Warnier et al. 2014).

These findings have important implications as a large proportion of individuals with COPD are at increased risk of mortality. It also highlights the benefit of HR as a therapeutic target in the COPD management and its value as a measure of risk that is not well utilised in risk stratification in this group.

Despite the availability of robust data linking resting HR to cardiovascular risk and mortality, there is a modest evidence supporting an independent association between HR and aPWV in several populations according to a systematic review (Cecelja and Chowienczyk 2009). However, several large epidemiological studies published after this review confirmed this association for example, the Whitehall II Study found a similar association in men (Johansen et al. 2012). Similarly, the Caerphilly Prospective Study, a population-based cohort study of all men followed for 20 years also concluded that aPWV was positively associated with HR at baseline (McEniery et al. 2010a). This association was also supported by the result of The Anglo-Cardiff Collaborative Trial (McEniery et al.) where HR positively and independently associated with a aPWV, in the adjusted multivariable model (McEniery et al. 2010b).

However, the relationship between aPWV and HR was not fully established in COPD due to the limited number of studies examining this relationship. In a recent study including a small sample of patients with COPD, higher aPWV was associated with increased resting HR (Sima et al. 2020). In contrast to their finding, no association between aPWV and HR was found in

the current study. A possible explanation is the increased prevalence of CVD and the high use of CVD medications that may have influenced this relationship.

The mechanism of the increase in HR in this population could be attributed to several haemodynamic responses seen in COPD. For example, increased HR has been reported in smokers (Linneberg et al. 2015) , a common risk factor in COPD (Jensen et al. 2011). Smoking increases HR through the effect of nicotine that promotes the activation of sympathetic nerves system and the release of epinephrine and norepinephrine from the adrenal medulla and terminal nerve endings (Benowitz 2003).

Heart rate may be related to airflow obstruction and increased work of breathing and activity increasing metabolic demands (Vogiatzis et al. 2012). On the other hand, higher heart rates might be an indicator of poor fitness or severity of COPD (Jensen et al. 2013; Byrd et al. 2018). Additionally, impaired cardiac function due to the decrease in left ventricular size and stroke volume that accompany worsening pulmonary functions can lead to higher HR in order to maintain cardiac output (Barr et al. 2010). Furthermore, increased sympathetic activity caused by autonomic dysfunction associated with COPD may lead to a significant reduction in HR variability that can promote arrhythmias and myocardial ischaemia (Roque et al. 2014). Moreover, some forms of long-acting bronchodilators used in the treatment of COPD have been shown to negatively affect the heart and increase the risk of CVD mortality (Agabiti and Corbo 2017). However, many studies assessing the impact of these inhalers on the heart are small and have their methodological limitations, so the evidence in this remains to be firmly determined (Laratta and van Eeden 2014).

3.5.5 CVD comorbidities

The number of cardiovascular comorbidities in patients with COPD in this study was higher than their age-matched controls, corresponding to previously published data (Mannino et al. 2008; Miller et al. 2013; Stridsman et al. 2013). Hypertension and high cholesterol were amongst the most common cardiovascular comorbidities in both groups.

In the present COPD patient group, the prevalence of diagnosed hypertension was 45% at baseline, which decreased to 39% at 6-year follow-up. This prevalence was comparable to what has been previously reported where hypertension was present in between 40-48% of COPD patients (Mannino et al. 2008; Stridsman et al. 2013; Vanfleteren et al. 2014). It seems possible that the reduction in hypertension in the COPD patients might be caused by the fact that individuals voluntarily taking part in research are more likely to comply with health recommendations, they improved medicines adherence as the study progressed, their BP medication changed as the study progressed and/or are more inclined to make positive lifestyle changes to maintain health. However, the prevalence of hypertension in the control group was 21% at baseline and was unchanged throughout the study duration. It may be that COPD participants benefitted from extensive medical follow up; hence, the effect of targeted BP control was more marked in those participants. However, as BP was adjusted for the analysis of aPWV hence it was not a confounder in this study.

The prevalence of high cholesterol was also higher in patients with COPD at baseline (41%) compared to the control group but declined to only 23% at the 6-year follow-up. This decline could be due to reporting bias as cholesterol level was confirmed by blood analysis at visit one and two but was self-reported at the last visit. Conversely, the decline might have also resulted from better control due to approximately 39% of patients with COPD were using statins as cholesterol-lowering medication at the third visit compared to only 15% at baseline. Similar high prevalence was also reported in a large group of patients with COPD (Putcha et al. 2014). There was no change in the prevalence of high cholesterol between baseline and visit three in the control group (20% and 21% respectively).

There is a large body of evidence supporting the beneficial effect of statins on the vasculature and its role in the prevention of CVD and was supported by several guidelines (Zhou and Liao 2009; Duerden et al. 2015; Bibbins-Domingo et al. 2016; Catapano et al. 2016). Statins use for more than two years had been associated with a 39% decreased risk of all-cause death in COPD patients independent of age, duration of COPD, total serum cholesterol and cardiovascular covariables (Lahousse et al. 2013). However, the effectiveness of statins in reducing the risk of CVD or mortality had been debated recently with a

systematic review including 35 randomised control trials involving non-COPD populations found no accompanying decline in this risk with statins use (DuBroff et al. 2020). Furthermore, use of statin resulted in some unfavourable lifestyle behaviours including higher BMI and reduction in physical activities, that may work against the preventive goals of statins (Kaestner et al. 2014; Korhonen et al. 2020). Hence, there is a need for redefining the risk thresholds and the selection criteria considering age, sex, and statin type to optimise the benefit of statins in the prevention of CVD (Yebyo et al. 2018).

The included COPD group were a mixed group in terms of the prevalence of CVD and the use of CVD medications. Therefore, it cannot be ascertained if aPWV was higher in these patients due to a history of CVD or the presence of the COPD alone. In addition, the impact of the different types of CVD medications on aPWV cannot be determined due to the small sample included. However, a systematic review concluded that increased aPWV is prevalent in COPD patients free from CVD even during moderate stage of the disease, suggesting the existence of maybe underlying vascular abnormalities apparent early in the disease course (Wang et al. 2017b).

3.5.6 aPWV and COPD characteristics

One of the key objectives of the study was to determine the relationship between aPWV and COPD characteristics including airflow obstruction as determined by FEV_1 and level of physical function assessed subjectively and objectively. The section below will address each of the fundamental factors associated with COPD severity and presentation.

3.5.7 aPWV and lung function

The present data showed no relationship between measures of airflow obstruction and aPWV despite seeing a trend of association, albeit insignificant, between aPWV and FEV_1 and between aPWV and the COPD Assessment Test (CAT). These findings confirm data from

Amaral and colleagues who also reported no association between FEV_1 and aPWV, in 108 participants with COPD (Amaral et al. 2015).

As expected, patients with COPD in this study had airflow obstruction as confirmed by a post-bronchodilator spirometry with the majority having moderate COPD as per the GOLD classification of COPD severity (GOLD). Moreover, FEV_1 declined significantly during the study duration by about 7%, in a manner similar to what had been reported previously (Bolton et al. 2009; Vestbo et al. 2011; Tantucci and Modina 2012; Bhatt et al. 2016).

Deterioration in lung function has been linked to increased risk of CVD and mortality (Sin and Man 2003; Sin et al. 2005). It was reported that for every 10% reduction in FEV_1, adjusted cardiovascular mortality rose by 28% in the Lung Health Study, a multicentre randomised clinical trial involving 5,887 smokers (Anthonisen et al. 2002). In the same way, the GOLD stages for COPD severity was shown to predict mortality with the median life expectancy reducing with increased COPD severity (Jensen et al. 2013).

According to the results of several studies in healthy individuals and individuals with COPD, airway patency and various measures of arterial stiffness including aPWV, AIx and Intima media thickness were inversely related (Iwamoto et al. 2009; Costanzo et al. 2017; Inomoto et al. 2017; Okamoto et al. 2019; Piccari et al. 2020).

Previously, arterial stiffness measured by brachial-ankle PWV was shown to inversely correlate with FEV_1 % in healthy individuals without COPD (Inomoto et al. 2017). In addition, the Whitehall II study measured the relationship between FEV_1 and aPWV over five years to determine if airflow obstruction accelerates the progression of arterial stiffness in 5342 participants (mean age 65.4). They reported that lower FEV_1 was associated with later higher aPWV however, the reduction in FEV_1 was not associated with the progression of aortic stiffness. Moreover, there was no strong evidence of a reverse connection as higher aPWV weakly associated with future impairment of FEV_1 after adjustment for cardiometabolic status (Okamoto et al. 2019). More recently, the recent work by Piccari and colleagues confirmed a correlation between FEV_1 and aPWV in patients with COPD, with history of CVD

(Piccari et al. 2020). In addition, significant associations between thickened intima-media thickness of the carotid artery and decreased FEV_1 % in smokers was established in 61 smokers with airflow obstruction (Iwamoto et al. 2009). In the same way, an inverse correlation between AIx and FEV_1 % was reported in a small number of COPD patients, even after accounting for potential confounders (Costanzo et al. 2017).

Likewise, Sabit and his group found an inverse relationship between aPWV and FEV_1 in 75 clinically stable patients with a range of severity of airflow obstruction (mean %FEV_1 was 57%) (Sabit et al. 2007). The study by Cinarka and colleagues included 62 COPD patients with varying severity levels also confirmed this relationship (Cinarka et al. 2013). A similar relationship was also reported by a recent study, including individuals with mild to moderate COPD (Mansour et al. 2020). The above evidence supports the clinical importance of considering airflow obstruction as an important marker in cardiovascular risk but also acknowledging the possibility of other factors in increasing stiffness beyond airflow obstruction.

Despite the growing interest investigating the relationship between airflow obstruction and increased arterial stiffness, the exact mechanism linking pulmonary function and arterial stiffness remains inconclusive (Sabit et al. 2007). A link to an alteration of elastolytic activity occurring in the pulmonary system and the vasculature has been suggested (McAllister et al. 2007). Moreover, FEV_1 was shown to inversely relate to some serum markers of endothelial dysfunction and inflammation (Duprez et al. 2013). In addition, FEV_1 has been associated with glycated haemoglobin (HbA1c) (Oh et al. 2015), a marker that was related to high aPWV in older individuals and hypertensive patients (Liang et al. 2012).

A possible explanation for the lack of the association between FEV_1 and aPWV in the current study might be that most of the participants had moderate COPD and the nature of this relationship may differ dependent on the patient's degree of airflow obstruction. Unfortunately, the small sample size in each GOLD category of airflow obstruction did not allow for a sub-analysis. On the other hand, due to the longitudinal study's nature, it is plausible that the loss of follow-up for those with greater airflow obstruction and

comorbidities was high and, therefore, underestimated the relationship between aPWV and airflow obstruction. On the other hand, the study showed that participants with COPD have more CVD and use more CVD medications that may have interfered with the relationship between aPWV and airflow obstruction.

3.5.8 Measures of physical function and aPWV

In the current study, the data showed a significant relationship between aPWV and measures of physical function including the 6MWT, TUG and the CGA in the COPD group. Physical inactivity and reduced exercise capacity had been associated with increased aPWV in community dwelling older adults (Nadruz et al. 2017; Orkaby et al. 2019) and in COPD (Stickland et al. 2013).

Any form of physical activity requires muscle contraction, which creates stress to homeostasis that necessitates a response from complex, multifaceted systems such as pulmonary, cardiovascular, and skeletal muscle systems. This response is distinctly modified in the elderly as a result of age-related loss in cardio-pulmonary function, leading to cardiac and ventilatory limitations and subsequently, limitation to exercise and function (Roman et al. 2016). Regardless of the expected age-related decline in function, a functional limitation is a key feature in individuals with COPD who have lower levels of physical activity and function than individuals without COPD (Watz et al. 2009; Spruit et al. 2010; Gimeno-Santos et al. 2014; Sievi et al. 2015).

Evidence increasingly supports the association between lower physical function and a higher risk of CVD and all-cause mortality in the general populations (Leon et al. 1997) and in individuals with COPD (Waschki et al. 2011; Garcia-Rio et al. 2012; Fermont et al. 2019). Besides, the coexistence of CVD and COPD reinforce the adverse outcomes on the quality of life and reducing exercise capacity in those patients (Stridsman et al. 2013; Garvey and Criner 2018; Fermont et al. 2019).

A possible explanation for the relationship between increased aPWV and functional limitations could be attributed to the progressive loss of pressure absorption in the vasculature due to increased arterial stiffness and hence affecting the microcirculation of different body organs including the skeletal muscles leading to impairments in physiologic reserve (O'Rourke and Hashimoto 2007).

In addition, there may be a vicious cycle linking aPWV to functional limitations in COPD where functional limitations may be exaggerated because of the presence of progressive airflow obstruction. Patients with COPD may tend to avoid activity that provokes symptom of dyspnoea and over time, become less active and deconditioned, which may also increase the risks of CVD. Nevertheless, further investigation is required to fully understand the mechanisms linking arterial stiffness to functional limitations in this group.

Several reports had shown that higher risk of CVD and mortality was associated with shorter walking distance measured by the 6MWT (Polkey et al. 2013; Yazdanyar et al. 2014; Fermont et al. 2019), a longer time in TUG (De Buyser et al. 2013; Chun et al. 2019; Son et al. 2020) and a weaker handgrip (Celis-Morales et al. 2018; Prasitsiriphon and Pothisiri 2018) in community-dwelling older adults. In patients with COPD, a similar conclusion was reached where higher CVD risk and mortality was associated with 6MWT (Cote et al. 2007; Casanova et al. 2008; Dajczman et al. 2015), hand grip strength (Burtin et al. 2016) and also with scales that incorporates functional measures such as the BODE index (Celli et al. 2004; Divo et al. 2012).

In addition, the COPD group in this study spent a longer time completing the TUG test compared to the control group, in a way that matches findings from other reported studies (Crisan et al. 2015; Haddad et al. 2015). It was proposed that TUG results of ≥10 seconds related to a 2.9-fold increase in cardiovascular mortality in women based on a study of more than 40,000 participants from the general population followed for about six years. This risk was also marked in individuals free from risk factors such as obesity, diabetes and smoking (Son et al. 2020) suggesting the role of a predictive yet simple tool that can be used in identifying individuals at risk of CVD or mortality. In addition, participants in the COPD group

had a higher score in the CGA, indicating a lower physical function and poor outcomes relating to their everyday activities that correspond well with their reduced level of physical function observed through objective measures such as the 6MWT and the TUG tests.

3.6 **Strength/limitations of the study**

The present study adds to the growing body of research in the cardiovascular risk in patients with COPD by confirming an increase in aPWV in patients with COPD followed longitudinally. The longitudinal study design provides valuable information about the course of the progression that cross-sectional studies lack. In addition, it provides a good overview of the time-course interaction between aPWV and COPD characteristics in this group. This study further benefited from having a control group who were age matched. Using the gold standard method to assess arterial stiffness and other factors linked to cardiovascular risk, these data provide additional information that may be of benefit in clinical practice and may be used to guide practice in CVD prevention and COPD management.

However, the study has some limitations that should be acknowledged and addressed in future research. In observational studies, it is essential to consider the possibility of attrition bias where there is the likelihood of loss of follow up for individuals with advanced stages of COPD or with increased comorbidities, especially in advanced age. The loss of follow up can result from worsening COPD symptoms, the impact of other health problems or severe functional limitations. In addition, an inherent limitation of observational studies is their inability to ascertain causality.

Furthermore, residual confounding factors can never be excluded from this type of study. Those participating in research are possibly more eager than the general population to make healthier choices; however, the current results were comparable to other studies in other settings; hence this may be insignificant. It can also be argued that the COPD group had overt CVD and are prescribed a number of CVD medications as part of routine treatment; however, these data illustrate that the present COPD group are a representative sample of the wider COPD population who present with increased comorbidities.

One of the points to consider when interpreting the result of the 6MWT in the current study is the use of 10 m walking course at the 6-year assessment to the 30 m walking course at baseline and the 2nd visit. The American Thoracic Society guidelines recommend using 30 m, and the established normative values were based on this distance (ATS-Statement 2002). However, space limitations in clinical or research settings make 30 m impractical hence the use of 10 m course is commonly used and was supported by some COPD guidelines (Gosselink et al. 2008). However, using a 10 m course may influence the walked distance due to the extra turns when using a shorter walking course. In addition, the result's interpretation using normative values based on a 30 m distance may lead to inconsistencies in estimating the true result. An experimental crossover study including forty-five patients with COPD reported a 49.5m shorter distance using the 10 m compared to the 30 m. The study also concluded that using existence reference equations for 6MWT result overestimated the predicted distance by 33% hence the outcome of the test based on the two different courses length are not interchangeable (Beekman et al. 2013).

The relatively small sample size of the study did not allow for subgroup analysis to investigate aPWV and its predictors in different COPD stages neither was the influence of different forms of CVD comorbidities or categories of cardiovascular acting medications examined. More research is needed to understand the role of cardiovascular acting medications classes and dose in arterial stiffness.

3.7 Conclusion

Patients with COPD represent a complex clinical population as they have higher CVD comorbidities, lower physical function and are using more cardiovascular acting medications than healthy controls. Patients with COPD encounter premature vascular ageing as the results showed a chronically increased aPWV measured over six years. The increase in aPWV in the COPD group was above the cut-off point linked to subclinical organ damage and increased risk of cardiovascular events as proposed by established guidelines that may explain the overt history of CVD in the studied group.

Chapter 4

Investigation of the relationship between MCAPI and central haemodynamics

4 A cross-sectional; investigation of the relationship between MCAPI and central haemodynamic

4.1 Introduction

Although Chronic Obstructive Pulmonary Disease (COPD) is primarily a pulmonary disease, evidence from the literature suggests that it is frequently associated with numerous extra-pulmonary conditions. The presence of other co-existing diseases in an individual is frequently referred to as comorbidity which has a significant impact on the health status of these patients in addition to increased mortality risk (Sin et al. 2006; Cavailles et al. 2013).

One of the most common comorbidities in COPD is Cardiovascular Disease (CVD), which encompasses the diseases of heart and blood vessels and includes Coronary Heart Disease (CHD), and cerebrovascular disease such as stroke and Transient Ischemic Attack (TIA). There is a significant amount of research about the diseases of heart and circulatory system in COPD but less so about cerebrovascular disease (Chen et al. 2015; Morgan et al. 2017; Kim et al. 2018).

The prevalence of cerebrovascular comorbidities is significantly higher in patients with COPD than in individuals without COPD (Yin et al. 2017). Patients with COPD have increased cerebral small-vessel disease and microbleeds, with presentation of such characteristics more prevalent in those COPD patients with severe airflow obstruction (Lahousse et al. 2013). Furthermore, a comprehensive analysis using data from primary care concluded that COPD patients have three times higher risk of stroke (Feary et al. 2010) and was estimated to affect between 6.9% to 9.9% of patients with COPD (Mullerova et al. 2013). More recent evidence showed that individuals with COPD have a 30% increased risk of developing stroke as per a meta-analysis (Kim et al. 2018). Also, the risk of stroke increases in acute exacerbation and accompanied by an increase in mortality risk post-stroke (Lin et al. 2017).

Despite the availability of several lines of evidence linking COPD to increased risk of stroke, the exact mechanisms that explains this link remains unclear. Factors such as increased age, smoking status and history, sedentary lifestyle and low socioeconomic status were identified as traditional risk factors associated with the development of both COPD and stroke, making it challenging to identify the specific mechanisms linking the two conditions (Maclay and MacNee 2013).

Ageing is associated with significant alterations in both the systemic and cerebral haemodynamic systems including elevations in central arterial stiffness, SBP and pulse pressure, reductions in mean and diastolic cerebral blood flow velocity CBFV, increases in pulsatility and cerebrovascular resistance (Tarumi et al. 2014).

It has been proposed and shown that pathological changes in COPD including increased systemic inflammation and increased oxidative stress markers were associated with vascular dysfunction (Mills et al. 2008). These changes also cause structural remodelling of cerebral vessels leading to disruption in the blood-brain barrier (Brassington et al. 2019). Additionally, cerebral perfusion was found to be significantly reduced in patients with COPD. The reduction in the cerebral perfusion may increase the risk of Transient Ischaemic Attack (TIA) or stroke even in those with stable and acceptable oxygenation level (Ortapamuk and Naldoken 2006). These factors could potentially explain the excess cerebrovascular morbidity and mortality associated with COPD.

4.1.1 Pulsatility Index (PI)

Transcranial Doppler (TCD) is a non-invasive, cost-effective and extensively utilised system in the assessment of hemodynamic states of cerebral circulation. It provides information on various parameters for structural, functional, and hemodynamic conditions of cerebral circulation, including blood flow velocity and pulsatility index PI (Aries et al. 2010). Furthermore, it indexes vascular health as higher PI reflects the downstream increase in vascular resistance and has been considered as a surrogate marker of small vessel disease

(Aries et al. 2010; de Riva et al. 2012; Kim et al. 2015; Kim et al. 2016; Webb 2019) and was associated with higher CVD risk (Pase et al. 2012)..

Considering that MCA is one of the largest cerebral artery supplying around two-thirds of the brain (Harris et al. 2018) and its critical location in brain autoregulatory site (Oppenheimer 1993), It is the most pathologically affected and studied cerebral vessel (Navarro-Orozco and Sánchez-Manso 2020).

As shown by the Rotterdam Study, a large population-based study, including 2022 participants (mean age 70.2), a strong and independent association between higher cerebral blood velocity at middle cerebral artery MCA and increased risk of stroke was reported. Particularly for ischemic stroke where for every 1 SD increase in the cerebral blood velocity at MCA, there was a 38% increase in the risk of ischemic stroke (Bos Michiel et al. 2007). This provides evidence of the utility of cerebral blood flow parameters in predicting the risk of stroke or the extent of ischemic injury.

4.1.2 The link between MCAPI and aPWV

There is evidence that supports the interplay between central and cerebral hemodynamics and the impact of the loss of large arterial compliance on the downstream flow dynamics. For example, research showed a connection between aPWV and MCAPI in some populations (Webb et al. 2012; Sanahuja et al. 2016). However, the evidence investigating this relation in COPD is not well established.

4.1.3 Study justification

As increased MCAPI is linked to SVD and stroke is a manifestation of SVD, it can be postulated that MCAPI is also linked to risk of stroke. However, because of the already established high aPWV levels of COPD patients in the previous chapter, it is unknown if such a relationship exists in COPD patients. There is also a lack of clarity as to the exact role of arterial stiffness in increasing stroke risk in this group, and it is not fully understood if this association is direct or

secondary due to alteration in the cerebral blood flow pattern. Neither is there consensus across studies as to what determines the association between COPD and increased risk of stroke. The study of pulsatility in the MCA and how it relates to arterial stiffness and COPD characteristics may provide an insight into this relationship.

4.1.4 Study aim, objectives, and hypothesis

4.1.4.1 The aim of the study

Therefore, this study aimed to investigate the relationship between central arterial stiffness measured by aPWV and the cerebral flow downstream pulsatility as measured by MCAPI in patients with COPD and aged-matched controls. It aims to address the following research objectives:

4.1.4.2 The objectives of the study

1. To evaluate the difference in aPWV and MCAPI in patients with COPD and controls.

2. To establish the relationship between aPWV and MCAPI in patients with COPD and controls.

3. To establish the relationship between MCAPI and COPD characteristics as measured by FEV_1, FEV_1/FVC, CAT and SGRQ in patients with COPD.

4. To establish the relationship between MCAPI and health status measured subjectively by the Comprehensive Geriatric Assessment (CGA) and objectively by the 6 Minute Walk Test (6MWT) and Time up and Go test (TUG) in patients with COPD and controls.

5. To identify the predictors of MCAPI in patients with COPD and controls.

4.1.4.3 The hypothesis of the study

Null hypothesis:

1. There is no difference in aPWV and MCAPI in patients with COPD and controls.

2. There is no relationship between aPWV and MCAPI in patients with COPD versus controls.

3. There is no relationship between MCAPI and COPD characteristics as measured by FEV_1, FEV_1/FVC, CAT and SGRQ in patients with COPD.

4. There is no relationship between MCAPI and health status measured subjectively and objectively in patients with COPD and controls.

5. There are no predictors of MCAPI in the COPD group.

Alternative Hypothesis

1. There is a difference in aPWV and MCAPI in patients with COPD and controls.

2. There is a relationship between aPWV and MCAPI in patients with COPD.

3. There is a relationship between MCAPI and COPD characteristics as measured by FEV_1, FEV_1/FVC, CAT and SGRQ in patients with COPD.

4. There is a relationship between MCAPI and health status measured subjectively and objectively in patients with COPD and controls.

5. Aortic PWV or CPP will independently predict MCAPI in the COPD group.

4.2 **Method**

4.2.1 Design setting and recruitment

This study was a cross-sectional study to evaluate aPWV and MCAPI in patients with COPD and age-matched controls from the 6 year follow up data from the ARCADE study, NCT 01656421 (Gale et al. 2014). The inclusion and exclusion criteria are detailed in the methods chapter (Chapter 2.1.1). Previously assessed ARCADE participants had a postal invitation, and the study was fully explained before considering participation and consenting to take part in the study.

4.2.2 Measurements

4.2.2.1 Participant's characteristics

Height, weight, waist circumference and body composition were measured in all participants. Self-reported history of CVD and use of CVD medications were also recorded. Lung function was measured using the Vitalograph ALPHA 6000 (Vitalograph Ltd. UK) for both groups. A reversibility test was completed in the COPD group to confirm COPD status (Chapter 2.3.3).

4.2.2.2 Vascular measurements

4.2.2.2.1 Blood Pressure (BP)

Blood pressure was measured using a semi–automated oscillometric sphygmomanometer (Omron M2 HEM-7121-E, OMRON Healthcare UK Ltd) as detailed in (Chapter 2.3.2.1). Two measurements were taken with the average of the two readings was recorded. In the case of variation by >5mmHg between the two readings, a third reading was taken. The systolic and the diastolic blood pressure were recorded in mmHg.

4.2.2.2.2 Aortic pulse wave velocity (aPWV)

Arterial stiffness was measured using the SphygmoCor system (AtCor Medical, Sydney, Australia). Measurements of aPWV were obtained as per the protocol endorsed by Expert consensus document on arterial stiffness (Laurent et al 2006). The recorded measurements were AIx, Central systolic/diastolic BP, Heart Rate, and Mean Arterial Pressure (MAP), Central Pulse Pressure (PP) and aPWV (Chapter 2.3.2.2).

4.2.2.2.3 Middle cerebral artery pulsatility index (MCAPI)

Middle cerebral artery pulsatility Index (MCAPI) was assessed by Transcranial Doppler (DWL Doppler-Box™ X, Compumedics, Germany) (Figure 4.1).

Figure 4.1: Transcranial Doppler Box X

Participants were positioned in the supine position. The assessor was positioned either at the side of the participants' head or behind their head. Aquatic coupling gel was applied to the tip of the TCD probe, and the TCD probe was gently positioned over different acoustic windows of the skull. The temporal window was used to insonate the Middle Cerebral Artery (MCA) (Figure 4.2). An established reference values for depth, and flow direction for each vessel was used to help with identifying and measuring this artery (Kassab et al 2007). Then, blood flow velocity waveforms for the MCA artery were recorded. Essential parameters

including the Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV), and Mean Velocity were all used to calculate Pulsatility Index (PI).

4.2.2.3 Measures of health status

Objective measures and subjective measures of health status were evaluated in all participants, with some measures specific to the COPD group.

4.2.2.3.1 Objective measures of physical function

This included handgrip strength using the Grip Strength Dynamometer GRIP-D/ T.K.K.5401 (Takei Scientific Instruments Co Ltd, Tokyo Japan) with an average of two measurements for each hand was recorded in kg. Participants also completed the Timed Up and Go test (TUG test) and the Six-Minute Walk Test (6MWT). Heart rate, SaO2 and Borg Rating of Perceived Exertion Scale were recorded prior and after 6MWTtest completion. The distance covered by the end of the six minutes were measured and recorded. The details of the protocols for each of these tests are detailed in (Chapter 2.3.5).

4.2.2.3.2 Subjective measures of physical function

Subjective measures of physical function included the generic Comprehensive Geriatric Assessment (CGA). In addition, the COPD specific questionnaires including the COPD Assessment Test (CAT) and the St George's Respiratory Questionnaire (SGRQ) (Chapter2.3.4). The questionnaires were self-administered by participants on the assessment day before commencing the measurements and assistance and clarifications were provided when needed.

4.2.3 Data analysis

Data were analysed using SPSS Statistics 25. With the P value set at $p < 0.05$ for rejection of Null hypothesis. Data included continuous (interval ratio) and categorical data. Descriptive data included mean and standard deviation for parametric data and median IQR frequencies

and percentages for nonparametric data. Interval ratio data were checked for normality using Shapiro Wilko test (p > 0.05) and inspection for outliers using a boxplot. Data that were not normally distributed were transformed, and homogeneity of variances was assessed by Levene's test for equality of variances. The independent-sample t-test was used to establish the difference between COPD and control groups for parametric data (Chi-Square test and Mann-Whitney U test for data with ordinal or multinomial variables). Pearson's correlation test was used to assess the relationship between MCAPI and other variables in both groups for parametric data (the non-parametric equivalent, Spearman test, was used for ordinal or multinomial data). Stepwise Multiple Regression Analysis was used to identify independent predictors of MCAPI.

4.3 Results

4.3.1 Participant' characteristics

Data for a total of 45 patients with COPD and 50 healthy age-matched controls who had a complete set of measurements of both aPWV and MCAPI were included in the analysis. The exclusion of those participants was mainly due to inadequate insonation window, which is discussed in detail later in this chapter. The mean age of participants in both groups was (71 ± 6.7 years in the COPD group and 70 ± 7.1 years in the control group, (p>0.05). In both groups, there was no difference in gender proportions (p>0.05) (Table 4.1).

As expected, the mean $FEV_1\%$ and FEV_1/FVC ratio were significantly lower in COPD than the control group (p<0.05). Mean FVC measured score in the COPD group (2.63 ± 1.04 L) was lower than the mean score in the control group (3.24± 1.11 L, P=0.007). Mean FVC percentage in the COPD group (82.82 ± 23.67%) was also lower than mean FVC in the control group (103.36 ± 27.75%, P= 0.001).

Table 4.1: Participants Characteristics

	COPD group	Control group	p-value
Age (Years)	70.6 ± 6.7	70.2 ± 7.1	0.790
Gender Female: Male	17: 28	22:28	0.538
Body Mass Index (Kg/m2)	27.8 ± 4.5	27.3 ± 3.9	0.561
FEV1measured (L)	1.43 ± 0.71	2.40 ± 0.80	**0.001**
FEV1 %	57.4 ± 20.85	96.38 ± 24.32	**0.001**
FVC measured (L)	2.63 ± 1.04	3.24 ± 1.11	**0.007**
FVC %	82.82 ± 23.67	103.36 ± 27.75	**0.001**
FEV1/FVC measured	0.53± 0.16	0.72± 0.16	**0.001**

Abbreviations:

FEV_1: Forced expiratory volume in 1 second
FVC : Forced vital capacity
p≤0.05 statistically significant

In general, patients with COPD had more self-reported comorbidities than the controls (p=0.032) (Figure 4.2). Cardiovascular disease was more prevalent in patients with COPD compared to controls. Approximately 38% of patients with COPD had a diagnosis of hypertension compared to only 25% in controls. Patients with COPD had more diagnosis of Angina/MI (9 %) than control (2%), and the diagnosis of AF was double in COPD compared to the control group. A small percentage in COPD but not in the controls had Chronic Heart failure (CHF), and Pacemaker fitted. Nonetheless, a raised cholesterol level seems to be a problem with equal weightage among both groups. Additionally, the prevalence of TIA/Stroke was higher in COPD (7%) compared to controls (4%). In the same way, the diagnosis of Diabetics Mellitus (DM) and cancer is almost doubled in patients with COPD.

Also, degenerative conditions including osteoarthritis (OA) and Rheumatoid arthritis (RA) were more common in COPD group (51%) compared to the control group (33%). In addition, the prevalence of osteoporosis was more than six times greater in the COPD group than in the control group.

Figure 4.2: The prevalence of self-reported comorbidities in the COPD and control group
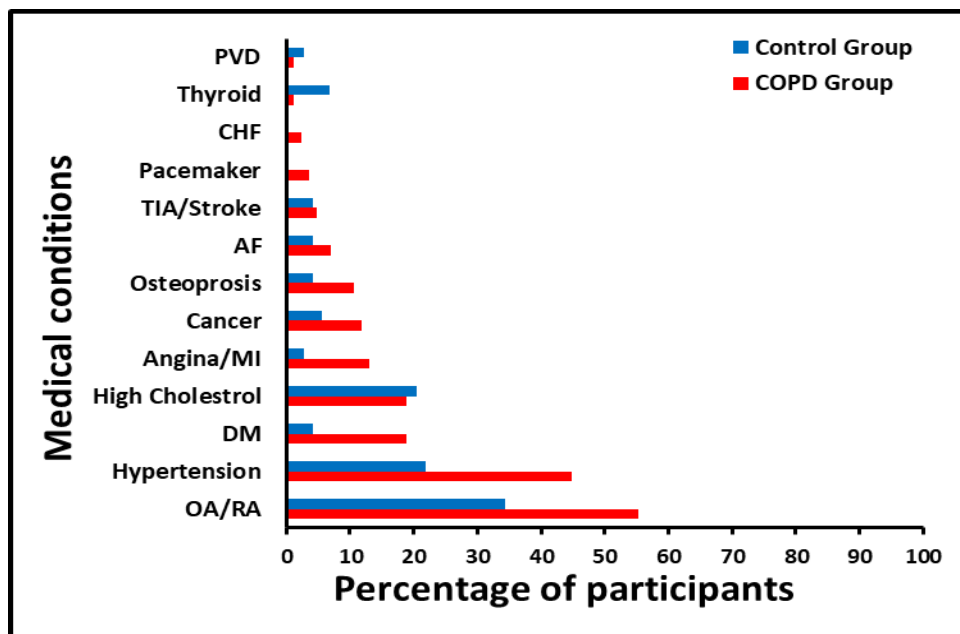
Abbreviations:
OA –Osteoarthritis, RA- Rheumatoid arthritis, DM- Diabetes mellitus, MI- Myocardial infarction,
TIA- Transient ischemic attack, CHF-Chronic heart failure, PVD-Peripheral Vascular Disease

Patients with COPD were prescribed more medications than the controls (Figure 4.3). Over 50% of patients with COPD were using some forms of cardiovascular medications compared to only 35% in the control group (p=0.013). These cardiovascular medications include angiotensin-converting-enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), beta-blockers, calcium channel blockers, diuretics, and statins. In general, patients with COPD were prescribed more anti-inflammatory, anti-diabetic, and anti-depressant medications than the control group.

Figure 4.3: Types of prescribed medications in the COPD and control group
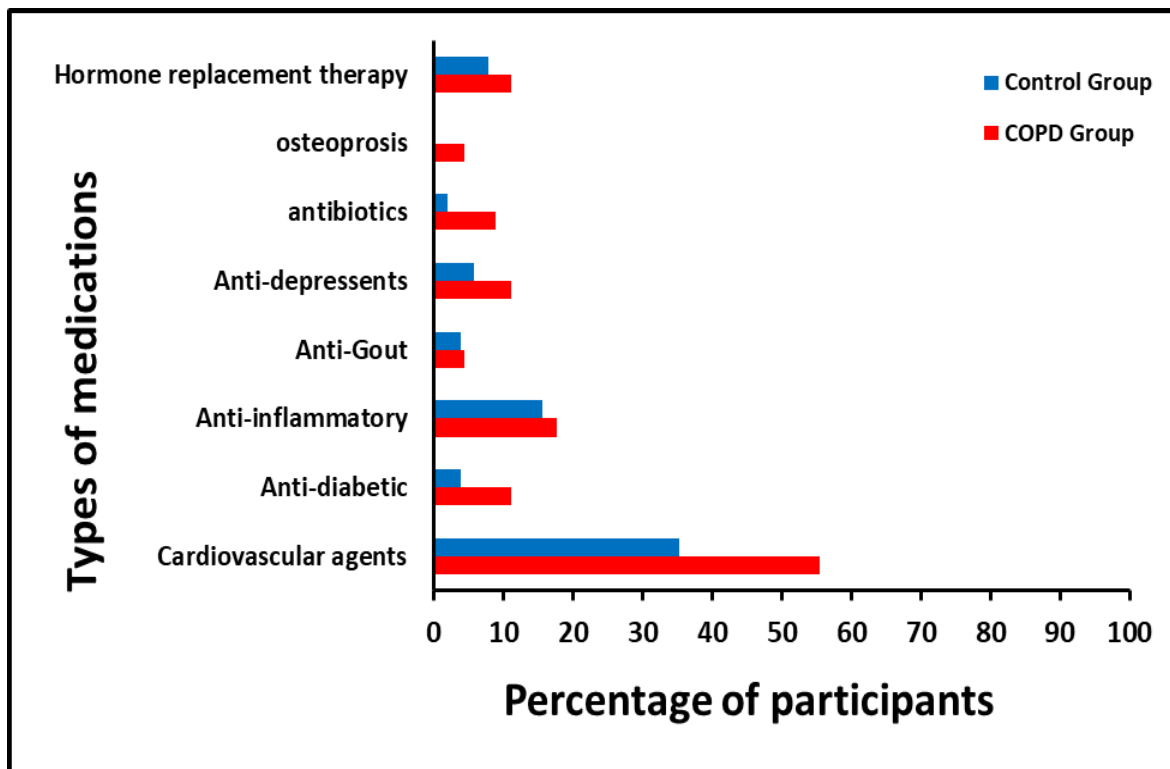
When looking at the breakdown of cardiovascular agents used among both groups (Figure 4.4), statins are the most used medications by both groups but higher use in COPD than the control group (40% and 26% respectively). The use of different blood pressure-lowering medications by patients with COPD is also higher than the controls except for the use of calcium channel blockers which is similar in both groups.

Figure 4.4: Cardiovascular acting agents used by participants in the COPD and control group
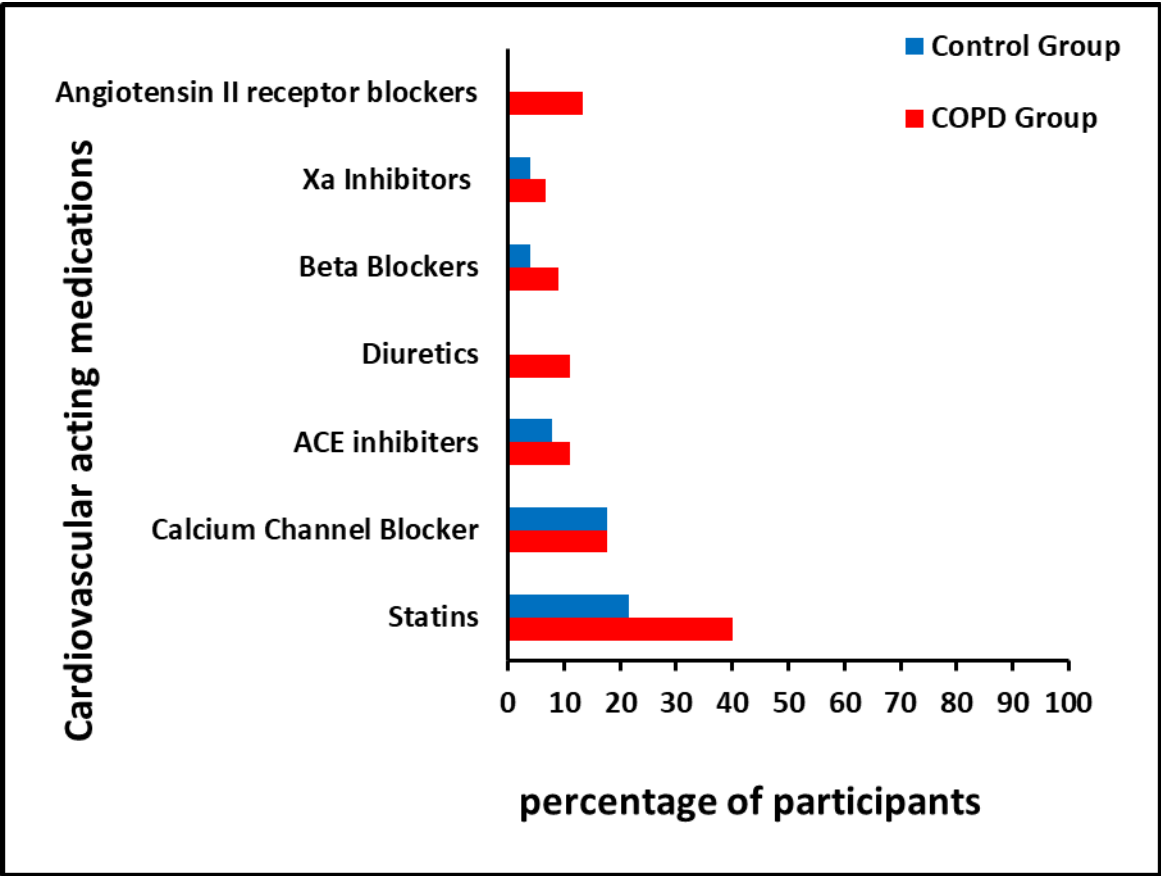
4.3.2 Vascular measurements in both groups

Patients with COPD had greater aPWV and HR than the control group (P<0.05). However, both groups were similar in terms of peripheral and central BP, AIx and MCAPI (Table 4.2).

Table 4.2: Differences in vascular measures between COPD and controls

Vascular measurements	COPD Group mean ±SD n=45	Control Group Mean ±SD n=50	p-value
Seated Peripheral Systolic Blood Pressure (mmHg)	145.8 ± 18.2	142.5 ± 15.4	0.365
Seated Peripheral Diastolic Blood Pressure (mmHg)	82.7 ± 9.8	81.5 ± 9.0	0.540
Seated Central Systole Blood Pressure (mmHg)	135.2 ± 20.6	131.2 ± 14.6	0.271
Seated Central Diastole Blood Pressure (mmHg)	83.7 ± 9.9	82.5 ± 10	0.528
Seated MAP (mmHg)	105.2 ± 12.3	102.7± 10.4	0.287
Seated HR (bpm)	70.8± 16.48	65.1± 8.3	**0.011**
Central Pulse Pressure (mmHg)	51.7± 18.3	49.0± 10.6	0.385
Peripheral Pulse Pressure (mmHg)	65.4± 22.2	61.4± 12.3	0.270
Augmentation Index	27.9± 9.7	27.9± 8.5	0.981
Supine Systole Blood Pressure (mmHg)	144.2 ± 17.7	140.6 ± 16.6	0.300
Supine Diastole Blood Pressure (mmHg)	82.9 ± 9.9	79.5± 9.8	0.091
Supine MAP (mmHg)	101.7 ± 14.9	99.1±12.4	0.353
Supine Pulse Pressure (mmHg)	61.0 ± 13.8	61.1±12	0.977
Arterial Pulse Wave Velocity (aPWV) (m/s)	10.7± 2.5	9.6 ± 1.9	**0.023**
Middle cerebral pulsatility Index (MCAPI)	0.86 ± 0.2	0.9 ± 0.1	0.661

4.3.3 Relationship between MCAPI and vascular measurements

In patients with COPD, MCAPI significantly correlated with seated peripheral and central diastolic pressure, seated central and peripheral pulse pressure, supine diastolic pressure, supine pulse pressure (All, P<0.05) (Table 4.3). However, MCAPI did not correlate with aPWV (Figure 4.5). Interestingly, in the control group, MCAPI strongly correlated with aPWV (Figure 4.6), peripheral systolic pressure, central systolic pressure, pulse pressures, supine systolic pressure, supine pulse pressure (P<0.05).

Table 4.3 : Relationship between MCAPI and vascular measurements in COPD and controls

Measurements	COPD Group (N=45)		Control Group (N= 50)	
	R	p =	R	p =
Seated Peripheral Systole Blood Pressure (mmHg)	0.223	0.141	0.412	**0.003**
Seated Peripheral Diastole Blood Pressure (mmHg)	-0.314	**0.036**	-0.074	0.612
Seated Central Systole Blood Pressure (mmHg)	0.228	0.131	0.407	**0.003**
Seated Central Diastole Blood Pressure (mmHg)	-0.313	**0.036**	-0.085	0.558
Seated MAP	-0.045	0.768	0.155	0.283
Seated HR (bpm)	-0.038	0.802	-0.019	0.894
Seated Central Pulse Pressure (mmHg)	0.459	**0.002**	0.653	**0.002**
Seated Peripheral Pulse Pressure (mmHg)	0.417	**0.004**	0.574	**0.004**
Augmentation Index	0.061	0.689	0.169	0.241
Supine Systole Blood Pressure (mmHg)	0.232	0.126	.489	**0.001**
Supine Diastole Blood Pressure (mmHg)	-0.392	**0.008**	-0.015	0.918
Supine Pulse Pressure (mmHg)	0.584	**0.001**	0.679	**0.001**
Supine MAP (mmHg)	-0.096	0.530	0.269	0.066
Aortic Pulse Wave Velocity (aPWV) (m/s)	0.220	0.146	0.452	**0.001**

Abbreviations:

R= Pearson correlation coefficient
P≤0.05 statistically significant

Figure 4.5: Relationship between MCAPI and aPWV in the COPD group
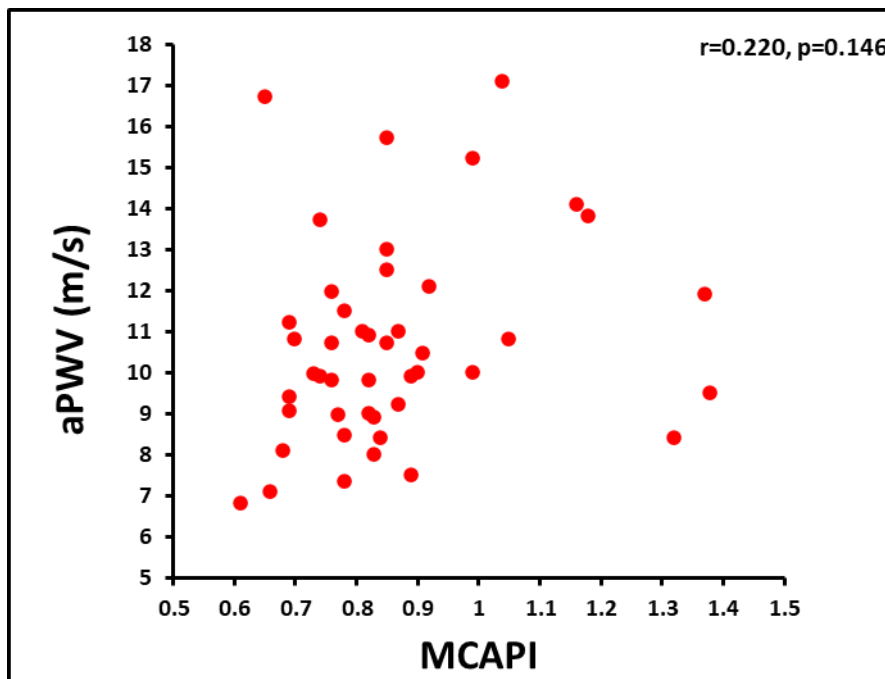
Figure 4.6 : Relationship between MCAPI and aPWV in the control group
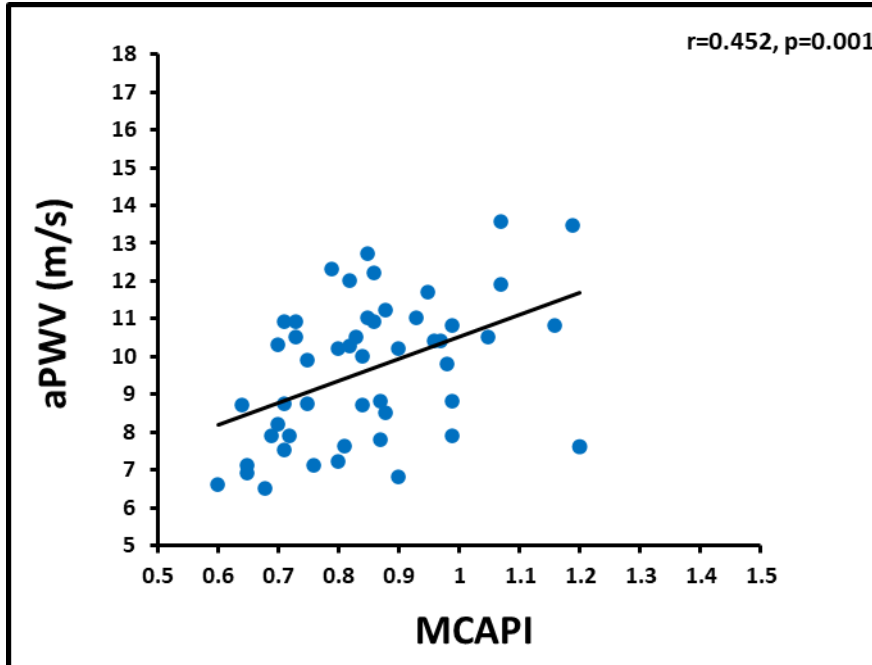
Figure 4.7: Relationship between MCAPI and CPP in the COPD group
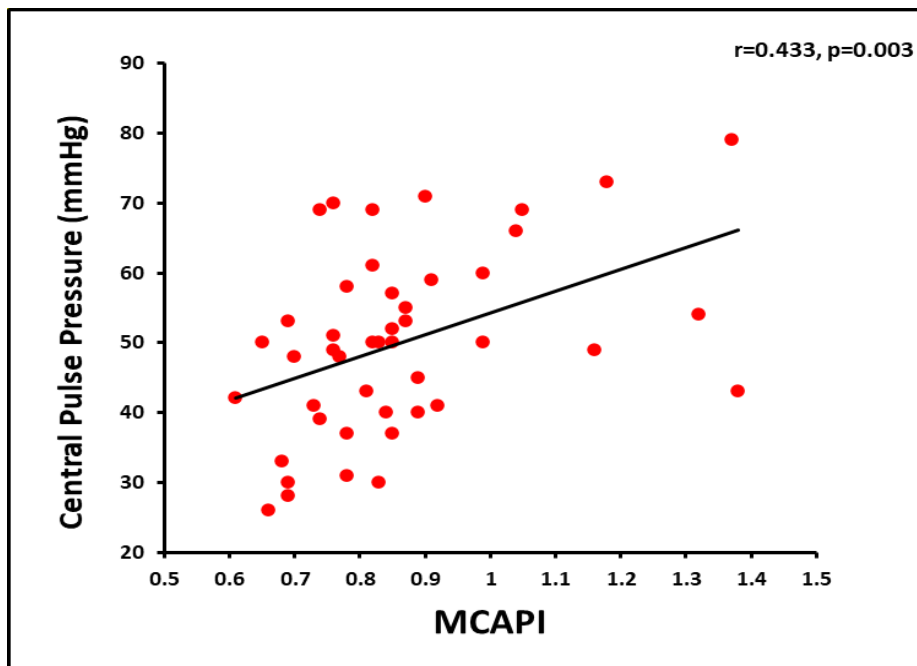
Figure 4.8: Relationship between MCAPI and CPP in the control group
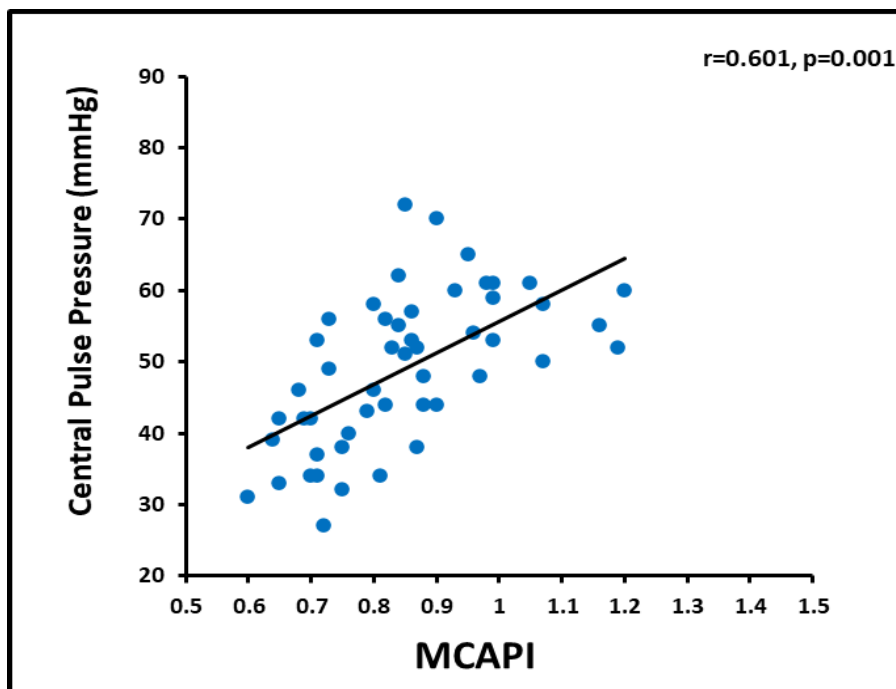
4.3.4 Relationship between MCAPI and comorbidity

MCAPI significantly correlated with comorbidity and cardiovascular agents' number in the control group but not in the COPD group (P<0.05) (Table 4.5).

Table 4.4: Relationship between MCAPI and comorbidities in the COPD and controls

Measurements	COPD Group N=45		Control Group N= 50	
	r_s	P	r_s	P
Comorbidity number	0.090	0.555	0.342	**0.015**
Medications number	-0.025	0.868	0.227	0.112
Cardiovascular Agents number	0.138	0.367	0.375	**0.007**
Abbreviations: r_s= Spearman's correlation coefficient P≤0.05 statistically significant				

4.3.5 Relationship between MCAPI and COPD characteristics

In patients with COPD, MCAPI correlated with FEV_1/FVC measured (r=0.330, P<0.05). MCAPI also correlated with activity domain obtained from the SGRQ (r=0.317, P<0.05) (Table 4.4).

Table 4.5: Relationship between MCAPI and COPD characteristics in the COPD group

Measurements		COPD Group N=45	
		R	P
Lung function test	FEV_1 predicted (L)	-0.394	**0.007**
	FEV_1 measured (L)	-0.289	0.054
	FEV_1 %	-0.215	0.156
	FVC measured	-0.265	0.079
	FVC %	-0.167	0.273
	FEV_1/FVC measured	-0.330	**0.027**
St George's Respiratory Questionnaire (SGRQ)	Symptoms Score	0.073	0.634
	Impact Score	0.257	0.088
	Activity Score	0.317	**0.034**
	Total Score	0.281	0.062
COPD Assessment Test (CAT)	Score	0.186	0.222
Abbreviations: R= Pearson correlation coefficient FEV1-Forced expiratory volume, FVC -Forced vital capacity, FVC -Forced vital capacity P≤0.05 statistically significant			

4.3.6 Relationship between MCAPI and level of physical function

MCAPI significantly correlated with walked distance during the 6MWT in both groups (P<0.05). It also significantly correlated with the resting SpO_2 and with the TUG time in the COPD group (P<0.05). No correlation was observed between MCAPI and CGA or handgrip strength in either group (Table 4.6).

Table 4.6: Relationship between MCAPI and level of physical function in patients with COPD and controls

Measurements		COPD Group		Control Group	
		R	P =	R	P =
Comprehensive Geriatric Assessment	Score	0.138	0.366	0.262	0.182
	Index	0.019	0.904	0.066	0.207
6 Minute Walk Test	Walked Distance (m)	-0.307	**0.040**	-0.294	**0.038**
	Pre-SpO$_2$ (%)	-0.341	**0.022**	-0.146	0.313
	Pre-Heart Rate (bpm)	-0.163	0.284	-0.004	0.978
	Pre-Dyspnoea Level	-0.105	0.491	-	-
	Post - SpO$_2$ (%)	-0.227	0.133	-0.160	0.268
	Post- Heart Rate (bpm)	-0.158	0.301	-0.157	0.276
	Post- Dyspnoea Level	0.053	0.727	0.100	0.489
Time Up and Go Test	Time (s)	0.313	**0.037**	0.143	0.321
Grip Strength	Right-hand Strength (kg)	-0.087	0.569	-0.168	0.245
	Left-hand Strength (kg)	-0.158	0.300	-0.186	0.196
Abbreviations: R= Pearson correlation coefficient SpO$_2$: peripheral capillary oxygen saturation P≤0.05 statistically significant					

4.3.7 Predictors of MCAPI

Stepwise Multiple Regression Analysis illustrated that only CPP remained an independent predictor of MCAPI (p=0.003), in a model which included age, aPWV, FEV_1/FVC and use of cardiovascular acting medications (Figure 4.7).

Table 4.7: Stepwise multiple regression analysis in the COPD group

Model	R square	Beta	95% CI	P
CPP	0.188	0.433	(0.002-0.10)	0.003
Excluded variables: age, aPWV, FEV_1/FVC, number of cardiovascular medications				

4.4 **Discussion**

The current study aimed to measure aPWV and MCAPI and establish the relationship between aPWV and MCAPI in a group of patients with COPD and aged-matched controls. This is the first study to investigate the relationship between MCAPI and aPWV in a well characterised group of patients with COPD, compared to controls. The results indicate that both groups were similar in their levels of MCAPI; thus, the null hypothesis of no difference in MCAPI between the two groups was accepted.

In addition, the study hypothesised that MCAPI would relate to aPWV. Interestingly, such a relationship did not exist in the COPD group despite having higher aPWV values, and therefore, the null hypothesis was accepted. However, an association was observed in the control group in partial support of the study hypothesis.

Importantly, CPP was independently associated with MCAPI, even when accounting for the use of cardiovascular acting medications, highlighting the value of using CPP in potentially monitoring cerebral flow dynamics associated with cerebral dysfunction. Therefore, the null hypothesis stating there are no predictors of MCAPI was rejected. As studies reporting MCAPI values in patients with COPD are lacking, the study provides novel knowledge by presenting values of MCAPI in this group that may be used to better understand cerebral pulsatility, progression of cerebrovascular dysfunction and its determinates in COPD.

Furthermore, the study also aimed to establish the relationship between MCAPI, and measures related to COPD and measures of health status in COPD patients compared to controls. An important novel finding of this study was the observed relationship between MCAPI and FEV_1/FVC and between MCAPI and physical function measures, including the 6MWT and the TUG test. Therefore, the null hypothesis stating no relationship between MCAPI and the previously mentioned measures was rejected. Although earlier studies showed a connection between a higher risk of stroke and impairment in lung function and lower physical function, data from this thesis provided a potential mechanistic link that may explain the connection between stroke and COPD through using novel, cost-effective and

short test such as MCAPI. This dataset supports the potential utility of MCAPI in predicting or monitoring the progression of cerebrovascular dysfunction and stroke outcomes in this population. Future use of MCAPI as a monitoring tool, applied early in the disease progression to address the causal links of the pulsatile flow transmitted to the brain of these patients, may be a viable option in the future.

4.4.1 Aortic Pulse Wave Velocity (aPWV)

Aortic PWV was significantly higher in the COPD group compared to the control group. The findings of increased aPWV in patients with COPD in the current study confirms the findings in chapter 3 and was consistent with other reports in the literature (Sabit et al. 2007; Mills et al. 2008; Gale et al. 2014; Ye et al. 2017; Fisk et al. 2018).

Several physiological and pathological mechanisms might contribute to vessel wall changes resulting from the process of arteriosclerosis and atherosclerosis, which both lead to vascular remodelling through different aetiology. Arteriosclerosis concerns the age-related increase in collagen resulting in vessels stiffness, whereas atherosclerosis relates to plaque accumulation within the vessels wall that can be triggered by inflammation (O'Rourke et al. 2002; Gimbrone and García-Cardeña 2016). These processes may be exaggerated in the COPD group due to the participants' age and the inflammatory nature of the COPD disease.

The current study did not assess inflammatory markers in the COPD group however, several cross-sectional studies which do not show causality reported an increase in the inflammatory markers in COPD patients including interleukin 6 and 8, TNF-α and fibrinogen (Maclay et al. 2007), CRP, interleukin 6 and Interleukin-1 receptor antagonist (Johansen et al. 2012) and Homocysteine levels (Avci and Avci 2016). In the same way, endothelial micro-particles were elevated suggestive of endothelial cell apoptosis even in individuals with mild COPD (Thomashow et al. 2013). However, (McAllister et al. 2007) found no association between aPWV and high level of CRP or between emphysema and high level of CRP in 157 COPD patients.

Patients with COPD in the current study were smokers or ex-smokers, which may explain the increased aPWV in this group. A key mechanism that is linked to alteration in the vasculature is smoking, which is recognised as one of the main risk factors in COPD and also shown to associate with systemic inflammation in those patients (Liew 2015). There is an association between increased central arterial stiffness and smoking in older individuals (Camplain et al. 2016). The chronic exposure to a toxic substance can cause hypertrophy of smooth muscle cells and faster degradation of lipids which can lead to thickening and loss of elasticity of vessel walls (Kool et al. 1993). In addition, blood viscosity, known to be increased in patients with COPD and smokers, is also an essential factor affecting the normal flow within blood vessels (Cakmak et al. 2013).

4.4.2 Middle Cerebral Artery Pulsatility Index (MCAPI)

The results of the study showed no difference in MCAPI between COPD and control group where the mean of MCAPI was 0.90 in both groups. There are no well-established reference values for MCAPI in adults. However, several studies reported the mean MCAPI in populations from the community and specific conditions and were estimated to range from 0.65 to 1.10 (Zhu et al. 2019) or between 0.5 to 1.19 (Naqvi et al. 2013). A higher index represents an increased transmission of pulsatile flow to distal cerebral small vessels (Kassab et al. 2007). It has been shown in animal models that ageing impairs the myogenic constriction in MCA that reduces the response to pulsatile pressure (Springo et al. 2015), increase blood-brain barrier disruption (Bors et al. 2018) and increase cerebral microhaemorrhages (Toth et al. 2015).

The MCAPI results in the current study is in line with what has been published previously. In healthy participants similar to the age of the present participants mean age (71 years), the mean of MCAPI was 0.89 (Zarrinkoob et al. 2016). In the same way, the mean MCAPI was 0.97 when measured in a group from the general population who were slightly younger (mean age 64 years) than the present cohort but shared a similar profile of hypertension and hypercholesterolemia (Vigen et al. 2020). However, in younger age groups, mean MCAPI was 0.81 in participants with a mean age of 55 years (Nevzorova et al. 2018) and 0.72 in

participants with a mean age 25 years. The progression of MCAPI with age supports the role of age in increasing cerebral pulsatility which is typically lower than 0.80 in younger individuals with a progression amplifying around the sixth decade (Tegeler et al. 2013; Tarumi et al. 2014).

However, studies reporting data of MCAPI in COPD are scarce. Nevzorova et al reported a significant difference in MCAPI between participants with COPD compared to an age-matched control with mean MCAPI 0.93 in COPD and 0.81 in controls (Nevzorova et al. 2018). The mean age of participants in their study was 55 years, notably younger than the age of COPD participants in the present study (mean age 72 years). Considering that the COPD group in the current study was older by almost two decades than the one studied by Nevzorova et al, it was expected to see a higher MCAPI as a result. It could be that the overt CVD history and the high number of CVD medications used by the cohort worked through lowering the age and diseases influence on the MCA flow pulsatility.

Despite the wide use of PI as a measure of increased transmission of pulsatile flow to distal cerebral small vessels, some evidence suggests applying caution when interpreting results in conditions that are characterised by changes in arterial gases levels, which can be present in COPD. The PI is derived from cerebral blood flow velocities (CBFV) measurement, where the latter is used as a surrogate for cerebral blood flow (CBF) with the assumption of constant vessel diameter (Tarumi and Zhang 2018). However, a study by Coverdale et al showed that using CBFV underestimated the true blood flow changes in the MCA when individuals were subjected to conditions that alter their arterial partial pressure of carbon dioxide ($PaCO_2$) (Coverdale et al. 2014) and consequently, may have impacted PI. Previous evidence acknowledges the role of $PaCO_2$ in changing global cerebral blood flow and flow velocities of the MCA through increasing or decreasing cerebral vascular resistance. It was shown that for each mmHg increase or decrease in $PaCO_2$, there is a 2% to 4% change in MCA flow velocity when $PaCO_2$ values are within 20 to 80 mmHg (Brian 1998).

4.4.3 The link between aPWV and MCAPI

The reported association between aPWV and MCAPI in the control group supports the evidence that increased arterial stiffness amplifies the transmission of high pulsatile energy into the cerebral circulation as signified by higher PI (Kwater et al. 2009; Mitchell et al. 2011; Webb et al. 2012; Xu et al. 2012; Tarumi and Zhang 2014; Kim et al. 2015).

Arterial stiffness, as measured by aPWV, has been linked to cerebral Small Vessel Disease (SVD), and SVD is a major cause of stroke (Mitchell et al. 2011). In addition, higher PI significantly predicted the occurrence of stroke death in a large group of hypertensive individuals followed longitudinally after adjusting for common cardiovascular risk factors (Laurent et al. 2003). As higher PI is associated with SVD (Birnefeld et al. 2020), it is plausible that PI can be regarded as an indicator of risk for stroke.

Several mechanisms can explain the association between increased aPWV and higher MCAPI. Arterial stiffness can reduce the sensitivity of baroreceptor function, which may contribute to the disturbance in the regulation of BP, CPP and eventually CBF (Tarumi and Zhang 2018). The high pressure leads to impedance matching between the aorta and peripheral arteries which facilitates the transmission and penetration of excess pulsatility into the downstream circulation (Mitchell et al. 2011) because of the low vascular resistance of cerebral vasculature (O'Rourke and Nichols 2005; Wohlfahrt et al. 2014).

On the other hand, the capacity to dampen of pulsations deteriorate with age (Zarrinkoob et al. 2016) which supports the widely accepted concept of "pulse wave encephalopathy", that describes the influence of high pulsatile flow in causing microvascular damage in the aged brain (Bateman 2004). Ageing alters the cardio and cerebrovascular regulatory mechanisms, such as baroreflex sensitivity and dynamic cerebral autoregulation (Xing et al. 2017). the disruption in these mechanisms may lead to the reduction in cerebral capillary density, microvascular wall distortion and endothelial dysfunction (Farkas and Luiten 2001; Iadecola and Gorelick Philip 2003) and elevation of pulse pressure (O'Rourke and Nichols 2005; Tarumi and Zhang 2018); which can lead to an increased cerebral pulsatility.

4.4.4 Role of Central Pulse Pressure (CPP)

The present study showed that MCAPI was associated with both aPWV and CPP in the control group, whereas MCAPI only associated with CPP in the COPD group. This link may be explained as high CPP is considered a measure of aortic pressure derived from a combination of both cardiac performance and arterial stiffness (O'Rourke and Nichols 2005), a better indicator for CVD than peripheral pressure (Benetos et al. 2002), and correlates with the extent of CBF pulsatility (Tarumi et al. 2014). In addition, literature has shown that both aPWV and CPP are significant measures of vascular haemodynamics used in predicting stroke (Laurent et al. 2003; Mattace-Raso et al. 2006).

The observed link between MCAPI with both aPWV and CPP in the control group are in keeping with previous literature. In a study including a large sample from the community reported that the upper quartile of MCAPI was associated with a higher pulse pressure (Vigen et al. 2020).

Another study including 334 participants referred for ambulatory blood pressure monitoring and were not on antihypertensive medications for at least two weeks (mean age, 50.9 years) evaluated the relationship between MCAPI and measures of central arterial stiffness including aPWV and CPP. The result showed a weak but significant association between MCAPI and aPWV. In addition, it showed that MCAPI was closely related to CPP (Xu et al. 2012). Likewise, Kwater and colleagues had also reported a relationship between CPP and MCAPI using multivariate-adjusted regression analysis (Kwater et al. 2009). A similar finding was also reported in a small group of individuals with and without metabolic syndrome after adjustment of age and sex (Robertson et al. 2019).

The stepwise multiple regression analysis showed that only CPP remained independently associated with MCAPI, even when accounting for the use of cardiovascular acting medications. A potential mechanism that explains how the CPP and MCAPI affects the brain may be due to the increase in CPP that impacts the cerebral circulation, which leads to abnormalities in the brain through increasing myogenic tone (Raignault et al. 2017) and

hypertrophic remodelling of cerebral arterioles (Baumbach et al. 1996). As CPP was a more robust correlate with MCAPI seen in this cohort and previous studies, it suggests that arterial stiffness may indirectly influence flow pattern in the cerebral circulation through increased CPP. In addition, this highlights the use of CPP as a simple tool to identify any cerebral changes in COPD patients. In addition, it highlights the limitations of aPWV in linking to flow velocity differences in the brain.

4.4.5 Role of Blood Pressure (BP)

The current study also showed a connection between MCAPI and blood pressure in both groups. It has been shown in middle-aged non COPD adults that an increase in SBP by 20 mmHg from the normal was associated with an increase in stroke mortality risk by more than two-fold (Lewington et al. 2002). In addition, a higher BP was also linked to larger infarctions and worse stroke outcome (Leonardi-Bee et al. 2002). The data of the current study confirm the impact of high BP in increasing cerebral pulsatility and potentially increasing cardiovascular risk. These findings were irrespective of the higher use of cardiovascular acting medications in patients with COPD.

Evidence supports the presence of a U-shaped relationship between BP and all-cause mortality and cardiovascular events in COPD (Byrd et al. 2018). Although no difference in BP amongst the studied groups was shown, it is worth pointing out that patients with COPD had a greater history of hypertension and reported higher use of hypertension therapy, which could have resulted in a better BP control. In fact, this BP control in the COPD group may have influenced the lack of associations observed between aPWV and MCAPI in the current study.

Fluctuations in BP occurs naturally with every cardiac cycle, but the cerebral regulation through vasoconstrictive or vasodilatory mechanisms constantly works to adjust the cerebral blood flow. However, with extreme changes in BP, these mechanisms can be impaired (Pires et al. 2013). Consequently, cerebral perfusion pressure can increase as a result of increased mean arterial pressure. As perfusion pressure increases, remodelling of cerebrovascular bed

occurs to prevent over perfusion that can potentially damage the sensitive brain tissue (Tarumi and Zhang 2018). Nonetheless, in chronic hypertension, the brain adapts to the increased pressure, although the exact mechanism of cerebral autoregulation in hypertension is not well understood, it may relate to myogenic tone alterations and inward vessel remodelling (Pires et al. 2013). This may also explain the lack of the association between MCAPI and aPWV in the COPD group of the current study. It may be that the influence of chronically increased MCAPI in the COPD group has already taken effect on the cerebral arteries and remodelled to impact on the flow dynamics. However, further studies are needed to evaluate cause and effect.

There has been evidence linking sympathetic overactivity to increased arterial stiffness in healthy populations and in hypertension which may explain the role of BP in arterial stiffness (Dinenno et al. 2000).

Evidence from a systematic review and meta-analysis that included data from 33 observational cohort studies and clinical trial cohorts assessing variability in blood pressure concluded that long-term variability was associated with cardiovascular and mortality outcomes, including increased stroke risk (Stevens et al. 2016). In addition, data from the Maastricht Study, including individuals from the general population, showed that greater BP variability was associated with greater aPWV (Zhou et al. 2018).

Acute BP fluctuations can occur in COPD in response to heart rate variability accompanying pulmonary symptoms such as dyspnoea, exercise intolerance or acute exacerbation (Camillo et al. 2008; Tseng et al. 2018). Research have shown that patients with COPD have more BP fluctuations than individuals without COPD (van Gestel et al. 2012). This fluctuation may be explained by sympathetic overactivity in COPD influenced by large intrathoracic pressure changes, hypoxia, systemic inflammation, and physical inactivity. However, the available evidence does not verify the cause-effect relationship (van Gestel et al. 2012).

Also, the evidence explaining the association between BP and increased arterial stiffness in COPD still lacks clarity, potentially due to the multiple cardiovascular risk factors. Therefore, the role of BP in increasing arterial stiffness and its potential in influencing the cerebral haemodynamic is yet to be established in COPD.

It has been shown that BP highly influences blood flow velocities at MCA causing a difference between normal BP to stage 2 hypertension of nearly 10 cm/s, diminishing the cerebral vessels' ability to adapt to these changes (Zhang et al. 2006). However, this can be reversible by lowering BP using antihypertension treatment. Antihypertensive treatment has shown significance in reducing the risk of recurrent stroke. Antihypertensive medications that primarily work as vasodilators were associated with reduced MCAPI despite having no reduction in pulse pressure as per a recent systematic review and meta-analysis (Webb 2019). In addition, antiplatelet medications can lower pulsatility index through the vasodilatory effect that reduces the vascular resistance in cerebral circulation (Han et al. 2014). Furthermore, a widely used CVD agent in this study such as statins have been linked to improvement in cerebral vasomotor reactivity (Forteza et al. 2012; Giannopoulos et al. 2012), through the upregulation of endothelial nitric oxide synthase activity and downregulation of superoxide production (Endres and Laufs 2004). Statins have been shown to affect the vasculature through its antioxidant, anti-thrombotic and anti-inflammatory effects in addition to the pleiotropic improvement of endothelial function which together can explain its position in stroke prevention (Fracassi et al. 2019). The systemic effect of stains on the vasculature may have prevented an increase in the MCAPI in the studied group.

On the other hand, patients with COPD use anti-inflammatory medications as part of COPD control. It was reported that some anti-inflammatory medications in chronic inflammatory diseases other than COPD had shown their benefit in reducing arterial stiffness through improving endothelial function and reduction of inflammation in the arterial wall (Maki-Petaja and Wilkinson 2009; Zanoli et al. 2020).

Despite the previously discussed established connections between central arterial stiffness and cerebral pulsatility, the increased aPWV found in the patients with COPD did not appear to impact the MCAPI as it was similar in both groups.

The previous evidence supports that BP influences arterial stiffness (The Reference Values for Arterial Stiffness Collaboration 2010) and blood flow velocities at the MCA (Zhang et al. 2006). Patients with COPD in the current study exhibited a higher prevalence of clinically diagnosed hypertension and reported higher use of antihypertensive medications. As already

stated, The high use of antihypertensive medications and a normal BP range when measured during the study may have influenced the relationship between aPWV and MCAPI in the current study. Therefore, the lack of the association between aPWV and MCAPI may relate to the adaptation of the brain to chronic high blood pressure and the number and types of CVD and COPD medications that may collectively have served as a protective mechanism against increasing pulsatility in the cerebral circulation.

4.4.6 Relationship between MCAPI and COPD characteristics

4.4.6.1 Relationship between MCAPI and lung function

In patients with COPD an inverse relationship between FEV_1/FVC and MCAPI was seen. Reduced lung function in patients with COPD, as indicated by a low FEV_1 and FEV_1 /FVC ratio is an independent risk factor for CVD mortality (Sin et al. 2005) and has been linked to the occurrence of stroke in long term follow-up of 26 years (Söderholm et al. 2012) and 40 years (Gulsvik et al. 2012). Similarly, increased risk of cerebral infarction and lesions to white matter was associated with the reduction in pulmonary function (Liao et al. 1999). This association suggests that COPD severity may be an indicator of stroke risk in patients with COPD. However, evidence directly linking airflow obstruction and MCAPI in COPD is lacking.

On the other hand, patients with COPD in the current study had lower resting SpO_2 (%), which was associated with higher MCAPI. The origin of such a relationship may relate to a key COPD manifestation which is the alterations in blood gases at pulmonary and circulatory levels. Patients with COPD frequently present with hypoxemia and hyperventilation, more in severe cases. In individuals with normal ventilatory capacity, the body reacts to episodes of hypoxia through hypoxic ventilatory response to prevent arterial oxyhaemoglobin desaturation. However, patients with COPD have reduced ventilatory reserve capacity due to airflow obstruction, alveolar-arterial gas exchange abnormalities and respiratory muscle weakness. Hence, they experience episodes of acute or chronic hypoxia which potentially lead to arterial oxyhaemoglobin desaturation (Beaudin et al. 2017) causing a disturbance in the regulation of cerebral blood flow (Willie et al. 2012; Tarumi and Zhang 2018).

Cerebrovascular sensitivity to hypoxia, assessed as the change in CBF during a hypoxic challenge, represents the capacity of cerebral vessels to respond to, and compensate for, a reduced oxygen supply, and is impaired or blunted in some conditions (Beaudin et al. 2017). Hypoxemia and hyperventilation may lead to a metabolic disturbance that can contribute to arterial wall changes. The metabolic disturbance may also induce cardiac arrhythmias including atrial fibrillation, a condition that is prevalent in COPD and has been linked to higher stroke risk (Dodd et al. 2012; Austin et al. 2016). In addition, patients with COPD have sympathetic nervous system overactivity that can impair the autonomic function and modifies the cerebral vasculature regulation and the arterial baroreflex (van Gestel et al. 2012).

The higher risk of haemorrhagic stroke compared to other types of stroke is in line with reports of increased cerebral small vessel disease seen in COPD where hypoxia and increased oxidative stress level play an essential role in the development of haemorrhagic stroke (Dodd et al. 2012; Austin et al. 2016).

4.4.6.2 Relationship between MCAPI and Level of physical function

The current study also showed that MCAPI was related to some measures of function in both groups. In patients with COPD, slow walking as indicated by a shorter distance in the 6MWT or a longer time in the TUG were associated with higher MCAPI. A similar relationship was also found between MCAPI and the activity score obtained subjectively via the St George's Respiratory Questionnaire (SGRQ). However, in the control group, MCAPI only related to the 6MWT.

The 6MWT provides information on the aerobic capacity and endurance of individuals (ATS-Statement 2002) and having an association with MCAPI may explain the potential role of the lack of ventilatory reserve and a limited ventilatory compensation in the increased pulsatility within the cerebral circulation. In contrast, the TUG test represents the global function with less ventilatory involvement where the poor outcome may relate to the number of comorbidities in this population.

4.4.7 Strengths / limitations of the study

The strength of this study is the collection of data derived from valid measurements that provide an insight into the nature of the interaction between central arterial stiffness and cerebral pulsatility in patients with COPD and age-matched control. It also examined this connection in light of COPD's specific characteristics taking a holistic approach filling a gap in the literature. It included a well-characterised group of patients with COPD, confirmed using the gold standard spirometry and controls free from respiratory diseases similar in age and gender representative of community-dwelling COPD. In addition, using gold non-invasive measures of arterial stiffness and a novel measure of MCAPI with reliability and validity previously established as part of this thesis.

However, there are a number of limitations which should be considered when interpreting the results. First, the cross-sectional nature of the study hinders establishing the causal relationship between central arterial stiffness and cerebral pulsatility. Second, the overt history of CVD, the use of cardiovascular acting agents in patients with COPD and the lack of control for smoking history may have affected the relationships observed in this study. However, the current study attempted to account for aspect of this limitation by adjusting the multiple regression analysis to take cardiovascular medications into account. Nevertheless, since CVD comorbidities are highly prevalent in patients with COPD, the participants were a representative sample from the wider COPD population. In addition, recruitment and attrition bias cannot be excluded where loss of follow up of those with worse health status is possible. Additionally, the sample was relatively small hence investigating the relationship between MCAPI and aPWV in a larger sample will enable a comparison of those with CVD to those without, in addition to stratifying them by COPD severity to observe independent associations in multivariate analysis. There is also the risk of selection bias, as data of individuals whose MCAPI measurement was not obtainable because of the poor temporal window were excluded.

Transcranial doppler (TCD) is beneficial in recording cerebral blood flow velocities non-invasively from the intracranial arteries (Aries et al. 2010). However, it has its technical

limitations which can affect the obtainability and the accuracy of the measurements. These limitations can result from inadequate insonation window, restlessness, or anxiety. In the current study, MCAPI measurement was obtainable in 68% of the control group which is very similar to the success rate in a study including 3008 participants (67%) and another study including 331 older adults (69%) from the general population (Bos Michiel et al. 2007; Kang et al. 2019). Inadequate insonation window due to an increase in temporal bone thickness with advanced age may have caused this failure (Lee et al. 2020). However, the success rate was lower in patients with COPD (53%), which correspond to the health status of this group. They often experience breathless, restless, and may have persistent coughing, which in turn, provoked intolerance for remaining still during the measurement session. The problems of restlessness and anxiety during TCD measurement were also reported in at least 8% of the participant in a previous study (Bos Michiel et al. 2007).

Also, one of the key points that hinder a full understanding of the connection between aPWV and MCAPI is that PI measurement by TCD only evaluates the proximal segment of the cerebral vascular system. Therefore, measuring vascular structure and function of the carotid artery may provide a link between the aorta and cerebral arteries. In addition, a detailed study using MRI may provide a better understanding of the structural and functional changes in the cerebral circulation in this group.

4.4.8 Conclusion

The current data showed no difference in MCAPI between patients with COPD and controls. However, studies reporting data of MCAPI in COPD are scarce hence the present data describe novel knowledge by presenting values of MCAPI in patients with COPD. In addition, there was no relationship between MCAPI and aPWV however, CPP was independently associated with MCAPI, even when accounting for the use of cardiovascular acting medications. Although earlier studies showed a connection between higher risk of stroke and impairment in lung function and lower physical function, the data of this thesis had provided a mechanistic link that may explain the link between stroke and COPD, through using novel and cost-effective measures such as MCAPI. This supports the potential utility of MCAPI in

predicting stroke outcomes in this population through using MCAPI as monitoring measurement early in the disease progression, to address the causal links of the pulsatile flow being sent to the brain of these patients.

Chapter 5

An exploratory study investigating the

relationship between vascular

haemodynamics and cerebral volumes

5 An exploratory study investigating the relationship between vascular haemodynamic and cerebral volumes

5.1 Introduction

The brain is the most metabolically demanding organ in the body, requiring about 20% of the body's resting energy consumption (Clarke and Sokoloff 1999). It is also highly susceptible to excess pulsatile flow (Mitchell et al. 2011) hence, maintaining a constant cerebral blood flow regulation is necessary to preserve the brain function and to prevent ischaemic injury (van Mook et al. 2005).

The critical function of the lungs is to provide oxygen and eliminate carbon dioxide from the blood so that sensitive organs such as the brain can function correctly. Hence, any disorder that impairs the lung capacity may adversely affect the structure and function of the brain. This effect can be further augmented with advanced age in which the brain reserve capacity is reduced (Bishop et al. 2010; Sibille 2013).

Chronic obstructive pulmonary disease is linked to a high prevalence of extra-pulmonary complications, including cerebrovascular comorbidities (Yin et al. 2017). Furthermore, a large body of evidence showed that individuals with COPD have a 30% increased risk of developing stroke as per a recent meta-analysis (Kim et al. 2018) and increase risk of stroke in certain subsets of patients with COPD, and for certain types of stroke when compared to individuals from the general population (Morgan et al. 2017).

Despite the availability of several lines of evidence linking COPD to increased risk of stroke, the exact mechanisms that explain this link remains unclear. There is now increasing interest in studying the lung-brain interaction using neuroimaging technologies such as magnetic resonance imaging (MRI) that enable a detailed examination of the structure and function of the brain. This technology allows for the quantification of several metrics associated with cerebrovascular health, including the measurement of cerebral volumes such as White

Matter (WM), Grey Matter (GM) and Cerebrospinal Fluid (CSF) In addition, it allows for evaluations of outcomes related to stroke including white matter lesions, lacunar infarcts and microbleeds (Lahousse et al. 2015).

The most commonly known lesions affecting the white matter is known as white matter hyperintensities (WMHs) , also referred to as leukoaraiosis and can be detected in MRI or CT scans (Wardlaw et al. 2015). Alteration in the WM in the brain is a sign of cerebral small vessels diseases (SVD), and a major cause of stroke (Mitchell et al. 2011). Although WM occupies 50% of the total brain volume, it has a similar metabolic rate to that of GM (Dewar et al. 1999; Goldberg and Ransom 2003). It also receives a disproportionately small blood supply and little collateral circulation, making it susceptible to ischaemic damage (Dewar et al. 1999; Arai and Lo 2009) and has been associated with triple the stroke risk (Debette and Markus 2010). These lesions can result from degenerative or inflammatory processes as well as ischemia related to reduce blood flow leading to permanent demyelination and axonal destruction (Pantoni and Garcia 1997; Yamamoto 2017).

White matter hyperintensities are associated with vascular risk factors including increased age, with a prevalence of 10% - 20% in individuals aged 60 years old, however, this has been shown to increase up to almost 100% in individuals older than 90 years (Smith et al. 2017). Moreover, WMH are also associated with hypertension, but this relationship is controversial as it is not clear if hypertension affects the brain directly or indirectly through systemic vascular stiffening that affects brain white matter (Brisset et al. 2013). Evidence also suggests that the impact of WMHs extend to other structures in the cortex, including the grey matter. New research showed that local WMH influence the grey matter in remote areas leading to secondary brain atrophy (Wang et al. 2020).

In COPD, a large body of evidence showed an increased volume and size of WMH and impaired WM integrity (Liao et al. 1999; Dodd et al. 2012; Zhang et al. 2012; Ryu et al. 2013; Taki et al. 2013; Spilling et al. 2017). As WMH is major cause of stroke, this may explain the increased stroke risk in COPD (Mitchell et al. 2011). However, whether these structural changes reflect changes in cerebral volumes is controversial. Several neuroimaging studies

have shown associations between COPD and decreased total WM and GM volumes in several brain regions (Zhang et al. 2013; Wang et al. 2017a; Yin et al. 2019). In contrast, other studies reported no significant differences in global GM and WM volumes between patients with COPD and controls (Esser et al. 2016; Spilling et al. 2017). Additionally, the evidence relating to CSF volume changes in COPD is limited with one study showing no difference in CSF volume in 27 patients with COPD compared to 23 aged matched individuals. (Spilling et al. 2019). Nonetheless, the measurement of CSF volume had shown its benefits in some conditions. For example, the measurement of the ratio of intracranial CSF volume to intracranial volume (ICV) in individuals with suspected ischemic stroke presenting within 9 hours of the onset of neurological deficits had supported the added value of CSF/ICV in predicting MCA infarction which allows for early management and reducing the high rate of mortality associated with MCA infarction (Kauw et al. 2019).

Despite the availability of evidence supporting the loss of cerebral volumes that accompany ageing (Raz et al. 2005; Taki et al. 2004; Tang et al. 1997), the deterioration in brain volumes in COPD were more significant than the decline reported in normal ageing (Aljondi et al. 2019; Scahill et al. 2003; Taki et al. 2011).

Literature showing the use of neuroimaging to investigate specific alterations in structure and function of the brain in COPD patients has partly conflicting results. Potential reasons for this conflict were differences in sample size, the inclusion of diverse COPD cohorts with differences in cardiovascular risks, different smoking exposure, variations in defining and categorisation of COPD severity and the use of different imaging methodologies.

5.1.1 Arterial stiffness and cerebral volumes

Increased central arterial stiffness in patients with COPD is well documented in the literature (Mills et al. 2008; Ye et al. 2017; Fisk et al. 2018) and can be estimated non-invasively by the measurement of aPWV and CPP (Mancia et al. 2007; Townsend 2017).

Evidence shows that the loss of compliance in the central arteries affects the downstream flow. Increased aPWV augments the transmission of high pulsatile energy into the cerebral circulation as signified by higher pulsatility index PI (Webb et al. 2012; Kim et al. 2015), and this relationship is well established in patients with Diabetes (Sanahuja et al. 2016) and leukoaraiosis (Webb et al. 2012).

In a meta-analysis of observational studies, aPWV has shown its extended role in predicting stroke beyond the traditional risk factors (Ben-Shlomo et al. 2014) in addition to its role in predicting stroke death (Laurent et al. 2003). In addition, aPWV has been associated with cerebral SVD and WM lesions (Mitchell et al. 2011; Austin et al. 2016; Silva and Faraci 2020).

Also, recent large-scale research including 1484 older adults (mean age 76±5 years) from the Atherosclerosis Risk in Communities Neurocognitive Study measuring WM microstructural integrity using diffusion tensor imaging showed that increased aPWV was associated with low cerebral white matter microstructural integrity. It was shown that each 1-m/s higher aPWV was associated with lower overall fractional anisotropy and higher overall mean diffusivity, which are important measures used to map white matter tractography in the brain (Wei et al. 2020).

5.1.2 Study justification

As already shown in chapter 3, patients with COPD have premature vascular ageing, compared to matched controls, as indicated by increased arterial stiffness measured by aPWV. As higher middle cerebral artery flow pulsatility index (MCAPI) is associated with SVD (Birnefeld et al. 2020), it is plausible that MCAPI can also be regarded as a risk factor for stroke. However, as shown in chapter 4, a cross-sectional analysis of the present data did not support the connection between MCAPI and aPWV in the COPD group, whereas there was an association observed between MCAPI and CPP. The lack of association between MCAPI and aPWV may have been obscured by the overt CVD history and the use of multiple forms of CVD medications in this group. Therefore, the direct measurement of cerebral volumes, measures of arterial stiffness and middle cerebral artery flow dynamics in a cohort of patients

not on cardiovascular acting medication may provide a clearer insight of underlying large artery and cerebrovascular abnormalities associated with COPD.

Although, alteration in the WM of the brain is a sign of cerebral SVD, and SVD is a major cause of stroke (Mitchell et al. 2011), the predictive value of cerebral volumes in predicting outcomes related to stroke can be of important clinical value (Kauw et al. 2019). Hence, the assessment of these volumes can be useful in identifying early changes in the brain in COPD.

In order to understand the degree of cerebral dysfunction, measurement of cerebral volumes and specific volumetric analyses of the brain and brain regions, may provide information about early changes before irreversible, overt hyperintensities take place (Breakey et al. 2017). The assessment of cerebral volumes changes before structural changes may be of benefit and can prevent irreversible, permanent damage to the brain structures, and potentially preventing adverse outcomes (Wardlaw et al. 2015).

5.1.3 Study aim, objectives, and hypothesis

5.1.3.1 The aim of the study

The aim of this pilot study was to measure the global cerebral volumes, including the global WM, GM and CSF volumes in patients with COPD and age and gender matched controls using magnetic resonance imaging MRI technique.

5.1.3.2 The objectives of the study

1. To compare the cerebral volumes including the global WM, GM and CSF volumes in patients with COPD versus healthy controls.

2. To establish the relationship between vascular measures including aortic Pulse Wave Velocity (aPWV), Central Pulse Pressure (CPP), Middle Cerebral Artery Pulsatiltiy Index (MCAPI) and the cerebral volumes as measured by White Matter (WM), Grey Matter (GM) and Cerebrospinal Fluid (CSF) volumes.

3. To establish the relationship between cerebral volumes including the WM, GM and CSF volumes and airflow obstruction as measured by Forced Expiratory Volume in one second (FEV_1) and the ratio of Forced Expiratory Volume in one second /Forced Vital Capacity (FEV_1/FVC).

5.1.3.3 The hypothesis of the study

Null Hypothesis:

1. There will be no difference in White Matter (WM), Grey Matter (GM) and Cerebrospinal Fluid volumes between patients with COPD and controls.

2. There will be no relationship between White Matter (WM), Grey Matter (GM) and Cerebrospinal Fluid volumes and central arterial stiffness measures including aortic Pulse Wave Velocity (aPWV), Central Pulse Pressure (CPP) and Middle Cerebral Artery Pulsatility Index (MCAPI).

3. There will be no relationship between cerebral volumes including the WM, GM and CSF volumes and airflow obstruction as measured by Forced Expiratory Volume in one second (FEV_1) and Forced Expiratory Volume in one second /Forced Vital Capacity (FEV_1/FVC).

Alternative Hypothesis:

1. There will be differences in White Matter (WM), Grey Matter (GM) and Cerebrospinal Fluid volumes between patients with COPD and controls.

2. White Matter (WM), Grey Matter (GM) and Cerebrospinal Fluid (CSF) volumes relate to aortic Pulse Wave Velocity (aPWV), Central Pulse Pressure (CPP) and Middle Cerebral Artery Pulsatility Index (MCAPI).

3. White Matter (WM), Grey Matter (GM) and Cerebrospinal Fluid (CSF) volumes relate to airflow obstruction as measured by Forced Expiratory Volume in one second (FEV_1) and Forced Expiratory Volume in one second /Forced Vital Capacity (FEV_1/FVC).

5.2 **Methods**

5.2.1 Design setting and recruitment

A cross-sectional pilot study was used to assess the relationship between arterial stiffness and cerebral volumes in a group of patients with COPD, compared to a group of age and gender-matched controls. A subset of participants from the ARCADE study were invited to take part in this study. The study was approved (HMOTs; REF: Sta-439) (Appendix 11). Participants had a postal invitation, and the study was fully explained before considering participation and consenting to take part in the study. The study was conducted during two visits, completed within a week. Both visits were conducted in the mornings with individuals fasting from midnight. They were also requested to refrain from consuming caffeine, exercising, or taking medications, including respiratory inhalers on the day before the test.

The first visit included vascular measurements conducted at the health assessment suite, School of Sport and Health Sciences, Cardiff Metropolitan University.

The second visit involved the assessment of brain structure using magnetic resonance imaging MRI conducted at Cardiff University Brain Research Imaging Centre (CUBRIC). All potential participants underwent thorough screening to ensure the MRI related safety measures were verified and approved.

Participants' recruitment inclusion and exclusion criteria can be seen below (Table 5.1).

Table 5.1: Participants' inclusion and exclusion criteria for the COPD and controls

	COPD Group	Control Group
Inclusion criteria	• Aged between 35-80 years old • Diagnosed with COPD with FEV_1: FVC <0.70 • Able to give informed consent and willing to participate. • No history of cardiovascular disease • Not on any cardiovascular acting medications at the time of recruitment • Smokers or ex-smokers	• Aged between 35-80 years old • Don't have a diagnosis of chronic respiratory disease including COPD and asthma • Able to give informed consent and willing to participate. • No history of cardiovascular disease • Not on any cardiovascular acting medications at the time of recruitment • Smokers or ex-smokers
Exclusion criteria	• Unable to give informed consent • Pregnancy • History of malignancy in the last five years. • Participants with any other diseases identified as having an inflammatory or metabolic component, e.g. rheumatoid diseases or active endocrine disorders • History of cardiovascular disease • Using cardiovascular acting medications at the time of recruitment	• Unable to give informed consent • Pregnancy • History of malignancy in the last five years. • Participants with any other diseases identified as having an inflammatory or metabolic component, e.g. rheumatoid diseases or active endocrine disorders • History of cardiovascular disease • Using cardiovascular acting medications at the time of recruitment
MRI-specific exclusion criteria	• Experienced dizziness, fainting, or desaturations • Pregnancy or have given birth in the last six weeks • History of epilepsy • Mechanical/electrical/or magnetically operated devices in/on the body, e.g. pacemaker • Metal implant (pins, rods, screws, clips, stents, plates, wires, nails, joint replacement, contraceptive coil, dental work, tattoos, cochlear implant, metal fragments).	

5.2.2 Measurements

5.2.2.1 Visit1:

5.2.2.1.1 Participant's characteristics

Height, weight, body mass index was measured in all participants. Lung function test was measured using the Vitalograph ALPHA 6000 (Vitalograph Ltd. UK) for both groups (Chapter 2.3.3). A reversibility test was completed in the COPD group as per the guidelines (GOLD 2018).

5.2.2.1.2 Vascular measurements

Vascular measurements included the measurement of BP, aPWV, CPP and MCAPI.

5.2.2.1.2.1 Blood Pressure

Blood pressure was measured using a semi–automated oscillometric sphygmomanometer (Omron M2 HEM-7121-E, OMRON Healthcare UK Ltd). Measurements of systolic and diastolic blood pressure were taken twice in supine position. The average of the two measurements was recorded and used in the analysis (Chapter 2.3.2.1).

5.2.2.1.2.2 Aortic Pulse Wave Velocity (aPWV) and Central Pulse Pressure (CPP)

Arterial stiffness was measured using the SphygmoCor system (AtCor Medical, Sydney, Australia). Measurements of aPWV was obtained as per the protocol endorsed by the expert consensus document on arterial stiffness (Laurent et al. 2006) using the c-f PWV method as detailed previously. The measurement of central pulse pressure (PP) was derived from the measurement of PWA (Chapter 2.3.2.2).

5.2.2.1.2.3 Middle cerebral artery pulsatility index (MCAPI)

The measurement of MCAPI was assessed by Transcranial Doppler (DWL Doppler-BoxTM X, Compumedics, Germany) as detailed previously (Chapter 2.3.2.4).

5.2.2.2 Visit 2:

5.2.2.2.1 MRI measurement

All MRI data were acquired using a Siemens MAGNETOM Prisma (Siemens Healthcare GmbH, Erlangen) 3T clinical scanner with a 32 channel receive head coil.

A 3D magnetization-prepared rapid gradient-echo (MP-RAGE) sequence was acquired (1mm slice thickness, 1 x 1 mm image resolution, TR/TE = 2100/3.2 ms) and A 3D T2-weighted Fluid Attenuated Inversion Recovery (FLAIR) sequence was acquired (1mm slice thickness, 1 x 1 mm image resolution).

Analysis of the FLAIR images was performed implementing the winning method in MICCAI 2017 WMH segmentation challenge created by (Li et al. 2018). The method uses a 2D Convolutional Neural Network Architecture based on U-net to segment White Matter Hyperintensities (WHM). Grey matter, white matter and CSF volumes were obtained using the fully automated FMRIB's Automated Segmentation Tool (FAST).

5.3 **Data analysis**

Data was analysed using SPSS Statistics version 25. All data were checked for normality using Shapiro Wilko test (p > 0.05) and inspection for outliers using a boxplot. Data that were not normally distributed were transformed where possible and results were presented as means and standard deviations. The independent sample t-test was used to establish the difference in the cerebral volumes and vascular measurements between COPD and control group. The Pearson's correlation test was used to assess the relationship between vascular measures including aPWV and MCAPI and cerebral volumes in all participants.

5.4 **Results**

5.4.1 Demographics and groups differences

After screening 30 COPD patients for this specific study, only fourteen patients with COPD met the inclusion criteria and were recruited into the study. However, eight participants had to be excluded due to the following reasons: two cases with MRI contraindications that were not detected at the initial assessment, one case of desaturation, one case of investigation for lung cancer, one case underwent abdominal surgery, two cases of claustrophobia, and one case of unusable MRI data due to movements.

Also, approximately 40 aged, matched controls were screened to check eligibility, but 21 were excluded as they did not meet all initial inclusion criteria (diagnosis of CVD, cancer, recent surgery, on statins). Then, further 13 were excluded due to MRI contraindications (metal implants, claustrophobia, unusable scans due to movements).

Ultimately, six participants with COPD and six aged and gender matched controls completed the two parts of the study. As per matching study design, both groups were matched across demographic characteristics with no significant difference in age, gender and BMI (p>0.05). Participants with COPD had poorer lung function than controls (p<0.05) and had moderate airflow obstruction as signified by the results of FEV_1 % predicted (70 ± 5.6%) (Table 5.2). There was no significant difference between the two groups in term of vascular measurements, including blood pressure, aPWV, central PP and MCAPI. However, it should be noted that the difference in aPWV observed between the COPD and control group was deemed clinically different and representative of the difference reported between the groups in previous chapters. In addition, MRI data showed no differences in the WM, GM and CSF volumes between the two studied groups as assessed using the T1 –weighted MRI (Figure 5.1).

Table 5.2: Participants' characteristics and main results

	COPD (n=6) Mean ±SD	Controls (n=6) Mean ± SD	P=
Age (Years)	72 ± 5.2	70 ± 4.9	0.618
Gender (male/female)	3 F 3 M	3 F 3 M	1.00
Body Mass Index (Kg/m^2)	25 ± 2.9	25 ± 3.5	0.860
FEV$_1$ % predicted	70 ± 5.6	112 ± 18.9	**0.002**
FEV$_1$/FVC measured (L)	0.56 ± 0.07	0.75 ± 0.05	**<0.001**
Supine Systole Blood	140 ± 18.3	129 ± 19.1	0.326
Supine Diastole Blood	86 ± 10.1	79 ± 10.3	0.295
Supine Pulse Pressure (mmHg)	47 ± 9.3	45 ± 10.7	0.312
Supine MAP (mmHg)	104 ± 12.3	96 ± 13.2	0.737
Arterial Pulse Wave Velocity	10.1 ± 1.8	8.6 ± 1.2	0.123
Adjusted aPWV (m/s)	9.7 ± 1.5	9 ± 0.5	0.268
Middle Cerebral	0.8 ± 0.2	0.8 ± 0.1	0.943
Cerebral Spinal Fluid Volume (cm^3)	359 ± 50	352 ± 40	0.780
Grey Matter Volume (cm^3)	550 ± 58	585 ± 44	0.255
White Matter Volume (cm^3)	491 ± 78	509 ± 46	0.636
Data are mean ±SD. P≤0.05 statistically significant			

Figure 5.1: T1 -weighted GM, WM and CSF segmentation of the same brain shown at (a) Axial (b) Coronal (c) Sagittal angles achieved using FSL fast. White matter (blue), grey matter (pink), and CSF (green).

A
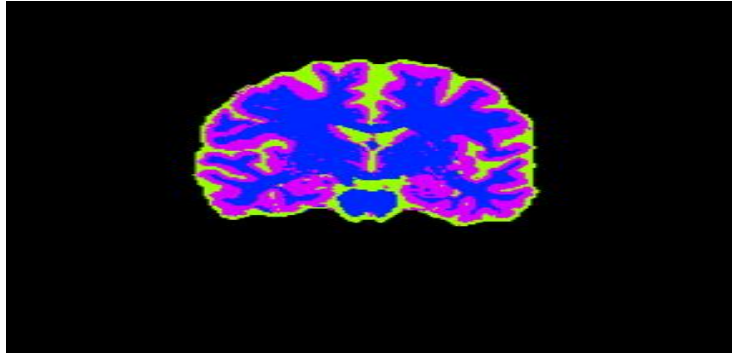
B
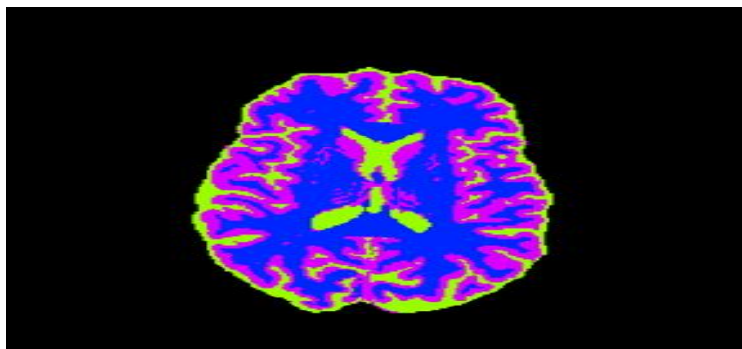
C
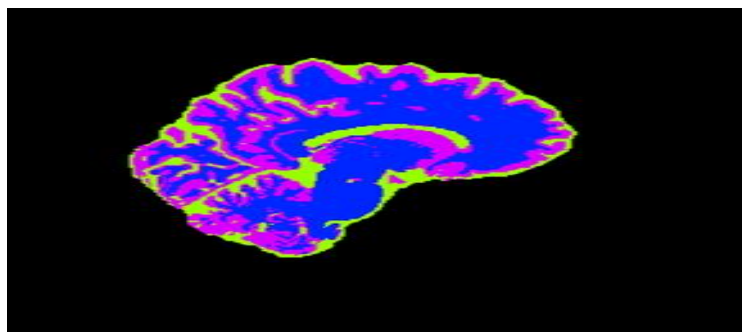
5.4.2 Relationship between vascular measures and cerebral volumes

This pilot study showed no relationship between the global volumes of WM, GM and CSF and vascular measures. As the sample size was very small, a graphical exhibition using scatter plot was utilised to explore any trends in this association.

5.4.2.1 Relationship between aPWV and cerebral volumes

The exploratory scatter plots showed no connection between aPWV and the cerebral volumes (GM, WM and CSF) (Figures 5.2-5.4).

Figure 5.2: Relationship between aPWV and White Matter Volume in patients with COPD and control group

Figure 5.3: Relationship between aPWV and Grey Matter Volume in patients with COPD and control group

Figure 5.4: Relationship between aPWV and Cerebrospinal Fluid Volume in patients with COPD and control group
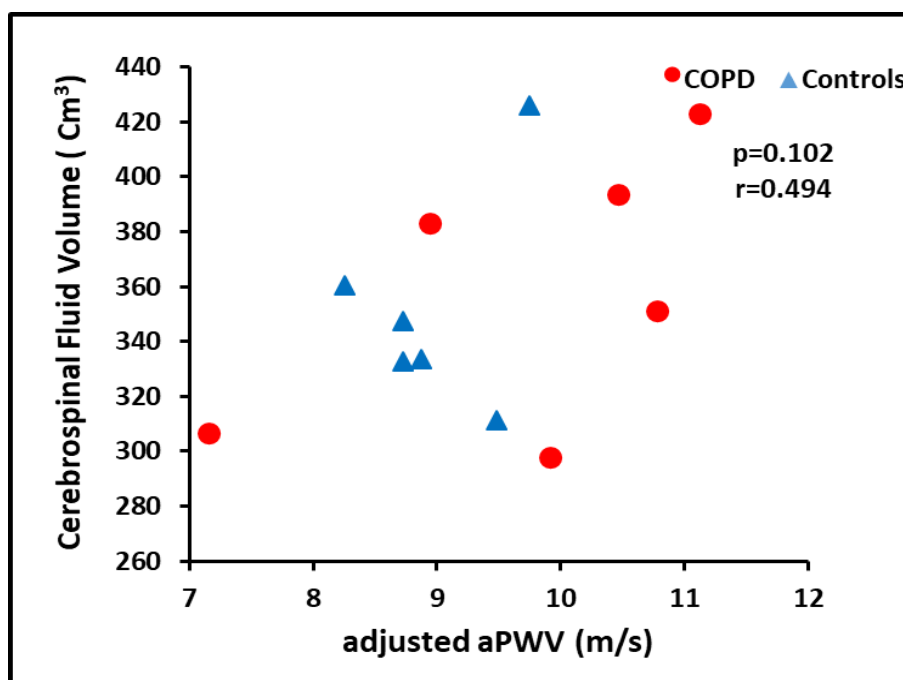
5.4.2.2 Relationship between CPP and cerebral volumes

The exploratory scatter plots showed no connection between CPP and the cerebral volumes (GM, WM and CSF) (Figures 5.5-5.7).

Figure 5.5: Relationship between CPP and White Matter Volume in patients with COPD and control

Figure 5.6: Relationship between CPP and Grey Matter Volume in patients with COPD and control group
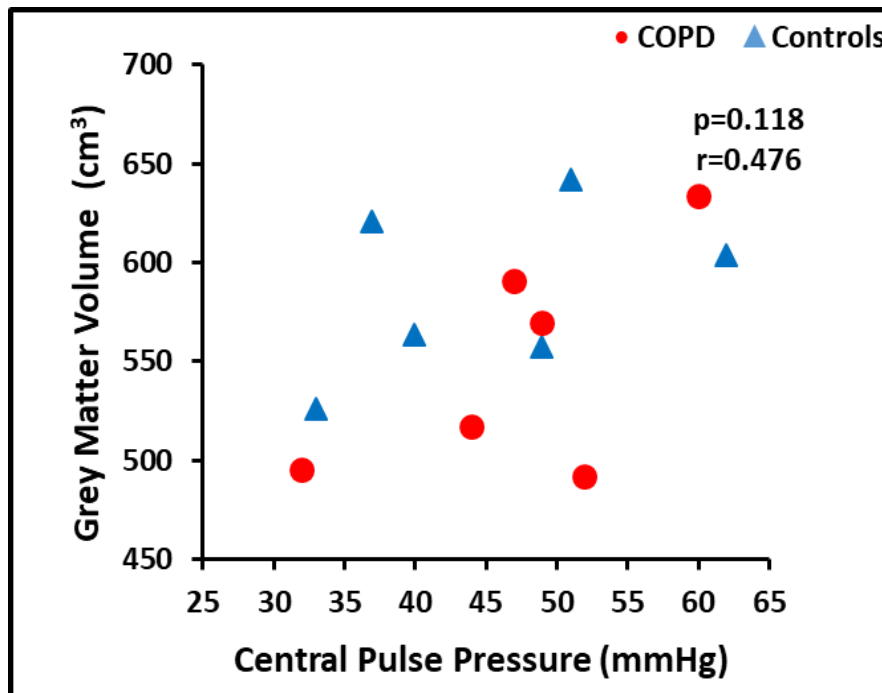
Figure 5.7: Relationship between CPP and Cerebral Spinal Fluid Volume in patients with COPD and control group
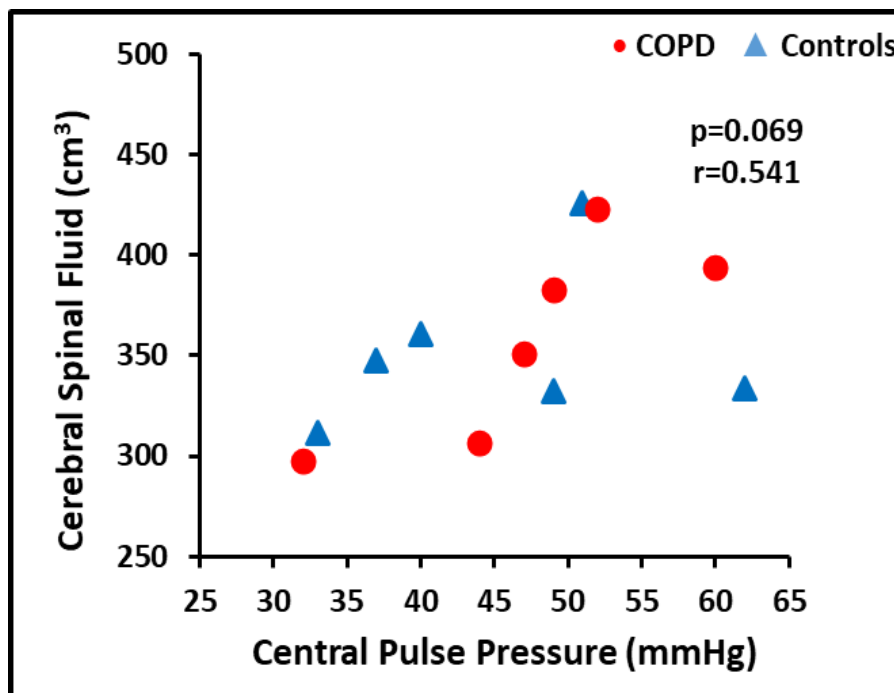
5.4.2.3 Relationship between MCAPI and cerebral volumes

There was no corelation between MCAPI and WM volume (Figure 5.8). However, there was a significant correlation between MCAPI and GM volume (r=0.620, p=0.04) (Figure 5.9) and between MCAPI and CSF volume (r=0.780, p=0.005) (Figure 5.10).

Figure 5.8: Relationship between MCAPI and White Matter Volume in patients with COPD and control group
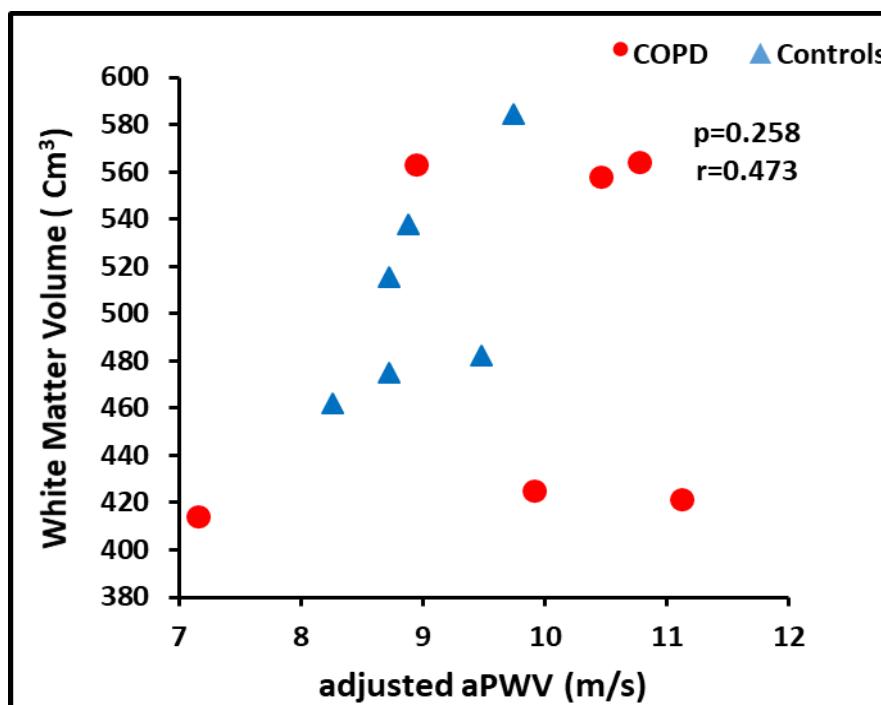
Figure 5.9: Relationship between MCAPI and Grey Matter Volume in patients with COPD and control group

Figure 5.10: Relationship between MCAPI and Cerebral Spinal Fluid Volume in patients with COPD and control group

5.4.3 Relationship between lung function and cerebral volumes

There was no relationship between severity of COPD as measured by FEV_1% of predicted or FVE_1/FVC and the cerebral volumes (Figures 5.11-5.15).

Figure 5.11: Relationship between FEV_1% of predicted and White Matter Volume in patients with COPD and control group
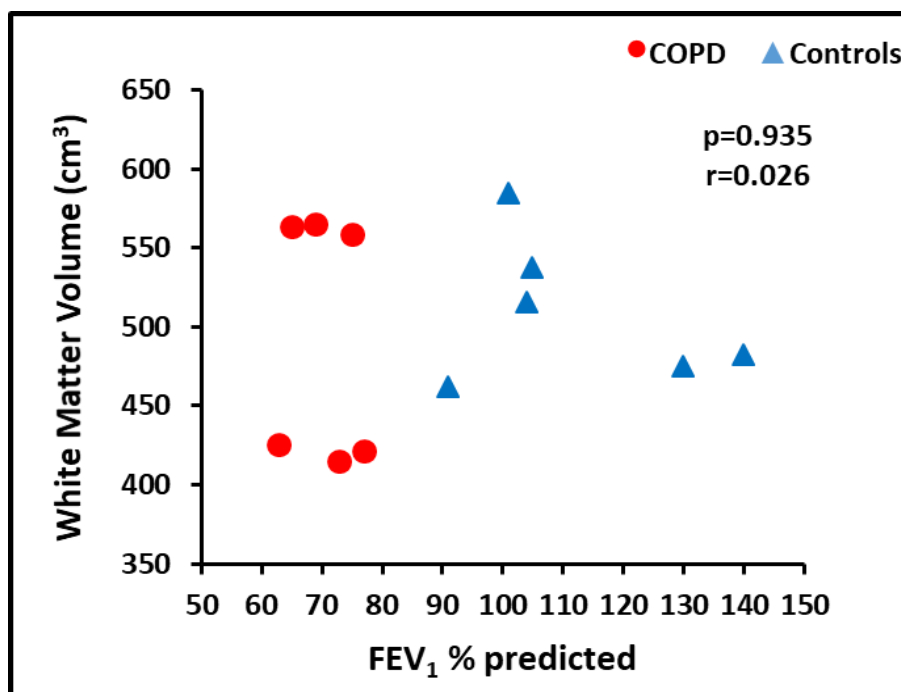
Figure 5.12: Relationship between FEV_1% of predicted and Grey Matter Volume in patients with COPD and control group
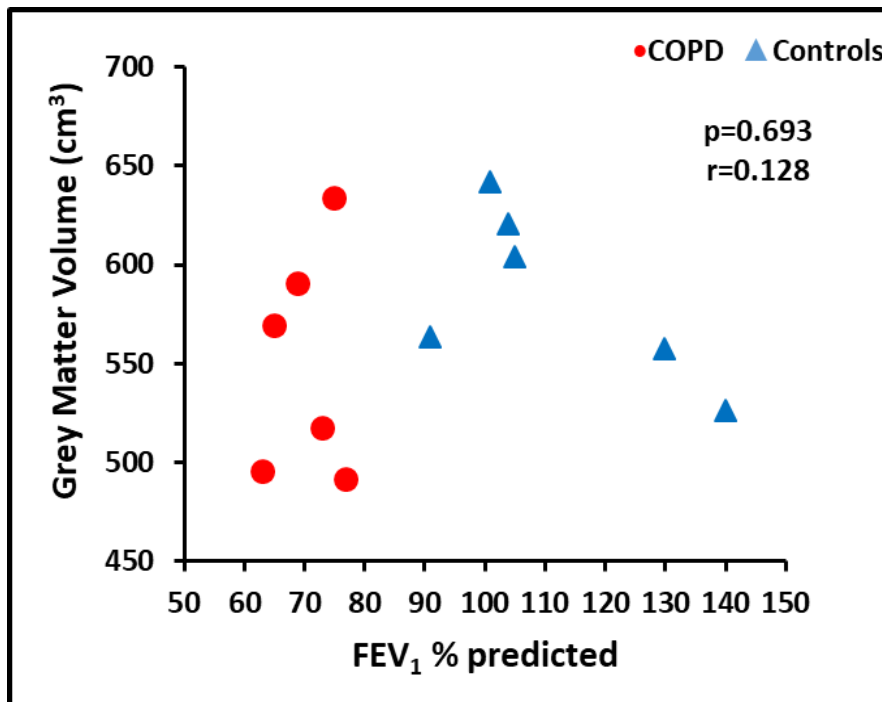
Figure 5.13: Relationship between FEV_1% of predicted and Cerebral Spinal Fluid Volume in patients with COPD and control group
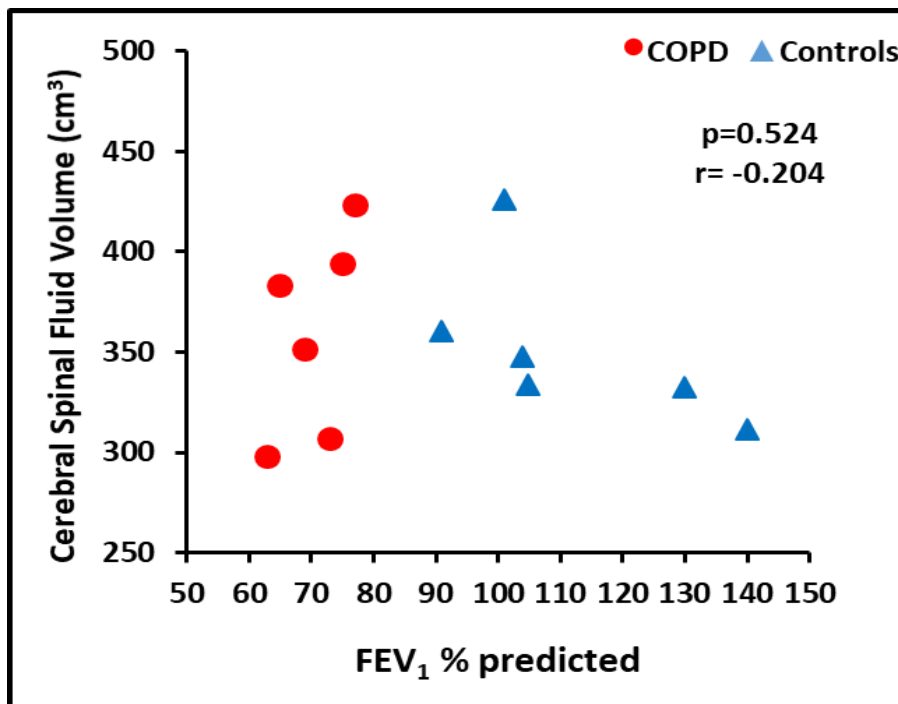
Figure 5.14: Relationship between FEV$_1$/FVC and White Matter Volume in patients with COPD and control group
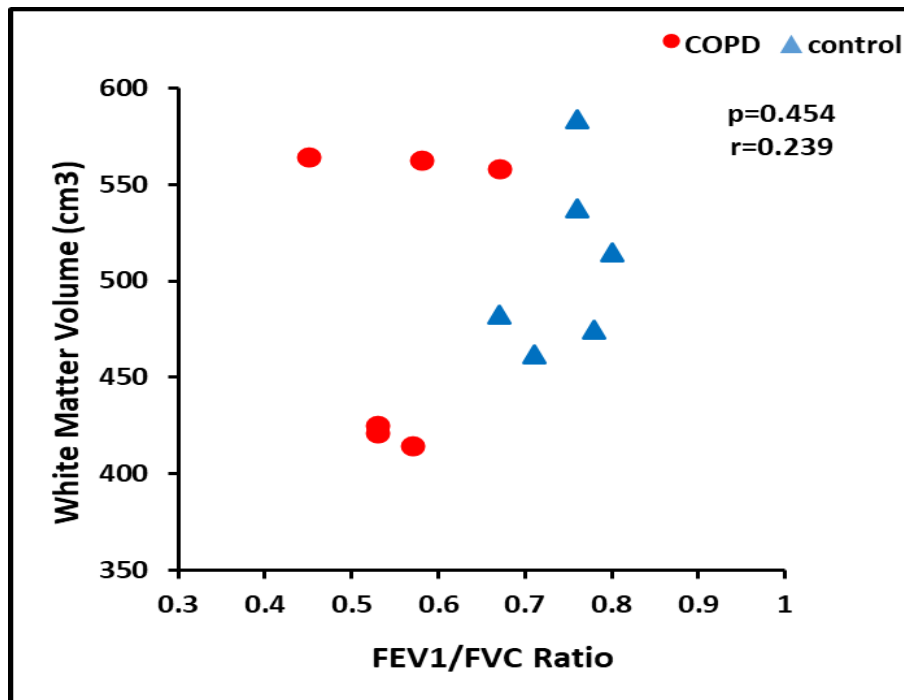
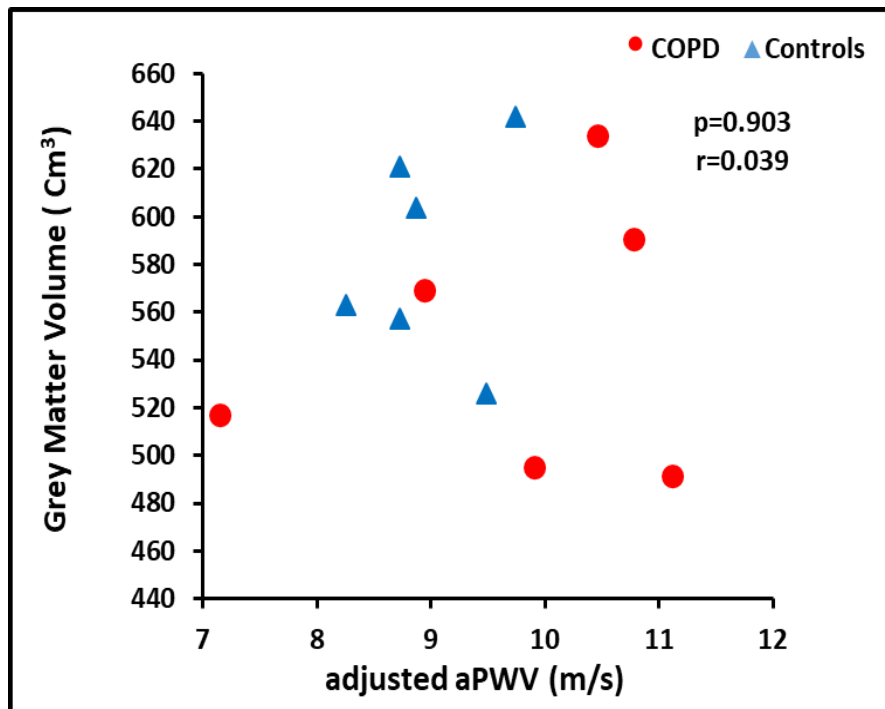Figure 5.15: Relationship between FEV$_1$/FVC and Grey Matter Volume in patients with COPD and control group
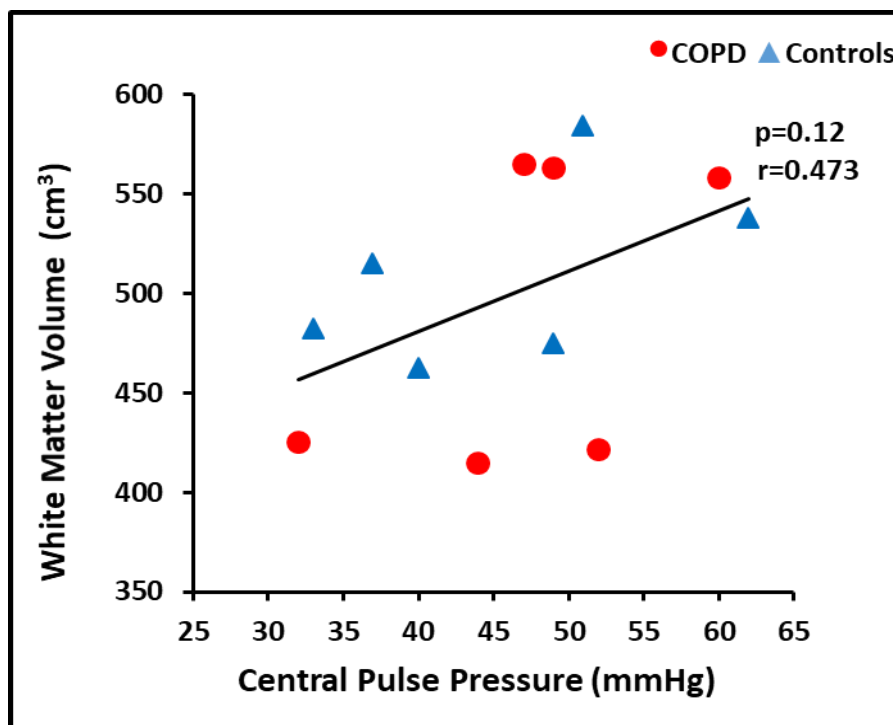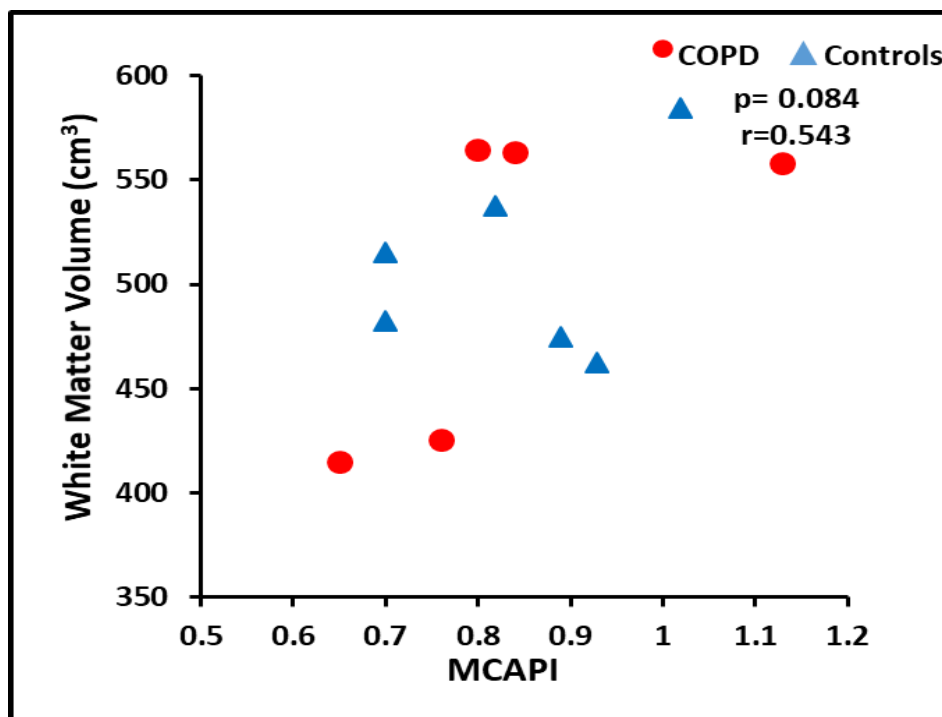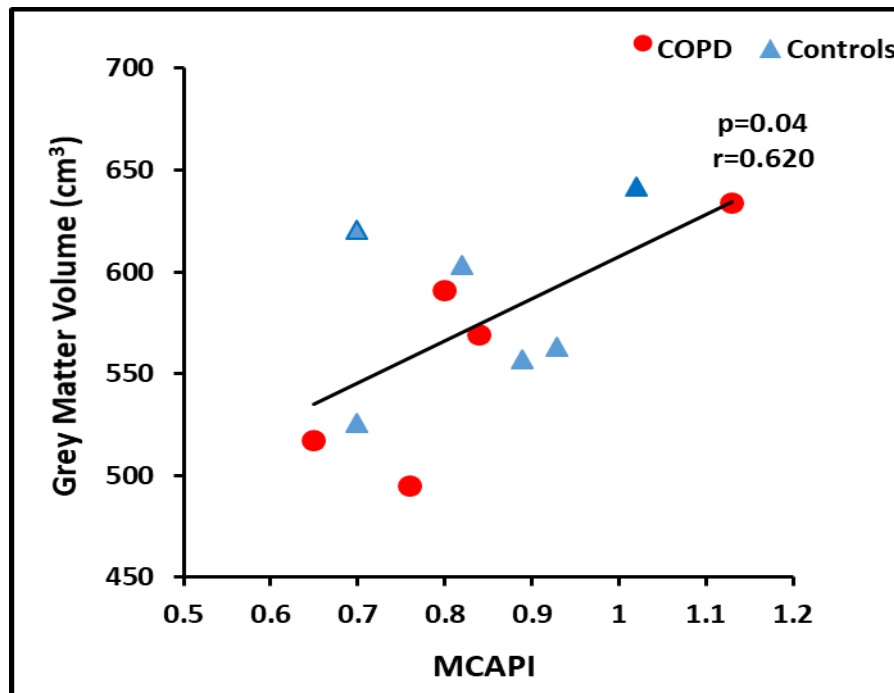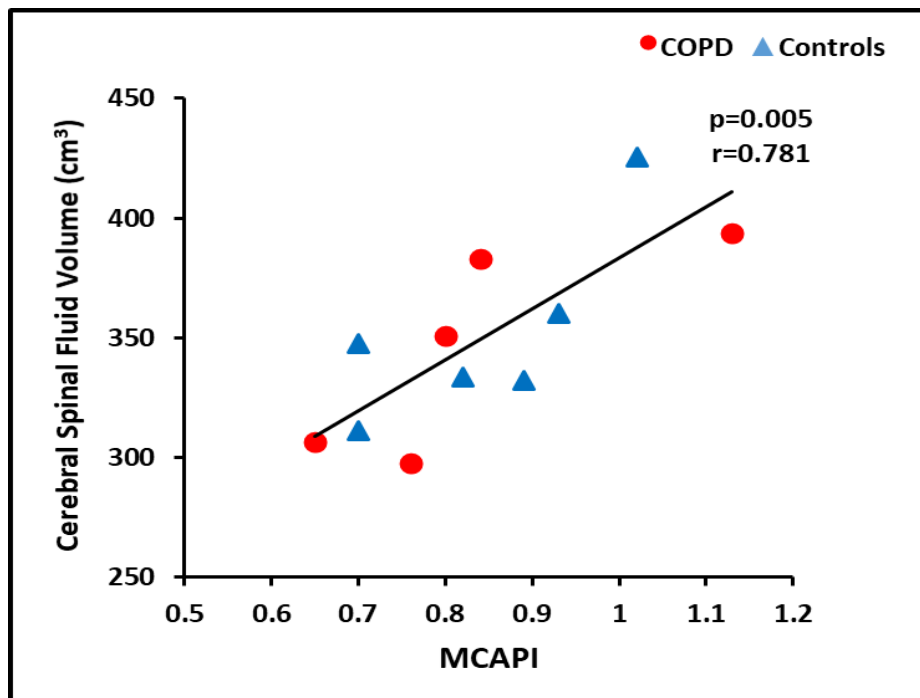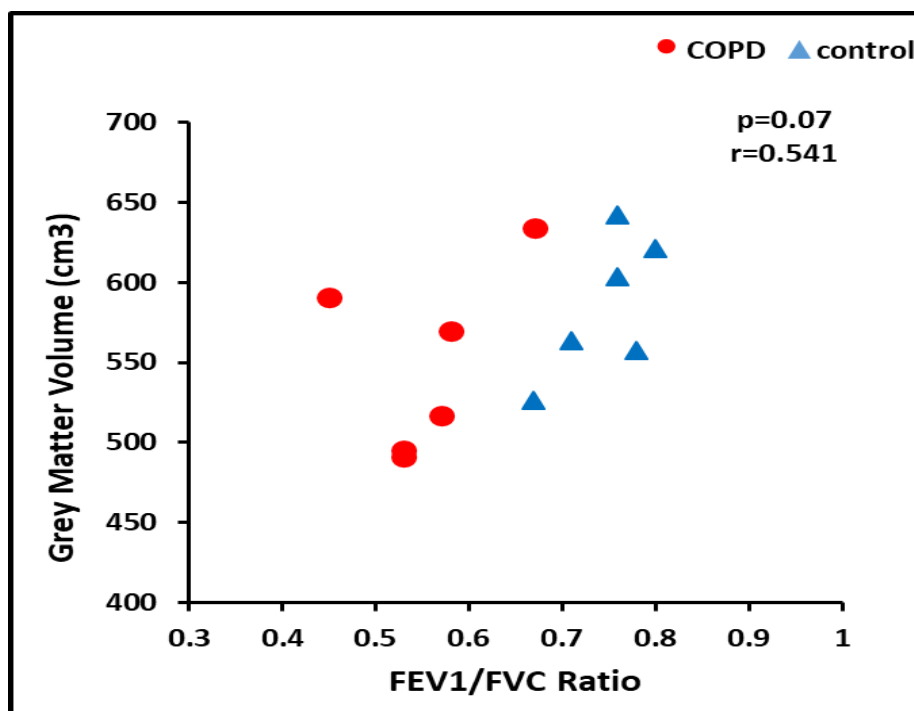
Figure 5.16: Relationship between FEV_1/FVC and Cerebral Spinal Fluid Volume in patients with COPD and control group
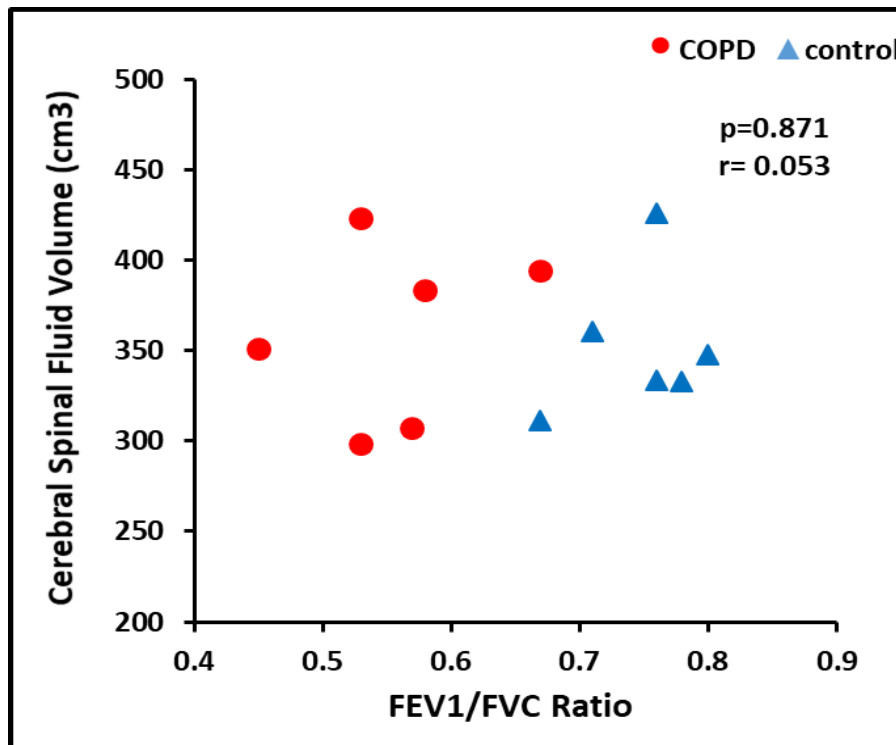
5.5 **Discussion**

This pilot study aimed to investigate differences in global cerebral volumes, including the WM, GM and CSF volumes between patients with COPD and age and gender-matched controls using MRI technique. In addition, the study aimed to explore the relationship between measures of central haemodynamics, including aPWV, CPP and MCAPI and cerebral volumes in patients with COPD and controls. Furthermore, the study aimed to investigate the relationship between lung function as measured by FEV_1 and FEV_1/FVC and cerebral volumes.

This was the first study to measure cerebral volumes using MRI in association with central and cerebral haemodynmics in a well characterised group of patients with COPD and controls. Data showed no differences in cerebral volume in COPD patients compared to controls and therefore, the null hypothesis stating no difference in cerebral volumes between the groups was accepted. Importantly, no associations were found between central haemodynamic parameters (aPWV and CPP) with cerebral volumes, thus the null hypothesis was accepted. However, MCAPI was significantly associated with GM and CSF volumes suggesting a connection between higher pulsatility at the small vessel level and changes in the cerebral volumes. The association between MCAPI and cerebral volume maybe because they are closely aligned in their measurement locations, which is an important finding suggesting the potential use of MCAPI as a practical tool to determine and monitor cerebral volumes in the future may be warranted. Finally, there was no association between cerebral volumes and measures of lung function (FEV_1 and FEV_1/FVC); hence, the null hypothesis describing no relationship between cerebral volumes and lung function was also accepted.

While a greater number of data points and sample size is needed to confirm these relationships, implementing the detailed study protocol supports the feasibility and tolerability of the MRI protocol in this group whom recruitment in these types of study is challenging.

5.5.1 Differences in cerebral volumes

The result of this pilot study showing no difference in the cerebral volumes in patients with COPD compared to controls is in line with previous research. An MRI study measured cerebral volumes in 31 patients with moderate COPD free from CVD (mean age 67 ±6 years; FEV1% of predicted 52±21) and 24 age, gender-matched controls. The result showed no significant differences in the global, regional, or local measures of WM and CSF volumes but significant reductions in normalised whole-brain GM volume between patients and controls (p=0.009). Although patients with COPD had an increased volume and size of WMLs that were linked to worse lung function (approached significance, p=0.06) (Spilling et al. 2017). Similarly, the measurement of WM and GM volumes using MRI in 30 in patients with COPD (mean age 66±9 years; mean FEV_1% of predicted 50±12) and 30 healthy controls (mean age 65±8) from the Vanderbilt Memory and Ageing Project reported no difference in the total volume of WM or GM between the groups. However, there was a grey matter reduction in brain areas relevant for the processing of dyspnoea and fear, which was related to longer COPD disease duration (Esser et al. 2016). In addition, Chen and colleagues conducted a detailed surface-based morphometrically study using MRI scans in 25 stable COPD patients (mean age 69±8; mean FEV1 % predicted 43±16) and 25 age-matched controls. The result showed no difference in the WM or GM volumes (Chen et al. 2016).

In contrast, the measurement of GM volume using 3D T1-weighted MRI in 25 stable patients with COPD (mean age 69.2 ± 8 years; FEV1% of predicted 43±16) and 25 controls (mean age 68.0 ± 8.0 years) matched for age, gender and smoking history showed GM volume reductions in several brain regions in COPD. This reduction was correlated with arterial blood PO_2 values, suggesting that low blood oxygenation may play a role in the reduction of GM volume in COPD (Zhang et al. 2013).

5.5.2 Relationship between vascular measures and cerebral volumes

One of the aims of the current study was to establish the relationship between aPWV, CPP and MCAPI and indices of cerebral volumes. No associations were observed between cerebral volumes and aPWV, CPP, possibly due to the small sample size. However, MCAPI was related to GM and CSF volumes. However, it should be noted that no inferences can be made at this point, but a larger sample may confirm or dispute these relationships in the future.

5.5.2.1 Relationship between Aortic Pulse Wave Velocity (aPWV) and cerebral volumes

Studies linking aPWV to structural changes in the brain in COPD are limited. Recent work showed no significant main effect of aPWV on brain structure assessed by MRI in a study including 27 stable COPD patients (age: 63 ±9 years, FEV_1: 58% predicted) (Spilling et al. 2019). The included COPD cohort in Spilling study was younger and had more airflow obstruction than participants in this pilot study. However, both cohorts were similar in terms of their CVD free history and had similar aPWV. The result suggests that factors other than arterial stiffness may influence the brain structural properties or that the study was not powered enough to detect this relationship.

There are several mechanisms proposed to explain the relationship between aPWV and changes in brain structure and function, including systemic inflammation, smoking, sedentary lifestyle, and hypoxia. However, investigating these mechanisms were beyond the scope of the current preliminary study but this warrants future investigations.

5.5.2.2 Relationship between Middle Cerebral Artery Pulsatility Index (MCAPI) and cerebral volumes

However, the exploratory scatterplots showed a significant relationship between MCAPI and both GM and CSF volumes. These findings support previous evidence that MCAPI predict

WMH in patients with stroke and TIA (Webb et al. 2012; Xiong et al. 2013; Birnefeld et al. 2020) .The connection between increased cerebral arterial pulsatility and alteration in brain structure and function may be explained by increased arterial stiffness that causes impedance matching leading to the transmission of high pulsatile flow into cerebral microcirculation (Mitchell et al. 2011; Webb et al. 2012). The increased pulsatility was suggested to cause hypertrophic remodelling and lumen narrowing in cerebral arterioles (O'Rourke and Hashimoto 2007; Mitchell 2008).

A possible explanation for the connection between MCAPI and cerebral volumes is that volumes influence MCAPI because they are closely aligned in the location of measurement. This is why we are seeing these associations and not with aPWV and CPP, as they are more peripheral measurements than MCAPI. Furthermore, PI may be responsible for cerebral volume differences through the interaction with blood-brain barrier permeability (Garcia-Polite et al. 2017).

5.5.2.3 Relationship between lung function and cerebral volumes

The current study showed no relationship between severity of COPD as measured by FEV_1% of predicted and the cerebral volumes. However, evidence from previous research including larger sample linked the reduction in lung function to changes in cerebral volumes. A study involved 99 elderly participants reported a significant positive correlation between FEV_1 and cerebellum regional WM volume after the adjustment for age, sex and cerebral volume (Taki et al. 2013). Likewise, cerebral diffusion indices that provide information about WM integrity were negatively correlated with FEV_1 in a study including 84 patients with COPD with various COPD severity compared to 31 individuals without COPD (Yin et al. 2019). In addition, lower FEV_1/FVC was related to lower normalised WM volume in COPD patients (p=0.047) but not in non-COPD smoker controls (p=0.461) (Spilling et al. 2019) suggesting a causal connection between COPD and WM volume changes beyond smoking, a known risk factor for COPD and CVD. The outcomes of these studies provide evidence of the negative impact of the airflow obstruction in the brain structure. Although the measurement of emphysema by CT scanning was found to be more closely related to impaired vascular function than the degree of airflow

obstruction (Barr et al. 2010), studies investigating the mechanistic link between lung function and brain structure in COPD are still lacking.

5.5.3 Mechanisms of the link between COPD and structural changes in the brain

Several mechanisms may explain the connection between structural changes in the brain and COPD and may include systemic inflammation, smoking, sedentary lifestyle and hypoxia, a common characteristic in COPD.

5.5.3.1 Smoking

Several lines of investigation have shown a connection between smoking and cerebral structural changes including WM volumes (Elbejjani et al. 2019), with FEV_1 playing a role in this relationship (Karama et al. 2015). In addition, smoking was also linked to increased WMH severity (Longstreth et al. 2005; Kim et al. 2012) and progression (Power et al. 2015).

One of key mechanism linking smoking and cerebral structural changes is inflammation. Smoking is associated with increased inflammatory markers, leading to vascular remodelling and calcification (Boutouyrie et al. 2008; Arnson et al. 2010). Moreover, smoking alters lipid metabolism, which contributes to the increased thickness of intima-media and changes in the arterial wall structure (Chambless et al. 1997; Campbell et al. 2008). Smoking has been linked to a loss of filtration rate in the kidney, leading to collagen accumulation and calcification of arteries (Safar et al. 2004).

5.5.3.2 Lifestyle

It is well established that COPD is associated with a reduction in physical function and physical activity as shown by systematic reviews (Bossenbroek et al. 2011; Thorpe et al. 2012; Gimeno-Santos et al. 2014) and is supported by data from this thesis (Chapter 3 and 4).

Evidence supports a connection between physical activity and cerebral microstructure integrity in a wide range of populations. For example, there was an association between physical activity level and the cerebral white matter microstructural integrity in patients with cerebral small vessels disease (Gons et al. 2013) and community-dwelling healthy individuals (Johnson et al. 2012). In addition, increased cardiorespiratory fitness was associated with an increase in WM volume (Colcombe et al. 2006; Sexton et al. 2016) and GM volume (Erickson et al. 2014; Hall et al. 2018; Wittfeld et al. 2020) and a reduction in WMH (Tseng et al. 2013). The association between higher cardiorespiratory fitness and better brain outcomes may relate to the role of improved cardiorespiratory fitness in lowering risks for cardiovascular disease (Gupta et al. 2011; Myers et al. 2015). It was shown that aerobic exercises increase the growth of new capillaries in the brain (Rhyu et al. 2010) and improve the connections between neurons, resulting from several growth factors making the brain more adaptive to changes (Cotman and Berchtold 2002).

5.5.3.3 Inflammation

It is widely accepted that COPD is characterised as chronic inflammatory diseases. Systemic inflammation release proinflammatory cytokines and activate T cells and monocytes, in the vascular wall leading to microvascular and macro-vascular dysfunction (Rocha and Libby 2009) which may provoke neuronal injury and result in several adverse clinical outcomes (Barnes 2010).

In SVD, numerous process such as lipohyalinosis, fibrinoid degeneration or atheroma formation can cause segmental arteriolar wall disorganisation, which can lead to increased risk of haemorrhagic stroke (Lahousse et al. 2015). On the other hand, there is also evidence showing a high prevalence of carotid artery plaque formation and rupture in COPD which may lead to ischemic stroke (Barr et al. 2012; Lahousse et al. 2013). A multivariate analysis of data involving stable non-hypoxemic COPD patients showed that stroke risk partially explained the observed WM changes and fully accounted for the GM changes (Dodd et al. 2012). However, well-controlled evidence from longitudinal studies is required to establish the exact mechanisms of increased risk of different stroke subtypes in COPD.

Hypoxia, a common feature in COPD, may partly cause the morphologic changes that happen in the brain of patients with COPD. Alterations in blood gases at pulmonary and circulatory levels may be damaging to the oxygen-dependent and sensitive brain structures (Erecińska and Silver 2001). It was shown in COPD that hypoxia leads to a reduction in cerebral metabolic and perfusion rate (Shim et al. 2001; Ortapamuk and Naldoken 2006) that may promote changes in brain structure. Evidence showing a relationship between arterial oxygen level measured by PaO_2 or SaO2 and GM volume loss (Li and Fei 2013; Zhang et al. 2013; Chen et al. 2016) and periventricular WM lesions (van Dijk et al. 2004). These findings were also found in patients with obstructive sleep apnoea (Macey et al. 2002; Torelli et al. 2011) and in high altitude residents (Zhang et al. 2010) supporting the role of oxygenation deprivation in altering cerebral volumes. In contrast, other studies reported no significant correlation between GM density and WM changes and PaO_2 or SaO_2 (Yin et al. 2019) and between SaO_2 and subcortical white matter lesions or lacunar infarcts (van Dijk et al. 2004). Also, in a small study including 18 patients with COPD (mean age 68.5 years) of whom nine were on long-term continuous oxygen and nine age and gender-matched healthy controls (mean age 68.2 years), there was no difference between indices of brain atrophy or WM hyper-intensities between the two groups. Although the oxygen-dependent patients had an 11% smaller mean hippocampal volume, but the difference was not statistically significant (Borson et al. 2008).

However, previous studies characterised COPD severity based on oxygenation level, which is prone to acute fluctuations and may not be representative of the degree of airflow obstruction. The established guideline in the diagnosis and categorisation of COPD severity recommends the use of FEV_1 and FEV_1/FVC (GOLD 2018) which was used to categorise COPD in other studies (Taki et al. 2013; Spilling et al. 2019; Yin et al. 2019).

5.5.4 Study strengths and limitations

The novelty of the study was in examining cerebral volumes and relating them to central arterial stiffness in a group of COPD cohort who had a detailed vascular, cerebral, pulmonary and measures of physical function acquired previously. It also benefited from including a

well-matched control group across demographic characteristics. It supported that implementing the study protocol using MRI is feasible, but greater numbers are needed to evaluate the group differences and establish any potential mechanisms linking central vascular changes to brain volumes associated with deteriorating cerebrovascular health.

However, the current study has several limitations that should be acknowledged. The very small sample size does not allow for further analysis or stringent adjustments. It is worth noting that most studies investigating brain integrity in COPD were limited by the modest sample size, which may represent recruitment challenges in this complex group.

Also, the cross-sectional design does not allow to establish the causal relationship between arterial stiffness and cerebral volumes. Hence, a longitudinal study with better statistical power is needed to address this limitation. It is also possible that various COPD severity may exhibit different structural changes in the brain hence the need to include individuals with a broader range of disease severity. Nonetheless, the study is exploratory, and it's protocol and preliminary data can be used to inform the methods and the feasibility of conducting a larger-scale study in the future.

Additionally, we excluded individuals with a history of CVD or those who use CVD medications, which may have reduced the generalisability of the results to the broader COPD population who have a high prevalence of CVD. However, the strength of our approach resides on the inclusion of participants without a history of CVD or using any cardiovascular acting medications, after data from the previous chapter highlighted that cardiovascular acting medications may affect the cerebrovascular interactions. Nevertheless, data from individuals without CVD can form a baseline measurement. However, an increased number of comorbidities may present a challenge in completing the study protocol as participants are required to lie supine for a quiet long time that may not be well tolerated by participants with several comorbidities or severe COPD. Hence, careful consideration and modification of the protocol may be required.

Patients with COPD in the current pilot study had stable, moderate COPD and were prescribed some forms of respiratory inhalers to manage their COPD. Although they were requested not to use these inhalers whenever possible in the morning of the two visits, it is unknown if the long-term effect of these medications had an impact on causing changes in the brain structure. This warrants an investigation and can be explored with enrolling participants with more severe COPD.

Finally, the presented pilot data was based on analysis derived from conventional MRI technique that dichotomises brain tissue into normal or abnormal tissue, although other measurements with more sensitive MRI metrics may inform the relationship between central arterial stiffness and brain structure and function in addition to volumes. In parallel to another ageing study, the investigation into the COPD cohort is important and only through further work involving larger sample size, can mechanistic links be explained.

5.5.5 Conclusion

The exploratory data found no difference in cerebral volumes including the WM, GM and CSF volumes between patients with COPD and aged matched controls. In addition, no association was established between cerebral volumes and aPWV in the studied population but interestingly, MCAPI was related to cerebral volumes. This suggest a potential use of MCAPI as monitoring measurement early in the disease progression, to address the causal links of the pulsatile flow being sent to the brain of these patients and changes in cerebral volumes.

Chapter 6

General Discussion and Conclusion

6 General discussion and conclusion

The results of this thesis adds novel information to the field of cardiovascular risk in patients with COPD by assessing aPWV longitudinally and establishing the connection between central and cerebral haemodynamics to provide a potential mechanism for the increased risk of stroke in COPD

This was achieved by investigating the central and cerebral haemodynamic interactions in a group of COPD cohorts with detailed vascular, cerebral, pulmonary, and functional measurements and a control group well matched across demographic characteristics. The thesis investigated these relationships using a comprehensive approach in which several studies employing different methodologies were performed. These studies are summarised below and included:

1 A longitudinal study assessing the magnitude of change in central arterial stiffness change over 6 years.

2 A cross-sectional study investigating the relationship between central arterial stiffness and cerebral pulsatility.

3 A pilot cross-sectional study investigating cerebral volumes using MRI and its relationship to arterial stiffness.

The following section highlights the key outcomes of these studies and their relevance. An outline of the study can be found in Figure 6.1.

6.1 Longitudinal study; longitudinal changes in aPWV and measures of health status in patients with COPD and controls

The result of this thesis adds novel information to the field of cardiovascular risk in patients with COPD by assessing aPWV longitudinally. A key result of this study is the higher aPWV in the COPD group compared to the controls that confirms the study hypothesis of increased arterial stiffness in patients with COPD. Importantly, both groups showed an increase in aPWV in a parallel fashion that can be explained by the progression in age. However, the higher baseline of aPWV in patients with COPD suggests this group encounter premature vascular ageing and this increase that was observed at baseline maintained over the 6 years follow up, even in the presence of additional cardiovascular acting medications. The increase in aPWV in the COPD group was above the cut-off point linked to subclinical organ damage and increased risk of cardiovascular events as proposed by established guidelines that may explain the overt history of CVD in the studied group. Also, patients with COPD had higher resting HR compared to controls, which also related to aPWV. Mounting evidence supports the presence of a linear relationship between HR and higher risk of CVD and mortality in the general population (Greenland et al. 1999; Seccareccia et al. 2001; Zhang et al. 2016). This highlights the benefit of HR as a therapeutic target in the COPD management and its value as a measure of risk that is not well utilised in risk stratification in this group.

In addition, patients with COPD showed a decline in physical function over the study period that may not be explained by age alone. It was also found that general measures of physical function including the CGA, 6MWT and TUG test were better related to aPWV than COPD related characteristics such as lung function or COPD specific questionnaires. These findings indicate the potential role of arterial stiffness in reduced physical function or a reverse association, but causality cannot be confirmed based on the current data. However, the physical function levels may be an important determinant of vascular damage that may link

to increased CVD risk. Nonetheless, the nature of the connections between arterial stiffness and physical function in patients with COPD merits attention.

6.2 Cross-sectional Study; investigation of the relationship between MCAPI and central haemodynamics

The study hypothesised that there will be a difference in MCAPI between patients with COPD and controls and that central arterial stiffness relates to and may predict cerebral haemodynamics in COPD. Hence, this cross-sectional study measured MCAPI, aPWV and CPP in patients with COPD and aged-matched controls.

The findings highlight that there was no difference in the MCAPI between patients with COPD and control group, despite patients with COPD having an increased aPWV. Studies reporting data of MCAPI in COPD are scarce hence the present data describe novel knowledge by presenting values of MCAPI in patients with COPD.

In addition, there was a relationship between aPWV and MCAPI in the control group in support of the original study hypothesis. However, such a relationship did not exist in the COPD group. Importantly, CPP, as measure of central arterial stiffness, was independently associated with MCAPI, even when accounting for cardiovascular acting medications. Nevertheless, the interaction between cardiovascular acting medications and the relationship between aPWV and MCAPI remains unclear and needs further investigations in a larger sample. These data highlight the use of CPP instead of aPWV as a tool to better identify and monitor any cerebral changes in flow pulsatlity in COPD patients.

6.3 Cross-sectional study; relationship between aPWV and cerebral volumes

The study presented a pilot data of cerebral volumes, including the WM, GM and CSF volumes measured using MRI in six patients with COPD and six age and gender-matched controls. The study also explored the relationship between vascular measures including aPWV, CPP and MCAPI and cerebral volumes. The preliminary data showed no difference in the GM, WM and CSF volumes between patients with COPD and controls. In addition, no association was established between cerebral volumes and either aPWV or CPP in the studied population.

Importantly, MCAPI was significantly associated with GM and CSF volumes suggesting a connection between higher pulsatility and changes in the cerebral volumes. However these pilot data did not show any relationship between measures of lung function and cerebral volumes.

It maybe that the small sample size may have resulted in a type II error; hence, this result is inconclusive. Studies linking aPWV to structural changes in the brain in COPD are limited by the modest sample size and the cross-sectional design. In addition, the MRI protocol may lead to the exclusion of individuals with severe COPD, or overt comorbidities hence may reduce the studied group's representativeness to the broader COPD population. Several mechanisms may explain the brain's structural changes and its relationship with aPWV, including systemic inflammation, smoking, sedentary lifestyle, and hypoxia, which were outside the scope of this current study but warrant future investigation.

6.4 Application to Theoretical Framework

It is well known that patients with COPD present a complex clinical population often with multiple comorbidities. As discussed in the theoretical framework the concept of comorbidity described by Feinstein (1970) acknowledges that the existence of two diseases with distinct aetiology and pathophysiology in an individual may occur by chance or has elements of co-occurrence that acknowledges the possibility of underlying causality between the two diseases (Krueger and Markon 2006). The multi-morbidity conceptual framework adopts the notion of a patient-centred approach where a specific disease and the co-occurrence of other diseases are considered along with patients' physical, social, and psychological status (Boyd et al. 2016). Hence, this thesis attempted to establish the connection between CVD and COPD, considering the impact of comorbidities to potentially improve outcomes using a patient centred approach. This was achieved through exploring this relationship in the context of disease outcomes, considering potential factors that may mediate such a relationship.

Several comorbidity models propose an explanation of the nature of the connections between two or more diseases present in an individual. However, the heterogeneity model may be the best to illustrate the nature of the relationship between COPD and CVD examined by this thesis. In the heterogeneity model, the risk factors for each disease may not be related, but each one of them can cause either disease (Valderas et al. 2009).

The results obtained from the three studies conducted in this thesis support this theoretical framework where shared risk factors such as age, physical function, and smoking may have elements of co-occurrence between the two diseases (Maclay and MacNee 2013). In addition, other shared risk factors were not assessed by this study, including inflammation, hypoxia and the interactions of COPD medications.

Age

Although older age is a shared risk factor between COPD and CVD, the results of the longitudinal study, which aimed to establish the time course of vascular changes in arteries, showed that both groups had an increase in aPWV in a parallel manner that can be explained by the progression in age. However, the higher baseline of aPWV in patients with COPD confirms the study hypothesis of increased arterial stiffness in patients with COPD and suggests this group encounter premature vascular ageing that may increase CVD risk. The accelerated biological ageing may instigate CVD development in patients with COPD which were associated with structural and functional changes of the cardiovascular system (Sharma et al. 2009). The elevated aPWV observed at baseline which was maintained over the six-year follow-up, even in the presence of additional cardiovascular acting medications, suggests that patients with COPD encounter high and premature CVD risk. The increase in aPWV in the COPD group was above the cut-off point linked to subclinical organ damage and increased risk of cardiovascular events as proposed by established guidelines that may explain the present of overt CVD in the studied group.

Physical function

It was also found that general measures of physical function which may be related to activity, including the CGA, 6MWT and TUG test, were significantly impaired in patients with COPD compared to the age-matched controls. Also, these measures were better related to aPWV and MCAPI than COPD related characteristics such as lung function or COPD specific questionnaires. These findings indicate the potential role of reduced physical function in increasing cardiovascular risk, but causality cannot be confirmed based on the current data. However, the physical function may be an important determinant of vascular damage that may link to increased CVD risk.

There may be a vicious cycle linking aPWV to lower physical function in COPD where functional limitations may be exaggerated because of progressive airflow obstruction. As a result, patients with COPD may avoid activity that provokes dyspnoea and, over time,

become physically inactive and deconditioned, consequently increasing the risks of CVD. Nevertheless, further investigation is required to fully understand the mechanisms linking arterial stiffness to functional limitations in this group.

Smoking

In the current study, patients with COPD and controls were either smokers or past smokers, although outcomes representing vascular health were negatively affected in the COPD group suggesting that factors other than smoking may be key in the process of increased CVD risk. A systematic review of evidence including 39 studies linked acute, chronic and passive smoking to increased arterial stiffness. However, the role of chronic smoking in increasing arterial stiffness is somewhat more controversial due to the high heterogeneity of the populations or methodologies of research examining this connection (Doonan et al. 2010).

6.5 Clinical implications and future research

The findings of this thesis add novel information for the understanding of the role of measures of arterial stiffness and cerebral haemodynamics in risk predictions and identification of subclinical vascular damage in patients with COPD. The measurement of arterial stiffness by aPWV may be used to identify subclinical changes and add a diagnostic and prognostic value to the CVD risk prediction in patients with COPD. Interestingly, the vital role of CPP in predicting cerebral dysfunction through its link with higher MCAPI was a key finding in this thesis. Therefore, measuring CPP in the clinic may provide an important predictive value for assessing cerebral dysfunction beyond traditional risk assessment. In addition, the study highlights the benefit of HR as a therapeutic target in COPD management and its value as a measure of risk that is not well utilised in risk stratification in this group. In addition, the assessment of cerebral volumes can be of important clinical value by identifying early changes in the brain in COPD and predicting outcomes related to stroke.

Interestingly, MCAPI was shown to be associated with cerebral volumes, potentially allowing for the use of MCAPI using TCD assessments to monitor cerebral volume changes in COPD patients in the future.

Furthermore, the observed link between levels of physical function with both aPWV and MCAPI highlights the importance of maintaining and improving physical function through pulmonary rehabilitation and strategies that address psychological, social, and physical constraints that contribute to the reduction in physical function.

However, several questions remain unanswered and can be addressed in future research, considering some of the limitations in this thesis. For example, future studies should include sufficiently large cohorts to elucidate the longitudinal contribution of arterial stiffness to cerebral pulsatility. The longitudinal assessment of aPWV and CPP and its relationship with MCAPI may help determine causality and the direction of causality that cross-sectional studies lack. In addition, understanding the influence of specific vascular acting medications on central and cerebral structure and function in longitudinal trials is essential moving forward in this field. The mechanistic link between arterial stiffness and cerebral structure and function can also be addressed using sensitive MRI metrics to better inform this

relationship. Furthermore, the current thesis measured aortic stiffness and related it to MCAPI, and future research assessing vascular structural and functional changes in the carotid artery may provide a more focused link than the aorta and the cerebral arteries. Finally, measuring morbidity and mortality outcomes in relation to the longitudinal changes in arterial stiffness and cerebral haemodynamic in patients with COPD may provide an understanding of the effect of vascular changes in morbidity and mortality outcomes.

6.6 Conclusion

This is the first study investigating vascular changes over six years in well characterised community-dwelling participants with COPD and aged-matched controls. The thesis provided additional novel and important knowledge to the field of cardiovascular risk in patients with COPD by examining how large artery stiffness and vascular haemodynamic affect measures of cerebral hemodynamic associated with cerebral health in patients with COPD and aged-matched controls. The previous evidence supports that ageing is vital in the role of increased risk of CVD, and as patients with COPD explicit the ageing process at a faster rate than non-COPD, they may be at higher risk for CVD. Arterial stiffness can possibly create the missing link between COPD and overt CVD in this group. In addition, the lack of association between aPWV and MCAPI may have been obscured by the greater use of cardiovascular medications by patients with COPD. However, the association between MCAPI and CPP suggests that cerebral pulsatility may be influenced by a widening pulse pressure rather than higher aPWV, at least in the studied population.

Furthermore, implementing the study protocol using MRI was feasible, but a larger number is needed to evaluate the group differences further and establish the relationships of interest. The present study reinforces the need for early detection of subtle but critically important measures of vascular health linked to CVD development in COPD patients. Understanding the systems physiological progression towards disease presentation may better inform clinicians of when to intervene, how to treat appropriately and reduce the burden of the disease in this patient group.

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Appendices
Appendix 1

ethics /amend-approved

Staff Research

Dear Applicant

Re: Application for Amendment to Existing Ethical Approval

Project Title: ARCADE Trial

Project Ref. Number: 8121

Amendment Number: 1

Your application to amend your existing ethics approval, as shown above, was considered by the Biomedical Sciences Ethics Panel on 13-12-17.

I am pleased to inform you that the requested **Amendment was APPROVED**, subject to the conditions listed below – *please read carefully*.

Standard Conditions of Approval

1. Please continue to quote the original Project Reference number on all documentation relating to work undertaken on the project (e.g. consent forms).

2. A revised **Risk Assessment** must be undertaken for this proposal if the amendment involves a change in the protocol, and be made available to the Committee if requested.

3. Any further changes in connection to the proposal or amendments as approved, must be referred to the Panel/Committee for consideration *without delay quoting your Project Reference Number*. Changes to the proposed project may have ethical implications that require further consideration.

4. Any untoward incident which occurs in connection with this proposal must be reported back to the Panel *without delay*.

5. If your project involves the use of **human samples**, your approval is given on the condition that you or your supervisor **notify the HTA Designated Individual** of your intention to work with such material by **completing** the form entitled *"Notification of Intention to Work with Human Samples"*. The form must be submitted to the PD (Sean Duggan), **BEFORE** any activity on this project is undertaken.

This amendment of approval expires on 13-12-18. Please set a reminder on your Outlook calendar or equivalent if you need to continue beyond this extended date.
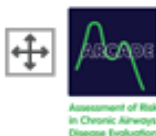
Yours sincerely

PLEASE RETAIN THIS LETTER FOR REFERENCE

Appendix 2

Dr Barry McDonnell
Senior Lecturer

/

*Cardiff School of Health Sciences,
University of Wales Institute,
Cardiff,
United Kingdom
CF5 2YB*

**Cardiff
Metropolitan
University**

PARTICIPANT INFORMATION SHEET FOR PATIENTS

"Assessment of Risk in Chronic Airways Disease Evaluation - ARCADE"

You are invited to take part in a research study. Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve. One of our research team members will go through this sheet with you and answer any questions you have. Please take time to read the following information carefully and discuss it with relatives, friends and your GP if you wish. We feel that this would take about 15 minutes to go through. Please ask us if there is anything that is not clear or if you would like more information. This study is funded for the first three years by a research grant from GlaxoSmithKline.

Why are we doing this study?
As we get older, our arteries generally stiffen over a period of time. It has been shown that individuals with chronic obstructive pulmonary disease (COPD) develop premature arterial stiffening. This leads to an increase in blood pressure and places them at greater risk of strokes and heart attacks. We are not sure how quickly their arteries stiffen, and whether this is related to the severity of COPD. Also, the reasons for arterial stiffening in COPD are not well understood. We now have the means to assess arterial stiffness and the aim of this study is to measure how quickly arteries stiffen over time in COPD, and what factors are associated with this.

Why have I been asked to participate?
You have been asked to participate because you fall into the following groups:
- Between 35-80 years
- Have a diagnosis of Chronic obstructive pulmonary disease (COPD)

Individuals who fall in the above categories have been similarly approached by a letter and we are looking to recruit 1500 people in this study.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. However, you are free to withdraw from the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?
Visit 0:
If you agree to take part, you will be asked to attend an initial visit lasting approximately 1 hour at a clinic at the University Hospital of Wales, Cardiff or your General Practitioner's surgery if it is one

of the participating research sites. Here we will go through the information sheet, consent form and obtain a detailed lifestyle and medical history from you in addition to a medical examination.

You will then be requested to make 3 visits over the next 5 years. All measurements made at these visits will be the same and each visit will last approximately 2 hours (please see figure 1 on page 5). Visit 1 will be baseline, visit 2 will be 2 years and visit 3 will be 5 years following your baseline visit.

Visit 1:
It would be preferable if you could refrain from smoking or using your inhalers for at least 6 hours prior to attending however you can use the inhalers if you feel necessary. The following measurements will be made:

- We will check your height, weight, and hip and waist circumference (using a tape).
- Blood pressure: After lying down for 15 minutes, you will have your blood pressure taken, just like at your General Practitioner's surgery.
- Arterial stiffness measurements: A small, pencil-like probe will be placed on the artery (blood vessel) at your wrist, neck and upper leg to calculate how stiff your arteries are. We will also record an electrocardiogram (ECG) with stickers on your chest to track small changes in your heart rate.
- Cardiac output: We will place some stickers on your chest and neck and measure how much blood is being pumped around with each heart beat.
- Lung function tests: You will then be asked to blow out as hard as you can into a tube after inhaling salbutamol (a safe drug using an inhaler with a spacer device) unless contraindicated to measure your lung function (the size of your lungs and how quickly you can empty them).
- Measurement of body composition: At this point we will check the proportion of muscle and fat tissue in your body by asking you to stand barefoot on a machine with hand grips. A tiny electric current which is not painful or harmful in any way is then passed and the measurements recorded. None of these measurements are painful.
- Hand-grip strength: This will be measured using a spring device.
- Bone density scan: We will then measure the bone density in your heels by using an ultrasound device which has no radiation.
- Blood sample: We will then ask you to give a blood sample of approximately 50 mls (5 tablespoons of blood) from your arm. This sample is used to measure various levels of naturally occurring substances in the blood, (eg cholesterol, glucose and inflammatory molecules). This blood sample will be disposed after the study is completed in 5 years.

  With your permission some blood will also be taken and stored for future analyses, which will examine the genes which are known or likely to be important in determining how stiff or elastic your arteries are. This sample taken for genetic analysis will be stored for 10 years in the custody of Dr Barry McDonnell at Cardiff Metropolitan University, Cardiff.

  Further ethical approval will be sought if any genetic testing is required beyond 5 years. As these genetic markers are mainly to establish a genetic link for the natural evolution of COPD it would not have any implications on inherited risk or insurance status.
  GlaxoSmithKline, the sponsors of the first three years of the study will have access to the genetic data, but you will not be identified individually to the sponsors or anyone else in respect of this data.

Will my GP be informed?

The doctor in charge of the study will inform your GP of your participation in the study with your consent. In addition with your permission, they will be informed of any clinically relevant results that may become available during the study.

What will happen to the study results?

The results of the study may be published in scientific journals or presented at medical conferences, a few months after completing the study. You will not be identified in any report or publication. An overall summary report (fully anonymised) will be sent to your General practitioner at the end of the study. You will be able to access this through your GP upon the completion of the study.

What happens if something goes wrong?

The doctors involved in the study, and the Cardiff Metropolitan University have suitable indemnity insurance if you are harmed due to someone's negligence. However, there are no special compensation arrangements for non-negligent harm. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

What will happen if I withdraw from the study?

If you withdraw from the study, this will in no way affect your future medical care. Data or tissue already collected up to the point of withdrawal will be anonymised and utilised for study purposes. The same would apply if capacity to consent is lost during the study. You will be withdrawn from the study and data or tissue already collected under consent will be anonymised and utilised for study purposes.

Will I be reimbursed for any travel expenses?

Travel expenses of up to £30 will be given as necessary for trips to hospital to cover bus, mileage and parking. Taxis can also be ordered from our department to pick you up or drop you off.

Who is organising this study?

This study is being organised by the Cardiff Metropolitan University. The investigator in charge is Dr Barry McDonnell.

Who has reviewed this study?

The South East Wales Research Ethics Committee has reviewed this study and given it a favourable opinion.

Contacts for research-related enquiries:

If you require more information about the study, then please contact any of the research team members namely, Dr Barry McDonnell (Senior Lecturer) or Mrs Margaret Munnery (Research Nurse) on Tel 02920686871. Alternatively, you may wish to speak to the Principal Investigators: -

Dr Barry McDonnell
Senior Lecturer
Cardiff Metropolitan University
Cardiff
0292041 6871

Thank you for reading this information sheet.

Appendix 3

Dr Barry McDonnell
Senior Lecturer

Cardiff School of Health Sciences,
University of Wales Institute,
Cardiff,
United Kingdom
CF5 2YB

PARTICIPANT INFORMATION SHEET FOR COMPARATOR SUBJECTS

"Assessment of Risk in Chronic Airways Disease Evaluation - ARCADE"

You are invited to take part in a research study. Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve. One of our research team members will go through this sheet with you and answer any questions you have. Please take time to read the following information carefully and discuss it with relatives, friends and your GP if you wish. We feel that this would take about 15 minutes to go through. Please ask us if there is anything that is not clear or if you would like more information. This study is funded for the first three years by a research grant from GlaxoSmithKline.

Why are we doing this study?
As we get older, our arteries generally stiffen over a period of time. This leads to an increase in blood pressure and may place us at a greater risk of strokes and heart attacks. A similar process occurs in patients with chronic obstructive pulmonary disease (COPD) causing premature arterial stiffening. However, we are not sure how quickly their arteries stiffen, and whether this is related to the severity of COPD. Also, the reasons for arterial stiffening in COPD are not well understood. We now have the means to assess arterial stiffness and the aim of this study is to measure how quickly arteries stiffen over time in COPD, and what factors are associated with this.

We are comparing these measurements in patients with COPD against those in subjects free of COPD who have a history of smoking.

Why have I been asked to participate?
You have been asked to participate because you fall into the following group:
- Between 35-80 years
- Have a history of smoking

Other individuals in this group have been similarly approached by a letter and we are looking to recruit 300 such subjects in this study to act as comparators for the 1500 patients with COPD.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. However, you are free to withdraw from the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?
Visit 0:
If you agree to take part, you will be asked to attend an initial visit lasting approximately 1 hour at a clinic at the University Hospital of Wales, Cardiff or your General Practitioner's surgery if it is one of the participating research sites. Here we will go through the information sheet, consent form and obtain a detailed lifestyle and medical history from you in addition to a medical examination.

You will then be requested to make 3 visits spaced over the next 5 years. All measurements made at these visits will be the same and each visit will last approximately 2 hours. Visit 1 will be baseline, visit 2 will be 2 years and visit 3 will be 5 years following your baseline visit.

Visit 1:
It would be preferable if you could refrain from smoking for at least 6 hours prior to attending. The following measurements will be made:

- We will check your height, weight and hip and waist circumference (using a tape).
- Blood pressure: After lying down for 15 minutes, you will have your blood pressure taken, just like at your General Practitioner's surgery.
- Arterial stiffness measurements: A small, pencil-like probe will be placed on the artery (blood vessel) at your wrist, neck and upper leg to calculate how stiff your arteries are. We will also record an electrocardiogram (ECG) with stickers on your chest to track small changes in your heart rate.
- Cardiac output: We will place some stickers on your chest and neck and measure how much blood is being pumped around with each heart beat.
- Lung function tests: You will then be asked to blow out as hard as you can into a tube after inhaling salbutamol (a safe drug using an inhaler with a spacer device) unless contraindicated to measure your lung function (the size of your lungs and how quickly you can empty them).
- Measurement of body composition: At this point we will check the proportion of muscle and fat tissue in your body by asking you to stand barefoot on a machine with hand grips. A tiny electric current which is not painful or harmful in any way is then passed and the measurements recorded. None of these measurements are painful.
- Hand-grip strength: This will be measured using a spring device.
- Bone density scan: We will then measure the bone density in your heels by using an ultrasound device which has no radiation.
- Blood sample: We will then ask you to give a blood sample of approximately 50 mls (5 tablespoons of blood) from your arm. This sample is used to measure various levels of naturally occurring substances in the blood, (ex: cholesterol, glucose and inflammatory molecules). This blood sample will be disposed after the study is completed in 5 years.

  With your permission some blood will also be taken and stored for future analyses, which will examine the genes which are known or likely to be important in determining how stiff or elastic your arteries are. This sample taken for genetic analysis will be stored for 10 years in the custody of Dr Barry McDonnell at Cardiff Metropolitan University, Cardiff.

  Further ethical approval will be sought if any genetic testing is required beyond 5 years. As these genetic markers are mainly to establish a genetic link for the natural evolution of COPD it would not have any implications on inherited risk or insurance status. GlaxoSmithKline, the sponsors of the first three years of the study will have access to the genetic data, but you will not be identified individually to the sponsors or anyone else in respect of this data.

What will happen to me if I take part?

Visit 0:

If you agree to take part, you will be asked to attend an initial visit lasting approximately 1 hour at a clinic at the University Hospital of Wales, Cardiff or your General Practitioner's surgery if it is one of the participating research sites. Here we will go through the information sheet, consent form and obtain a detailed lifestyle and medical history from you in addition to a medical examination.

You will then be requested to make 3 visits spaced over the next 5 years. All measurements made at these visits will be the same and each visit will last approximately 2 hours. Visit 1 will be baseline, visit 2 will be 2 years and visit 3 will be 5 years following your baseline visit.

Visit 1:

It would be preferable if you could refrain from smoking for at least 6 hours prior to attending. The following measurements will be made:

- We will check your height, weight and hip and waist circumference (using a tape).
- Blood pressure: After lying down for 15 minutes, you will have your blood pressure taken, just like at your General Practitioner's surgery.
- Arterial stiffness measurements: A small, pencil-like probe will be placed on the artery (blood vessel) at your wrist, neck and upper leg to calculate how stiff your arteries are. We will also record an electrocardiogram (ECG) with stickers on your chest to track small changes in your heart rate.
- Cardiac output: We will place some stickers on your chest and neck and measure how much blood is being pumped around with each heart beat.
- Lung function tests: You will then be asked to blow out as hard as you can into a tube after inhaling salbutamol (a safe drug using an inhaler with a spacer device) unless contraindicated to measure your lung function (the size of your lungs and how quickly you can empty them).
- Measurement of body composition: At this point we will check the proportion of muscle and fat tissue in your body by asking you to stand barefoot on a machine with hand grips. A tiny electric current which is not painful or harmful in any way is then passed and the measurements recorded. None of these measurements are painful.
- Hand-grip strength: This will be measured using a spring device.
- Bone density scan: We will then measure the bone density in your heels by using an ultrasound device which has no radiation.
- Blood sample: We will then ask you to give a blood sample of approximately 50 mls (5 tablespoons of blood) from your arm. This sample is used to measure various levels of naturally occurring substances in the blood, (ex: cholesterol, glucose and inflammatory molecules). This blood sample will be disposed after the study is completed in 5 years.

  With your permission some blood will also be taken and stored for future analyses, which will examine the genes which are known or likely to be important in determining how stiff or elastic your arteries are. This sample taken for genetic analysis will be stored for 10 years in the custody of Dr Barry McDonnell at Cardiff Metropolitan University, Cardiff.

  Further ethical approval will be sought if any genetic testing is required beyond 5 years. As these genetic markers are mainly to establish a genetic link for the natural evolution of COPD it would not have any implications on inherited risk or insurance status. GlaxoSmithKline, the sponsors of the first three years of the study will have access to the genetic data, but you will not be identified individually to the sponsors or anyone else in respect of this data.

Will my GP be informed?

The doctor in charge of the study will inform your GP of your participation in the study with your consent. In addition with your permission, they will be informed of any clinically relevant results that may become available during the study.

What will happen to the study results?

The results of the study may be published in scientific journals or presented at medical conferences, a few months after completing the study. You will not be identified in any report or publication. An overall summary report (fully anonymised) will be sent to your General practitioner at the end of the study. You will be able to access this through your GP upon the completion of the study.

What happens if something goes wrong?

The doctors involved in the study, and the Cardiff Metropolitan University have suitable indemnity insurance if you are harmed due to someone's negligence. However, there are no special compensation arrangements for non-negligent harm. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

What will happen if I withdraw from the study?

If you withdraw from the study, this will in no way affect your future medical care. Data or tissue already collected up to the point of withdrawal will be anonymised and utilised for study purposes. The same would apply if capacity to consent is lost during the study. You will be withdrawn from the study and data or tissue already collected under consent will be anonymised and utilised for study purposes.

Will I be reimbursed for any travel expenses?

Travel expenses of up to £30 will be given as necessary for trips to hospital to cover bus, mileage and parking. Taxis can also be ordered from our department to pick you up or drop you off.

Who is organising this study?

This study is being organised by the Cardiff Metropolitan University. The investigator in charge is Dr Barry McDonnell.

Who has reviewed this study?

The South East Wales Research Ethics Committee has reviewed this study and given it a favourable opinion.

Contacts for research-related enquiries:

If you require more information about the study, then please contact any of the research team members namely, Dr Barry McDonnell (Senior Lecturer) or Mrs Margaret Munnery (Research Nurse) on Tel 02920686871. Alternatively, you may wish to speak to the Principal Investigators: -

Dr Barry McDonnell
Senior Lecturer
Cardiff Metropolitan University
Cardiff
0292041 6871

Thank you for reading this information sheet.

Appendix 4

Dr. Barry McDonnell
Senior Lecturer

Cardiff School of Health Sciences,
Cardiff Metropolitan University,
Cardiff,
United Kingdom
CF5 2YB

PATIENT CONSENT FORM

LREC Reference Number:

Assessment of risk in chronic airways disease evaluation (ARCADE)

Name of Lead Investigators: Dr. Barry McDonnell

Please initial box

1. I confirm that I have read and understand the information sheet dated 13/06/17 (version 5) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected if I withdraw.

3. I agree that if my capacity to consent is lost during the study, I will be withdrawn from the study and data or tissue already collected under consent will be anonymised and utilised for study purposes.

4. I am willing that my GP is informed of my participation in the project as well as any clinically relevant results.

5. I understand that information held by the NHS and records maintained by The NHS Information Centre and the NHS Central Register may be used to help contact me and provide information about my health status.

6. I agree to take part in the above study and for the researchers to contact me again in the future

_____   _____   _____
Name of Research Subject   Date   Signature
(Please print)

_____   _____   _____
Name of Research Member   Date   Signature
(Please print)

3 copies required: top copy for researcher; one copy for patient; one copy to be kept with research subject's notes.

LREC Number 11/WSE02/7   Version 5   13/06/2017

Appendix 5

COMPARATOR CONSENT FORM

LREC Reference Number:

Assessment of risk in chronic airways disease evaluation (ARCADE)

Name of Lead Investigators: Dr. Barry McDonnell

Please initial box

1. I confirm that I have read and understand the information sheet dated 13/06/17 (version 4) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected if I withdraw.

3. I agree that if my capacity to consent is lost during the study, I will be withdrawn from the study and data or tissue already collected under consent will be anonymised and utilised for study purposes.

4. I am willing that my GP is informed of my participation in the project as well as any clinically relevant results.

5. I understand that information held by the NHS and records maintained by The NHS Information Centre and the NHS Central Register may be used to help contact me and provide information about my health status.

6. I agree to be contacted in 2 and 5 years time after the baseline assessment for a further follow-up visit.

7. I agree to take part in the above study and for the researchers to contact me in the future.

_____   _____   _____
Name of Research Subject   Date   Signature
(Please print)

_____   _____   _____
Name of Research Member   Date   Signature
(Please print)

3 copies required: top copy for researcher; one copy for patient; one copy to be kept with research subject's notes.

LREC Number 11/WSE02/7   Version 5 13/06/2017

Appendix 6

CGA-SF
Comprehensive Geriatric Assessment
Short Form

Patient ID

Completed by……………………………….. Date ………………

Motivation	circle one:	High	Usual	Low			

Self rated health	circle one:	Excellent	Good	Fair	Poor	Couldn't say

Cognition	circle one:	Normal	Mild cognitive impairment	Dementia

circle all that apply: Agitation/wandering   Delusions/hallucinations   Delirium

Emotional	Normal OR circle all that apply:	Anxiety	Bereavement	Depression	Fatigue

Sleep	Normal OR circle all that apply:	Poor or disrupted	Daytime drowsiness

Communication   circle one for each

Speech   Normal   Impaired   Hearing   Normal   Impaired   Vision   Normal   Impaired

Strength   Grip strength   Normal   Weak   Proximal muscle strength   Normal   Weak

Hemiparesis: Arm   Yes   No   Leg   Yes   No

Mobility	Transfer	Ind	Asst	Dep	
	Walking	Ind	Asst	Dep	Slow? Yes No
	Aid used?	Walking stick	Frame		

Balance	Balance	Normal	Impaired
	Falls	None	Yes

Elimination	Bowel	Cont	Occas accident	Incont
	Bladder	Cont	Occas accident	Incont

Nutrition	Weight change	Stable	Loss	Gain
	Appetite	Normal	Fair	Poor
	Weight	Normal	Under	Obese

ADLs	Feeding	Ind	Asst	Dep
	Bathing	Ind	Asst	Dep
	Dressing	Ind	Asst	Dep
	Toileting	Ind	Asst	Dep

IADLs	Cooking	Ind	Asst	Dep
	Cleaning	Ind	Asst	Dep
	Shopping	Ind	Asst	Dep
	Medications	Ind	Asst	Dep
	Driving	Ind	Asst	Dep
	Banking	Ind	Asst	Dep

Social Engagement
- ☐ Frequent
- ☐ Occasional
- ☐ Rarely

Medical history
- ☐ Hypertension
- ☐ COPD
- ☐ TIA/Stroke
- ☐ Angina/MI
- ☐ CHF
- ☐ Diabetes
- ☐ Cancer
- ☐ Alcohol excess
- ☐ Pressure sores
- ☐ Hip Fracture
- ☐ OA/RA
- ☐ Osteoporosis
- ☐ Parkinson's dis.

Notes:

Problems
1.
2.
3.
4.
5.
6.
7.

Current medication

Appendix 7

How is your COPD? Take the COPD Assessment Test™ (CAT)

This questionnaire will help you and your healthcare professional to measure the impact that COPD (Chronic Obstructive Pulmonary Disease) is having on your wellbeing and daily life. Your answers and test score can be used by you and your healthcare professional to help improve the management of your COPD and gain the greatest benefit from the treatment.

If you wish to complete the questionnaire by hand on paper, **please click here** and then print the questionnaire.

For each item below, place a mark (X) in the box that best describes your current situation. Please ensure that you only select one response for each question.

Example: I am very happy (0) (X) (2) (3) (4) (5) I am very sad

SCORE

I never cough	0 1 2 3 4 5	I cough all the time	
I have no phlegm (mucus) on my chest at all	0 1 2 3 4 5	My chest is full of phlegm (mucus)	
My chest does not feel tight at all	0 1 2 3 4 5	My chest feels very tight	
When I walk up a hill or a flight of stairs I am not out of breath	0 1 2 3 4 5	When I walk up a hill or a flight of stairs I am completely out of breath	
I am not limited to doing any activities at home	0 1 2 3 4 5	I am completely limited to doing all activities at home	
I am confident leaving my home despite my lung condition	0 1 2 3 4 5	I am not confident leaving my home at all because of my lung condition	
I sleep soundly	0 1 2 3 4 5	I do not sleep soundly because of my lung condition	
I have lots of energy	0 1 2 3 4 5	I have no energy at all	
		TOTAL SCORE	

Make sure you print your CAT before visiting your healthcare professional!

A COPD assessment test was developed by an interdisciplinary group of international COPD experts with support from GSK. GSK's activities in connection with the COPD assessment test are monitored by a supervisory council that includes external, independent experts, one of which is chair of the council.

Appendix 8

St. George's Respiratory Questionnaire
PART 1

Questions about how much chest trouble you have had over the past 3 months.

Emphasise to the patient that we are interested in how much chest trouble they have had over the last three months, eg "generally, how have you been over the last three months?". The exact calendar time is not important. Patients cannot reliably recall more than a few weeks. We are looking for an impression or perception of health.

Please tick (✔) one box for each question:

	most days a week	several days a week	a few days a month	only with chest infections	not at all
1. Over the past 3 months, I have coughed:	☐	☐	☐	☐	☐
2. Over the past 3 months, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3. Over the past 3 months, I have had shortness of breath:	☐	☐	☐	☐	☐
4. Over the past 3 months, I have had attacks of wheezing:	☐	☐	☐	☐	☐

Check that one box has been ticked for each question. Ticks may have been transposed to boxes either above or below the appropriate line.
Check for double entries.

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had?

Please tick (✔) one:

more than 3 attacks ☐
3 attacks ☐
2 attacks ☐
1 attack ☐
no attacks ☐

"Severe or very unpleasant attacks of chest trouble" can be further described as "whatever is a bad attack for you" (ie in the patient's own judgement, not bad as defined by doctor or nurse).
Check that only one box has been ticked.
Query double ticking.

UK/ English (original) version
with guidance notes

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continued...

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6. How long did the worst attack of chest trouble last?
   (Go to question 7 if you had no severe attacks)

   Please tick (✔) one:

   a week or more ☐
   3 or more days ☐
   1 or 2 days ☐
   less than a day ☐

Or "how long did the most severe attack of chest trouble last ?" This response should relate to Item 5.

If "no attacks" was the response for Item 5, then this item will be blank.

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had?

   Please tick (✔) one:

   No good days ☐
   1 or 2 good days ☐
   3 or 4 good days ☐
   nearly every day is good ☐
   every day is good ☐

The real meaning of this item is often misinterpreted because the polarity of the questions and responses is reversed compared to the previous items. The item refers to "how well have you been?" Make it clear that it is the number of good days (in an average week over the last three months) that we are interested in, ie we are asking for positive information, and not negative as in the previous questions.

It may help to give an example, eg "'None' means no good days in an average week, so you had the chest trouble all the time. 'Every day' means you were well every day of an average week, so you had hardly any chest trouble at all".

Check that the response to this item agrees with Items 1-4. Ensure that only one box has been ticked.

8. If you have a wheeze, is it worse in the morning?

   Please tick (✔) one:
   No ☐
   Yes ☐

If the patient does not have a wheeze no response will be given. Check that no response means no wheeze by asking the patient, or referring back to Item 4.

Patients should respond positively if their wheeze is worse in the morning compared to any other time of the day or night.

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 with guidance notes

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St. George's Respiratory Questionnaire
PART 2

Section 1

(Q 9) How would you describe your chest condition?

Please tick (✔) one:

The most important problem I have ☐

Causes me quite a lot of problems ☐

Causes me a few problems ☐

Causes no problem ☐

This item may be further explained by "is your chest trouble the most important problem you have in your life ?" or "do you worry about your chest trouble more than anything else?" Emphasise that "the most important problem" is worse than "causes me a lot of problems". The plurality here gives some patients difficulty.
Check that one box has been ticked.

(Q 10) If you have ever had paid employment.

Please tick (✔) one:

My chest trouble made me stop work altogether ☐

My chest trouble interferes with my work or made me change my work ☐

My chest trouble does not affect my work ☐

If "my chest trouble made me stop work" is ticked, ensure that giving up work permanently is what is meant. Patients often slip in a "has", so that their response means they have taken days off work or a period of time off work.
Retiring early because of health problems relating to chest trouble is an appropriate reason for responding to this item.
If "chest trouble interferes with my work" is ticked, appropriate reasons are:- having to take time off because of illness or frequent visits to the doctor, or changing jobs to one less physically demanding or less stressful.
Environmental factors may instigate a job change.
If the patient has never been in paid employment, then this item is left blank. Ensure that no response means "never employed".

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with guidance notes

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continued…

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Section 2

Questions about what activities usually make you feel breathless these days.

A major change in response style occurs here.
Up to now the patients have chosen one option from several. For this section (and most of the remaining sections) true or false must be ticked for each and every item. It is worth making this plain.
Questions apply to these days so "generally how are you at the moment?"

(Q 11) Please tick (✔) in **each box** that applies to you **these days**:

	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

Many patients do not engage in physical activity. However, it must be determined whether this is a genuine lack of inclination or a limitation because of chest trouble.
A response should be made if the patient would like to be able to play sports and games, but cannot because of their chest trouble.

UK/ English (original) version
with guidance notes

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Appendix 9

Modified Borg's Scale

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight (light)
3	Moderate
4	Somewhat severe
5	Severe (heavy)
6	
7	Very severe
8	
9	
10	Very, very severe (Maximal)

Appendix 10

![Cardiff Metropolitan University logo]

Cardiff School of Sport and Health Sciences

Dear applicant

Re: Application for Ethical Approval 'Does an individual's physical activity level impact cerebral blood flow and 24 hour aortic pulse wave velocity?'

Project Reference Number: UG-246

Your application for ethical approval of the above project was considered by Cardiff School of Sport and Health Sciences under the Cardiff Metropolitan University Ethics Framework, and I'm pleased to inform you that it was APPROVED on **24/10/2018**

Minor issues may still need addressing before you commence any work, and if so these will be listed below:

• **A3 - Indicate sample size**
• **Correct the consent form to indicate 2 copies required; one for the researcher and one for the PARTICIPANT**
• **Throughout the document ensure reference made to CSSHS and not CSHS**
• **Use version control for consent form and information sheet**
• **Supervisor to add date of declaration in signature box**

Where changes to the information sheet, consent form and/or procedures are deemed necessary you must submit revised versions through the CSSHS Ethics Process. If you are a student, your supervisor must do this on your behalf.

Note: Failure to comply with any issues listed above will nullify this approval.

Standard Conditions of Approval

1. Your Ethics Application has been given a Project Reference number, noted above. This **MUST** be quoted on all documentation relating to the project (e.g. consent forms, information sheets), together with the full project title.
2. All documents must also have the approved University Logo and the Version number in addition to the information highlighted in point 1.
3. A full **Risk Assessment** must be undertaken for this proposal, as appropriate, and be made available to the School if requested.
4. Any changes in connection to the proposal as approved, must be referred to the School for consideration **without delay, quoting your Project Reference Number**. Changes to the proposed project may have ethical implications therefore must be approved.
5. Any untoward incident which occurs in connection with this proposal must be reported back to the School **without delay**.
6. If your project involves the use of **human samples**, your approval is given on the condition that you or your supervisor **notify the HTA Designated Individual** of your intention to

work with such material by completing the form entitled "Notification of intention to Work with Human Material". This form must be submitted to the PD (Sean Duggan) **BEFORE** any activity on this project is undertaken.

7. It is the responsibility of the Principal Investigator to ensure the project aligns with Cardiff Metropolitan University's policies and procedures regarding GDPR compliance. For further information please click here.

This approval expires on**24/10/2019.** It is your responsibility to reapply/request extension if necessary.

Ethics Committee
Cardiff School of Sport & Health Sciences

sportethics@cardiffmet.ac.uk / healthethics@cardiffmet.ac.uk

Appendix 11

McDonnell, Barry
Cardiff School of Sport and Health Sciences

Dear applicant

Re: Application for Ethical Approval 'Health MOTs'

Project Reference Number: Sta-439

Your application for ethical approval of the above project was considered by Cardiff School of Sport and Health Sciences under the Cardiff Metropolitan University Ethics Framework, and I'm pleased to inform you that it was APPROVED on **14/11/2018**
Minor issues may still need addressing before you commence any work, and if so these will be listed below:

N/A

Where changes to the information sheet, consent form and/or procedures are deemed necessary you must submit revised versions through the CSSHS Ethics Process. If you are a student, your supervisor must do this on your behalf.
Note: Failure to comply with any issues listed above will nullify this approval.
Standard Conditions of Approval

1. Your Ethics Application has been given a Project Reference number, noted above. This **MUST** be quoted on all documentation relating to the project (e.g. consent forms, information sheets), together with the full project title.
2. All documents must also have the approved University Logo and the Version number in addition to the information highlighted in point 1.
3. A full **Risk Assessment** must be undertaken for this proposal, as appropriate, and be made available to the School if requested.
4. Any changes in connection to the proposal as approved, must be referred to the School for consideration **without delay, quoting your Project Reference Number**. Changes to the proposed project may have ethical implications therefore must be approved.
5. Any untoward incident which occurs in connection with this proposal must be reported back to the School **without delay**.
6. If your project involves the use of **human samples**, your approval is given on the condition that you or your supervisor **notify the HTA Designated Individual** of your intention to work with such material by completing the form entitled "Notification of intention to Work with Human Material". This form must be submitted to the PD (Sean Duggan) **BEFORE** any activity on this project is undertaken.
7. It is the responsibility of the Principal Investigator to ensure the project aligns with Cardiff Metropolitan University's policies and procedures regarding GDPR compliance. For further information please click here.

This approval expires on **14/05/2019.** It is your responsibility to reapply/request extension if necessary.

Ethics Committee
Cardiff School of Sport & Health Sciences
sportethics@cardiffmet.ac.uk / healthethics@cardiffmet.ac.uk