AGEING, INFLAMMATION AND CARDIOVASCULAR FUNCTION

by

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SYNOPSIS

Age is associated with the development of multi-system function loss including the musculoskeletal, immune and cardiovascular system, as well as body composition changes. These age-related alterations lead to frailty development and disease progression, reducing quality of life. A major lifestyle change that is evident in later years is a reduction in physical activity. The studies outlined in this thesis sought to examine the associations between physical activity and multi-system function loss in a cohort of elderly individuals, and to better understand the neural mechanisms underpinning the circulatory responses to exercise. It was observed that high daily physical activity levels attenuate some but not all of the age-related changes in elderly individuals. High physical activity was associated with superior physical functioning, lower total body fat and visceral adiposity, and plasma plasminogen activator inhibitor 1 (PAI-1) concentrations (a clotting agent). Left ventricular (LV) diastolic function was negatively associated with mean arterial pressure (MAP) and visceral adiposity, suggesting that elderly individuals with higher MAP and visceral adiposity may have inferior LV diastolic function. In terms of neural mechanisms related to circulatory responses to exercise, in models of metaboreflex over-activity whereby BP is elevated as observed in heart failure patients, left atrial systolic function is enhanced in order to maintain end-diastolic volume and SV.

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PUBLICATIONS ARISING FROM THIS THESIS

Full papers

(1) McNulty CL, Moody WE, Wagenmakers AJM, Fisher JP (2014) Effect of muscle metaboreflex activation on central hemodynamics and cardiac function in humans. Applied Physiology, Nutrition, and Metabolism. 39(8): 861-870.

Abstracts

(1) McNulty CL, Gorecki J, Kloss D, Knott L, Lee R, Robinson A, Moody WE & Fisher JP. Haemodynamic responses to muscle metaboreceptor activation in humans. 2012 Physiological Society Annual Meeting (Edinburgh, UK).

(2) McNulty CL, Bartlett DB, Crabtree N, Lord JM, Fisher JP & Wagenmakers AJM. Associations between physical activity, inflammation and cardiovascular function in an elderly cohort. 2013 American Ageing Association Annual Meeting (Baltimore, MD, USA).

ABBREVIATIONS

ACE	=	Angiotension converting enzyme
AT1	=	Angiotensin 1
ATII	=	Angiotensin II
ACh	=	Acetyl choline
ANOVA	=	Analysis of variance
BBS	=	Berg balance scale
BMI	=	Body mass index
BP	=	Blood pressure
CHF	=	Chronic heart failure
CKD	=	Chronic kidney disease
CO	=	Cardiac output
COPD	=	Chronic obstructive pulmonary disease
cPWV	=	Central pulse wave velocity
C-RP	=	C-reactive protein
CVD	=	Cardiovascular disease
DEXA	=	Dual-energy X-ray absorptiometry
EC	=	Endothelial cell

ECG	=	Electrocardiogram
EDV	=	End diastolic volume
EF	=	Ejection fraction
ELISA	=	Enzyme-linked immunosorbent assay
eNOS	=	Endothelial nitric oxide synthase
GP	=	General practice
HDL	=	High density lipoprotein
HR	=	Heart rate
ICAM-1	=	Inter-cellular adhesion molecule
IHG	=	Isometric handgrip
IL	=	Interleukins
IMT	=	Intima-medial thickness
LA	=	Left atrial
LV	=	Left ventricular
LVH	=	Left ventricular hypertrophy
MANOVA	=	Multivariate analysis of variance
MAP	=	Mean arterial pressure
MCP-1	=	Monocyte chemotactic protein 1

MIF	=	Macrophage migration inhibitory factor
MVC	=	Maximal voluntary contraction
MRI	=	Magnetic resonance imaging
NADPH	=	Nicotinamide adenine dinucleotide phosphate
NO	=	Nitric oxide
NOS	=	Nitric oxide synthase
PA	=	Physical activity
PAHA	=	Physical activity and healthy ageing
PAI-1	=	Plasminogen activator inhibitor 1
PAL	=	Physical activity level
PCT	=	Primary care trust
PEI	=	Post exercise ischaemia
p-QCT	=	Peripheral quantitative computed tomography
PWA	=	Pulse wave analysis
PWV	=	Pulse wave velocity
pPWV	=	Peripheral pulse wave velocity
RHG	=	Rhythmic handgrip
ROS	=	Reactive oxygen species

RPE	=	Rating of perceived exertion
SD	=	Standard deviation
SNP	=	Sodium nitroprusside
TDI	=	Tissue Doppler imaging
TNF-α	=	Tumour necrosis factor alpha
TPR	=	Total peripheral resistance
TIA	=	Transient ischaemic attack
TUG	=	Timed up and go
UK	=	United Kingdom
US	=	United States
VCAM-1	=	Vascular adhesion molecule 1
VEGF	=	Vascular endothelial growth factor
VSM	=	Vascular smooth muscle
VSMc	=	Vascular smooth muscle cell
VTI	=	Velocity time integral
WT-CRF	=	Wellcome Trust Clinical Research Facility

CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1:

INTRODUCTION

This thesis is predominantly concerned with the examination of physical activity status and physiological functioning in an ageing population. Physical activity is reduced with age, and parallels multi-system function loss, frailty development and age-related disease progression. Physical functioning i.e. walk speed and balance is reduced along with exercise capacity. Body composition changes including increased adiposity, particularly in central areas, and muscle mass decreases. Immune system alterations are also present which causes increased chronic plasma inflammatory cytokines, known to be detrimental to cardiovascular function. Age-related changes to the cardiovascular system include cardiac and vascular stiffening and dysfunction, leading to reduced exercise capacity and increased risk of cardiovascular disease development. Research has shown that physical activity is associated with superior functioning of these systems in elderly individuals, however little is known about the associations between habitual physical activity and multi-system function loss in a single elderly cohort.

The aim of this thesis is to investigate the associations between habitual physical activity, physical functioning, body composition, systemic inflammation and cardiovascular function in a single elderly cohort (Chapter 4). In addition, predictors of the variance in cardiac diastolic function will be examined in the same elderly cohort, including physical activity, body composition, systemic inflammation and cardiovascular risk factors (Chapter 5). Lastly, neural and cardiac mechanisms of the circulatory responses to exercise will be investigated in young individuals, to better understand neural cardiovascular control during exercise and to inform future related studies conducted in elderly individuals (Chapter 6).

CHAPTER 2:

LITERATURE REVIEW

2.1 Epidemiology of Ageing

2.1.1 Population Ageing

Population ageing is a major global issue (Egler 2000, Lloyd-Sherlock 2000, Shrestha 2000). Globally, the proportion of individuals aged ≥60 years will increase from 10% in 2000, to 21.8% in 2050 and incredibly 31.2% in 2100 (Lutz, Sanderson et al. 2008). Similarly, the United Nations indicated that by 2020 the global elderly population is expected to increase from 5.3% to 9.3% (Shrestha 2000) and Lakatta has stated that nearly one in four individuals will be 65 years of age or older by the year 2035 (Lakatta 2002).

Although overall global population ageing is occurring, the process is regionally heterogeneous. The United Kingdom (UK), along with Sweden, France, Norway, Denmark and Germany, were among the first to be confronted with the increase in longevity. As a result, very elderly populations (≥ 80 years of age) in these countries have grown and are now larger than other areas of the world such as Southern Europe (Jacobzone, Cambois et al. 2000). The reasons for this increase in longevity are manifold, but include public health interventions, and improvements in health care and education (Salomon, Wang et al. 2012).

The observed increase in life expectancy and longevity comes at a cost (Anderson 2000). Although the statistics indicate that modern medicine has successfully attenuated mortality, this has not been accompanied by a parallel reduction in morbidity (Egler 2000, Jacobzone 2000, Shrestha 2000, Fulop, Larbi et al. 2010). There is now a larger proportion of the population who are living in medicated and diseased states because as life expectancy has increased, the proportion of the population with morbities has also increased (Jacobzone 2000). As a consequence an individual spends an increased number of years in a frailty-related diseased state (Jacobzone 2000). Elderly individuals require healthcare more

frequently and thus, population ageing causes significant financial stress on Government healthcare spending (Egler 2000). According to the International Longevity Centre UK Think Tank, the rise in projected health care spending in the UK is parallel to the increase population ageing, forecasting a rise of £36bn between 2016/2017 and 2061/2062. Costs not only include those associated with medication and treatment, but also care home costs and disability support. Ideally, the best outcome for population ageing is morbidity compression, whereby the rise in life expectancy is maintained and accompanied with decreased morbidity (Jacobzone 2000). This would release the financial stress of Government costs and enhance quality of life. A potential strategy to facilitate morbidity compression is to increase physical activity. Physical activity is typically reduced with age, and physical inactivity has been estimated to cause 6% of the burden of disease from coronary heart disease, 7% of type 2 diabetes, 10% breast cancer, 10% colon cancer and 9% of premature mortality worldwide (Lee, Shiroma et al. 2012). Improved health can therefore be acquired through adopting an active lifestyle (Jacobzone 2000, Lee, Shiroma et al. 2012). It has been shown that elderly individuals who remain physically active have a better quality of life, maintained physiological function and reduced morbidity. Gill et al. (2006) conducted a randomized clinical trial of a home-based program designed to prevent functional decline in a high-risk group of physically frail, elderly persons who lived at home (Gill, Baker et al. 2002). They found that the home-based activity program was effective in preventing frailty-related functional decline. As such, increasing daily physical activity among the elderly population could be a successful, cost-effective strategy to prevent the functional decline observed with ageing.

2.1.2 Age-related Frailty

Age-related frailty is a term that has been used to loosely describe a range of conditions, such as general debility and cognitive impairment, occurring in older individuals (Lally and Crome 2007). In the elderly population (≥65 years old), 7% are frail with this figure increasing to 20% in individuals >80 years (Walston 2004). As stated in section 2.1.1, frailty is related to diseases observed in an ageing population. Age-related morbidities include degenerative conditions such as Alzheimer's disease, Parkinson's disease and sarcopenia, and other diseases such as cancer, kidney disease, diabetes, autoimmune disease and cardiovascular disease. (Khansari, Shakiba et al. 2009). Frailty and chronic disease often occur hand in hand, with one potentially resulting in the other (Bergman, Ferrucci et al. 2007). Although age-related frailty can occur without comorbidity, 'disease-free' frail individuals are still at increased risk of hospitalization (Fried, Tangen et al. 2001). The major difference between frailty and disease is that chronic disease requires appropriate diagnosis based on established guidelines, however frailty often remains undiscovered until disease is manifest, therefore lack of consensus on how to measure frailty still remains (Keevil and Romero-Ortuno 2015)The relationship between frailty and comorbidity has been investigated by a number of studies (Fried, Tangen et al. 2001, Boyd, Xue et al. 2005). It was concluded that frailty does not share the same characteristics as comorbidity, but is a risk factor for it, which eventually causes disability.

Due to the cross-over between chronic disease states and frailty the diagnosis of frailty has mostly been subjective (Wilson 2004). To resolve this problem, using data from the Cardiovascular Health Study (CHS) Fried et al. defined the phenotype of frailty as the

presence of 3 or more of the following components: unintentional weight loss (10 lbs in past year), self-reported exhaustion, weakness (grip strength), slow walking speed (lowest 20% of participants' time taken to walk 15ft), and low physical activity (lowest 20% of participants' time taken to walk 15ft), (Fried, Tangen et al. 2001). Frailty is a complex syndrome comprised of multiple interrelated physiological components (Buchner and Wagner 1992, Fried, Tangen et al. 2001, Walston 2004) (Figure 2.1). The general consensus is that frailty results from complex interactions between a number of physiological processes, rather than a single cause (Adams, Linke et al. 2005) . It is thought that the core feature of frailty is increased vulnerability to stressors, which is due to impairments in multiple, inter-related systems (Bergman, Ferrucci et al. 2007). A recent review by Keevil et al. defined frailty as the decline in an individual's homeostatic function, strength and physiologic reserve, leading to increased vulnerability (Keevil and Romero-Ortuno 2015). Frailty is associated with a loss of function from multiple systems in the body such as muscles, bones, brain (cognition), cardiovascular system including the heart and blood vessels, and the immune system. If these function losses are left unnoticed and untreated, the progression of the aforementioned age-related diseases may occur, resulting in poor quality of life, increased medication use and therefore increased financial stress. Given these detrimental outcomes, low cost frailty prevention such as increased physical activity participation in elderly individuals would be highly advantageous to the UK population. Figure 2.1 represents a cycle of frailty based on associations between multiple systems, however this model still remains theoretical.

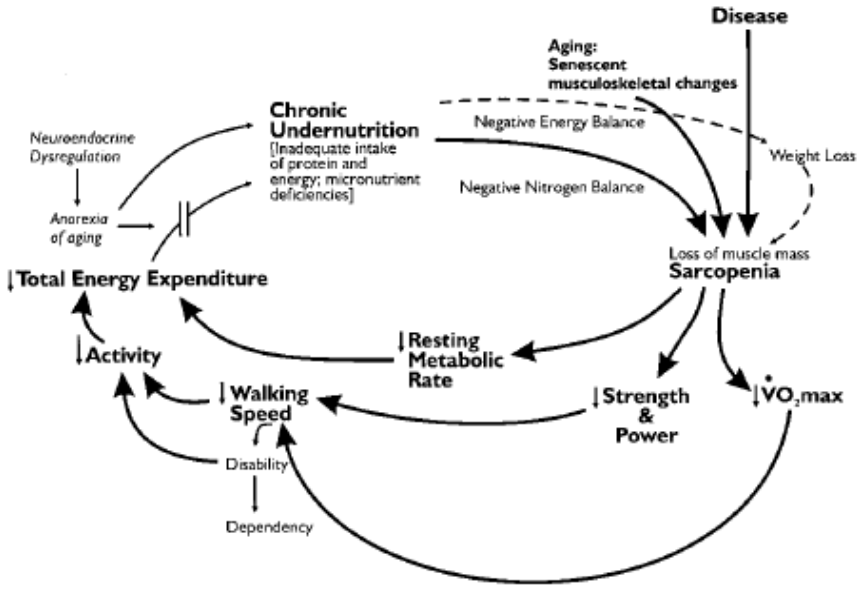


Figure 2.1: Cycle of Frailty (Fried et al. 2001)

2.2 Ageing and Physical Function

The development of age-related frailty is associated with a decline in physical function, i.e. mechanical performance, related to the musculoskeletal system and body composition changes (Buchner and Wagner 1992, Rockwood, Fox et al. 1994, Fried, Tangen et al. 2001). A loss of physical functioning results in disability onset and an increased dependence on others such as carers, impacting the quality of life. One of the attributes of age-related frailty identified by Fried et al. was slow walking speed, demonstrating inferior physical functioning, and was coupled with low physical activity (Fried, Tangen et al. 2001). Bendall et al. investigated the relationship between age, walking speed and calf strength in 67 women and 58 men aged 65-90 years, and found that walking speed was positively associated with calf strength and negatively associated with age (Bendall, Bassey et al. 1989). Physical activity measured via steps/day was also positively associated with walking speed, suggesting

beneficial and preventative effects of physical activity on aspects of functional decline observed with age. Pollock et al. performed a cross-sectional study in elderly cycling masters male and female athletes, with the aim to abolish any confounding effects of physical activity status in order to identify an independent marker of ageing (Pollock, Carter et al. 2015). However, the study failed to elucidate a physiological marker that could be used to reliably predict the age of any individual. It was confirmed that the physiological process of ageing is highly individualistic.

It has been postulated that a loss of physical function with age, such as walking speed and hand grip strength, could be related with an age-associated loss of muscle strength and mass (Fiatarone, O'Neill et al. 1994). Larsson et al. measured quadriceps muscle strength in 114 male participants aged 11-70 years old and found a significant decline in force generating capacity from the fifth decade (Larsson, Grimby et al. 1979). Aniansson et al. also observed marked strength loss over a period of only 5 years measured by knee extension, elbow extension and elbow flexion in 19 men and 21 women from the age of 70 years to follow up at 75 years (Aniansson, Sperling et al. 1983). The mechanism of muscle mass loss with increased age is not fully understood, however factors that play a role could include inflammageing (discussed further in Section 2.4.4), motorneuron death and fibre type change (Doherty, Vandervoort et al. 1993, Thomas 2007). One mechanism that is thought to play a role is a reduction of nutrient uptake of the skeletal muscles (Wagenmakers, Strauss et al. 2015). Inflammatory cytokines which are increased in the ageing process disrupt the signalling cascade involved in glucose and protein uptake, resulting in reduced muscle protein synthesis (Wagenmakers, Strauss et al. 2015). There is good evidence that maintaining physical activity can offset age-related decrements in muscle strength and mass. Fiatarone et al. examined the effects of a 10-week resistance exercise training regime on

frailty measures (i.e. muscle strength and stair climbing ability) in 100 frail nursing home residents with a mean age of 87.1 ± 0.6 years (Fiatarone, O'Neill et al. 1994). Exercise training significantly improved muscle strength, muscle cross-sectional area and stair-climbing power compared to the control participants, in whom muscle cross-sectional area actually decreased. It could be postulated that physical activity prevents age-related physical functional decline as a result of maintained neuromuscular control, muscle mass and strength.

In addition to muscle strength and mass loss, cardiac reserve and exercise capacity are also greatly attenuated in elderly individuals. Peak aerobic fitness (VO_2max) declines approximately 10% per decade after age 25-30 years in healthy sedentary adults (Tanaka and Seals 2008). Reduced VO_2max is attributable to both central and peripheral factors, i.e. lower maximal cardiac output (central) and reduced maximal oxygen extraction from skeletal muscle (peripheral) (Saltin and Calbet 2006). With increased age maximum heart rate (HR_{max}) falls, and in addition to structural and functional deteriorations of left ventricular (LV) function, causes a reduction in maximal cardiac output (Lakatta 2002, Lakatta 2003). As explained in more detail below (section 2.6) reduced maximal oxygen extraction can occur as a result of both structural and functional changes in the vasculature (Ehsani, Spina et al. 2003). However, the age-related decline in exercise capacity can be reduced with exercise training (Hagberg 1987, Ehsani, Spina et al. 2003). Elderly individuals who are able to perform activities of daily living with ease for longer are more able to live independently (Fries 1980, Fries 2002, Chakravarty, Hubert et al. 2008). Maintaining physical functioning as we age is therefore important to prevent disability onset and improve quality of life (Fries 1980, Fries 2002, Chakravarty, Hubert et al. 2008).

2.3 Ageing and Body Composition

With increased age there is a characteristic decline in skeletal muscle mass (sarcopenia) and an increase in central fat stores (visceral adiposity) (Baumgartner, Heymsfield et al. 1995, Gallagher, Ruts et al. 2000, Harris 2002, Wannamethee, Shaper et al. 2007).

2.3.1 Ageing and Sarcopenia

During the ageing process there is a significant loss of skeletal muscle mass and strength (Morley 2012). This progressive wasting of skeletal muscle is known as sarcopenia (Morley 2012). Sarcopenia is diagnosed when there is a less than expected muscle mass in an individual of a specified age, gender and race (Bauer and Sieber 2008). Specifically, sarcopenia is defined as an appendicular skeletal muscle mass divided by height in metres of more than 2 standard deviations below the mean value observed in young healthy individuals (Doherty, Vandervoort et al. 1993). Bauer et al. stated that the prevalence of sarcopenia has been calculated to be 7% for men and 11% for women above the age of 80 years in the US (Bauer and Sieber 2008). As muscle strength is proportional to skeletal muscle mass, sarcopenic individuals are often weak, which makes it more difficult to perform everyday activities. If activities of daily living are challenging to frail older individuals, loss of motivation to perform them can occur, escalating to a vicious negative feedback cycle of muscle and strength loss and reduced physical activity. If however, elderly individuals regularly participate in physical activity, muscle wasting can be at least partially reversed. Fiatarone et al. observed an improvement in stair climbing ability following a 10-week

resistance training intervention in elderly individuals, and also identified an increase in muscle strength and cross-sectional area (Fiatarone, O'Neill et al. 1994). They suggested that this increase in muscle strength and cross-sectional area may have aided advances in physical function.

2.3.2 Ageing and Visceral Adiposity

It is well established that with increased age there is a greater proportion of visceral adiposity. Song et al. composed a 2 year follow-up study examining body composition changes in elderly African American women (age at baseline 75.5 ± 5.1 years) with the use of Dual Energy X-ray Absorptiometry (DEXA) and found that with advancing age body fat increased and became centralized (Song 2004). Poehlman et al. conducted a large cross-sectional study on men and women aged 18-88 years, and measured body fatness and central adiposity using hydrostatic weighing and waist circumference (Poehlman, Toth et al. 1995). They concluded that older individuals possessed significantly higher body fatness and central adiposity, particularly in women. Age-related increases in visceral adiposity, located in the central area of the body, are associated with age-related diseases such as cardiovascular disease and insulin resistance (Prineas, Folsom et al. 1993, O'Leary, Marchetti et al. 2006). Sutton-Tyrrell et al. observed a significant positive association between body fat measured by DEXA and peripheral quantitative computed tomography (pQCT), and aortic stiffness measured via pulse wave velocity (PWV), which is a precursor for atherosclerosis, in 2488 elderly individuals with a mean age of 74 years (Sutton-Tyrrell, Newman et al. 2001).

As in non-elderly individuals (<60 years old), physical activity has proven to be an effective strategy to reduce increases in adiposity in ageing, as well as the progression of age-

related debilities such as reduced physical functioning and insulin resistance (Geffken, Cushman et al. 2001, O'Leary, Marchetti et al. 2006, Villareal, Chode et al. 2011). Villareal et al. conducted a 1-year interventional follow-up study examining the effects of an exercise intervention on physical functioning (walk speed, balance, VO_2 max) in 107 elderly obese adults (65-74 years old). The results yielded significant positive effects of the exercise intervention on fat mass and physical functioning. O'Leary conducted an exercise intervention study in 16 elderly obese men and women (mean age 63 ± 1 year) and found that central fat mass was significantly reduced, coupled with an improvement in insulin resistance (O'Leary, Marchetti et al. 2006). This suggests that by targeting central visceral adiposity in elderly individuals via physical activity, progression of age-related diseases could also be potentially attenuated. Conversely, a recent longitudinal study in middle-aged and elderly overweight or obese individuals with type 2 diabetes contradicts these findings (Look, Wing et al. 2013). The participants in this study underwent either a weight loss intervention comprising of calorie restriction and increased energy output or a control trial, and were followed up based on occurrence of a composite cardiovascular outcome (median = 9.6 years). Although the weight loss intervention group experienced greater weight loss (6%) compared to the control group (3.5%), cardiovascular morbidity rates were not significantly different. The expected benefits of increased physical activity via a weight-loss mechanism is not shown in this longitudinal interventional follow-up study, therefore care should be taken when discussing this potential explanatory pathway.

Visceral adipose tissue is a source of inflammatory cytokine production (interleukins [IL]) which have strong associations with the development of cardiovascular dysfunction (Hotamisligil, Arner et al. 1995, Purohit, Ghilchik et al. 1995, Yudkin, Kumari et al. 2000, Fain 2006), possibly via ROS production and cytokine infiltration in to arterial walls (See

Sections 2.4.4-2.4.6). Geffken et al. (2001) conducted an epidemiology study analysing data obtained from the Cardiovascular Healthy Study in 5,888 men and women aged >65 years old, investigating the associations between self-reported physical activity levels, inflammatory markers and body mass index (BMI) (Geffken, Cushman et al. 2001). They concluded that, following multivariate analysis, higher levels of physical activity were associated with lower levels of inflammation, which may be mediated by BMI. Indeed, one potential cause of increased age-related inflammation is this increase in visceral adiposity (Wu 2007).

2.4 Ageing, Immunity and Inflammation

2.4.1 The Innate Immune System

The innate immune system acts as the first line of defence against invading pathogens. Innate immunity includes several cell populations including but not limited to: monocytes, neutrophils, natural killer cells and mast cells, all of which function uniquely. Monocytes are one of the most fundamental cells of the innate immune system, their distinct role being to clear infection via chemotaxis to the site of infection. They perform this movement using cell surface receptors to detect a chemoattractant gradient. Monocyte chemotactic protein 1 (MCP-1) is the chemoattractant cytokine most commonly associated with monocytes. MCP-1 is detected by chemokine receptors and moves via adhesion to endothelial cells (Taylor and Gordon 2003, Shi and Pamer 2011). Here, receptor pathogen recognition occurs, inducing superoxide release from mitochondria and inflammation via cytokine production to kill bacteria (Shi and Pamer 2011). The secreting nature of monocytes not only induces inflammation, but also attracts more monocytes by releasing cytokines such as MCP-1.

However, this highly inflammatory and secretory action of monocytes may potentially be detrimental and harmful during the ageing process, due to the development of age-related immune dysfunction (Bruunsgaard and Pedersen 2003).

2.4.2 Ageing and Immunity

Immunosenescence describes the biological ageing of the immune system, whereby its functional role deteriorates (Caruso, Buffa et al. 2009, Panda, Arjona et al. 2009, Simpson 2009). Research suggests that age-related disease onset may be due to the development of immune dysfunction, causing increased infection and susceptibility to disease (Pawelec 2002, Caruso, Buffa et al. 2009, Simpson 2009). In addition to this, there is an increase in chronic levels of inflammation and oxidative stress observed in the ageing process, also related to disease onset (Stadtman 2006, Khansari, Shakiba et al. 2009).

2.4.3 Immunosenescence

Immunosenescence commonly occurs with age and describes an inability of the immune system to respond to new pathogens (Derhovanessian, Larbi et al. 2009). It is known to be most prevalent in the adaptive immune system (Gruver, Hudson et al. 2007), whereby there are a reduced number of naive T cells and increased number of memory T cells. There is therefore an increased demand on the innate immune system for a new adaptive response, producing an increased number of monocytes (Gruver, Hudson et al. 2007). A greater monocyte population causes higher basal levels of inflammatory cytokines such as Interleukin (IL)-1, IL-6 and tumour necrosis factor-alpha (TNF-α) due to constant cytokine

secretion at low levels without stimulation (Sadeghi, Schnelle et al. 1999, Hearps, Martin et al. 2012). This age-related increase in inflammation is also known as "inflammageing". The overall age-related immune dysfunction leads to the increased susceptibility to infection, malignancy and disease onset (High 2002).

2.4.4 Inflammageing

Inflammageing describes an increased pro-inflammatory status in the elderly (Caruso, Buffa et al. 2009, Derhovanessian, Larbi et al. 2009, Panda, Arjona et al. 2009). This chronic low-grade systemic inflammation is a result of low-level infection, increased innate immune activation and inflammatory cytokine release (IL-6, IL-1, TNF-α) (Bruunsgaard and Pedersen 2003, Forsey, Thompson et al. 2003, Ershler 2007). Chronic inflammation such as inflammageing, is associated with cardiovascular disease, sarcopenia, metabolic syndrome and long-term tissue damage, and is strongly related to increased disability in frail elderly individuals (Pawelec 2002, Panda, Arjona et al. 2009). An increased number of cytokine secreting immune cells at areas of endothelial damage causes atherosclerotic plaque formation, potentially resulting in major coronary events such as myocardial infarction or stroke (Merino, Buendia et al. 2011). Inflammatory cytokines (IL-6 and TNF-α) are known to produce reactive oxygen species (ROS) (Panda, Arjona et al. 2009, Metsios, Stavropoulos-Kalinoglou et al.), which have been implicated in the cell death associated with myocardial infarction (Sorescu and Griendling 2002). IL-6 has frequently been shown to increase with age and frailty (High 2002, Ershler 2007), however whether age increases TNF-α release is equivocal. Despite this, many reports suggest an increased basal TNF-α secretion (Delpedro,

Barjavel et al. 1998) and respiratory burst (Antonaci, Jirillo et al. 1984) as a result of heightened innate immunity and increased monocytes.

2.4.5 Other Mechanisms of Inflammageing

As mentioned in section 2.3.1, another potential cause of increased age-related inflammation in addition to heightened innate immunity is an increase in visceral adiposity (Wu 2007). One of the many detrimental effects of increased visceral adipose tissue is the production of inflammatory cytokines and markers of cardiovascular risk (IL-6, IL-8, TNF-α, plasminogen activator inhibitor 1 [PAI-1], and macrophage migration inhibitory factor [MIF]) (Hotamisligil, Arner et al. 1995, Purohit, Ghilchik et al. 1995, Yudkin, Kumari et al. 2000, Fain 2006).

2.4.6 Inflammation and Cardiovascular Function

There is mounting evidence that higher levels of circulating inflammatory cytokines and ROS with age may be associated with the development of cardiovascular dysfunction. Low-grade chronic systemic inflammation in healthy elderly individuals has been directly linked with arterial stiffness and vascular dysfunction, and harmful coronary events (Metsios, Stavropoulos-Kalinoglou et al.). ROS production via inflammatory cytokines such as TNF-α, has been shown to contribute to the development of endothelial dysfunction, via the suppression of endothelial-dervied NO (Figure 2.6) (Goodwin, Pendleton et al. 2007, Metsios, Stavropoulos-Kalinoglou et al.).

2.4.7 Physical Activity and Inflammation

Long-term habitual exercise has been associated with reduced chronic low-grade inflammation observed in the elderly frail population (Mattusch, Dufaux et al. 2000, Taaffe, Harris et al. 2000, Geffken, Cushman et al. 2001). Exercise is thought to mobilize T cells from the immunological compartment in to the periphery, leading to the generation of naive T cells and increasing the naive T cell repertoire (Simpson 2009). Ford conducted a large cross-sectional study in 13,748 US citizens (age ≥ 20 years old), examining physical activity status and plasma C-reactive protein (C-RP) levels, which rise in response to inflammation (Ford 2002). Those participants who were more physically active had significantly lower C-RP levels across all age ranges. In addition to the potential changes within the immune compartment itself, habitual exercise has modulatory effects on body fat distribution that are known to change with age, whereby an increase in central adiposity occurs. Geffken et al. analysed data obtained from the Cardiovascular Healthy Study of 5,888 men and women aged >65 years old to investigate the associations between self-reported physical activity levels, inflammatory markers and BMI (Geffken, Cushman et al. 2001). Following multivariate analysis, higher levels of physical activity were associated with lower levels of inflammation, which have been mediated by BMI. Exercise reduces adipose tissue, which secretes IL-6 and TNF-α. A reduction in the production and release of inflammatory cytokines would result in a reduction in the production of ROS. A consistent finding in the literature is that regular exercise relates to decreased systemic inflammation in both healthy and diseased populations, and results in prevention of endothelial dysfunction and atherosclerosis (Simpson 2009), presented in Figure 2.2.

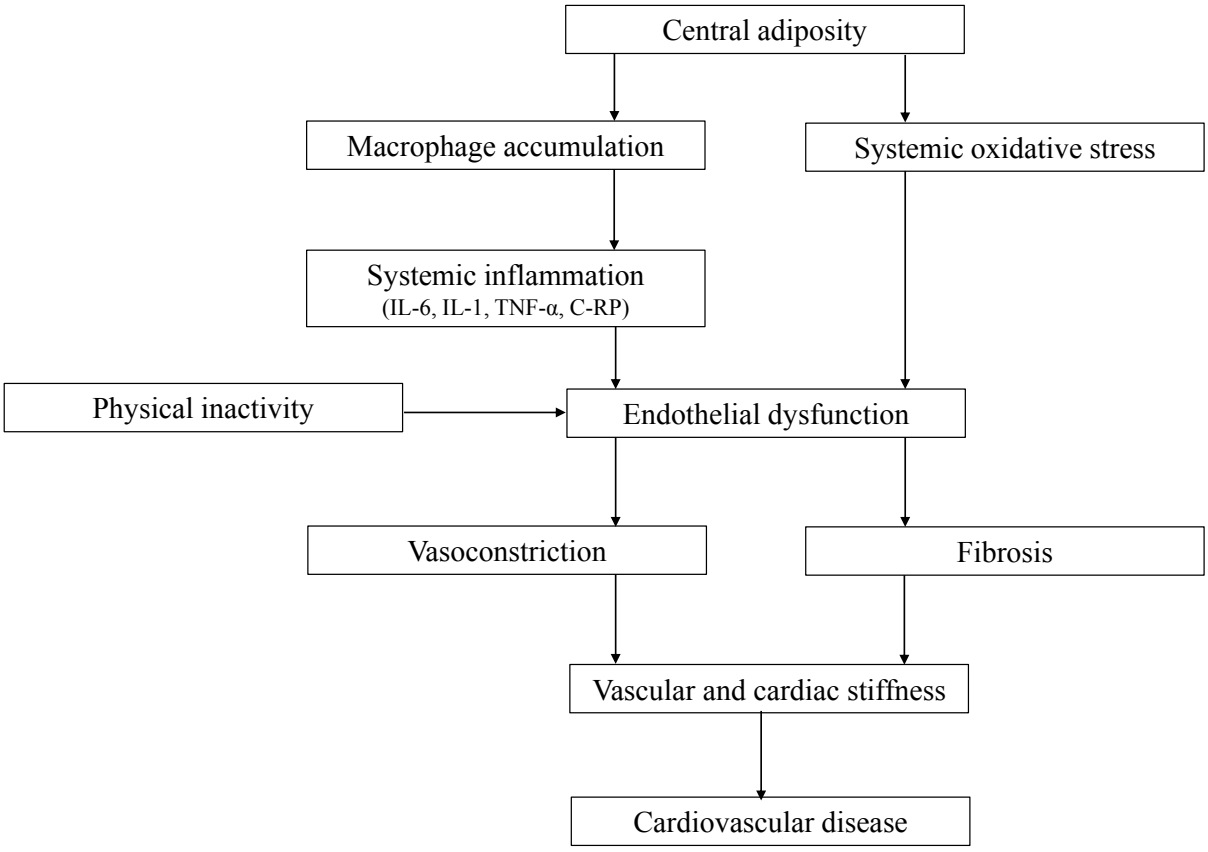


Figure 2.2: Schematic diagram highlighting the mechanisms between adiposity, inflammation and cardiovascular disease

2.5 Ageing and the Cardiovascular System

The prevalence and incidence of cardiovascular disease increases with advancing age, including diseases such as hypertension, stroke, atherosclerosis and chronic heart failure (CHF) (Lakatta 2002). In 2010, National Statistics for Death in the US showed the leading cause of mortality were diseases of the heart, where 24.2% of all individuals who died during that year, died due to heart disease (Murphy, Xu et al. 2013).

2.6 The Heart

With age the heart undergoes a number of structural, functional and regulatory alterations that may in part account for the age-related increase in cardiovascular risk (Lakatta and Levy 2003).

2.6.1 Age-related Structural Changes of the heart

The heart increases in mass by an average of 1g/yr in men and 1.5g/yr in women between the ages of 30-90 years. These changes include an increase in wall thickness, heart mass, chamber dimension, cardiomyocyte dimension and collagen, and occur in healthy elderly individuals without the presence of hypertension (Lakatta 2002, Lakatta and Levy 2003). Left ventricular hypertrophy (LVH) is caused by myocytes becoming larger with ageing (Song, Yao et al. 1999). Song et al. studied older hospitalized patients without diagnosed or apparent cardiovascular disease and observed at autopsy that the cardiac myocyte number was decreased and the size enlarged compared to young patients (Song, Yao et al. 1999). The decrease in myocyte number is thought to be caused by apoptosis and results in a stretching of the existing cardiac cells, the functional consequence of which is a prolongation in ventricular contraction duration (Lakatta and Levy 2003).

In addition to cardiomyocyte hypertrophy, alterations in the connective tissue layers of the heart, which contain the matrix protein collagen, also occur. These include the epimysium which surrounds the whole cardiac muscle, the perimysium which surrounds groups of cardiac muscle fibres, and the endomysium which enwraps individual cardiomyocytes (Figure 2.3) (Biernacka and Frangogiannis 2011). The development of

fibroblasts to myofibroblasts in these connective tissue layers leads to perivascular, endomysial and perimysial fibrosis, collectively known as cardiac fibrosis (Song, Yao et al. 1999, Lakatta 2003, Orlandi 2004, Burkauskiene, Mackiewicz et al. 2006, Biernacka and Frangogiannis 2011). This age-related increase in cardiac collagen results in a progressive increase in myocardial stiffness. LV compliance is therefore compromised and can impair LV diastolic function (Biernacka and Frangogiannis 2011). Attenuated LV relaxation and prolonged contraction duration leads to impaired LV filling and a reduction in cardiac output (CO) at high heart rates, which can limit exercise tolerance in healthy elderly individuals and lead to premature fatigue (Vanoverschelde, Essamri et al. 1993, Kitzman, Gardin et al. 2001, Lakatta 2003). As discussed in section 2.2, this age-related reduction in CO plays a vital role in physical function loss in elderly individuals, reducing quality of life.

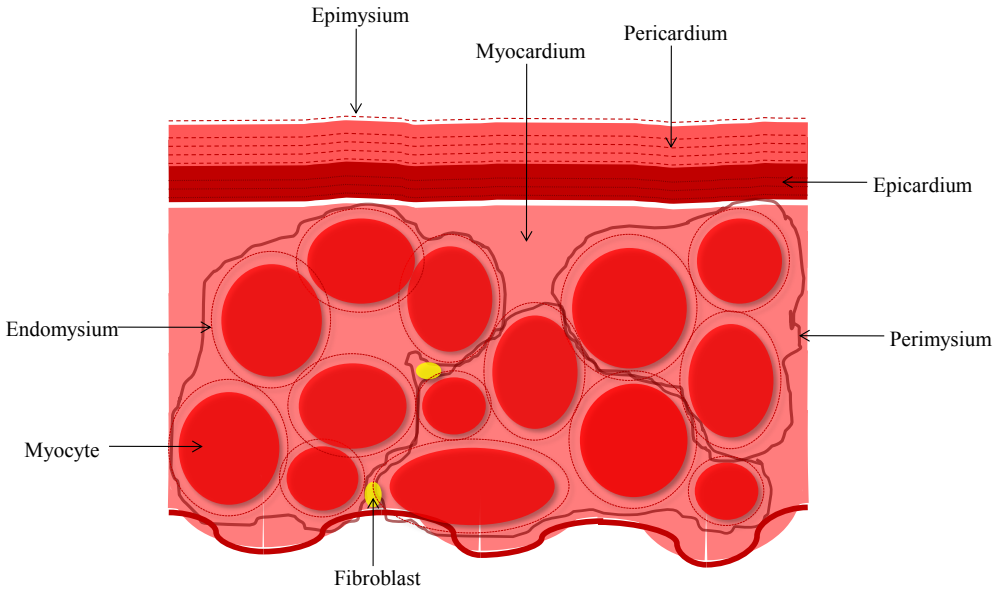


Figure 2.3: Diagram representing physiology of the cardiac muscle

2.6.2 Age-related Functional Changes of the heart

By the age of 80 years, early LV diastolic filling rate is reduced by approximately 50% (Lakatta 2002). Studies have shown this by using the well-established technique of Doppler echocardiography (Spirito and Maron 1988, Arbab-Zadeh, Dijk et al. 2004). Over the last three decades, Doppler echocardiography has emerged as the diagnostic modality of choice for the assessment of diastolic function (Garcia, Firstenberg et al. 2001). Two-dimensional (2D) echocardiography has become well accepted as a reliable, reproducible and practical noninvasive method for both diagnosis and longitudinal follow-up for patients with diastolic dysfunction (Nishimura and Tajik 1997).

Diastolic function comprises an interrelated complex sequence of events. Factors including ventricular relaxation, diastolic suction, erectile coronary effect, viscoelastic forces of the myocardium, pericardial restraint, ventricular interaction and atrial contribution, all determine how the LV fills with blood. However, simplistically we can divide LV diastole in to four separate processes. These are: isovolumetric relaxation, rapid filling, slow filling and atrial contraction (Little and Downes 1990).

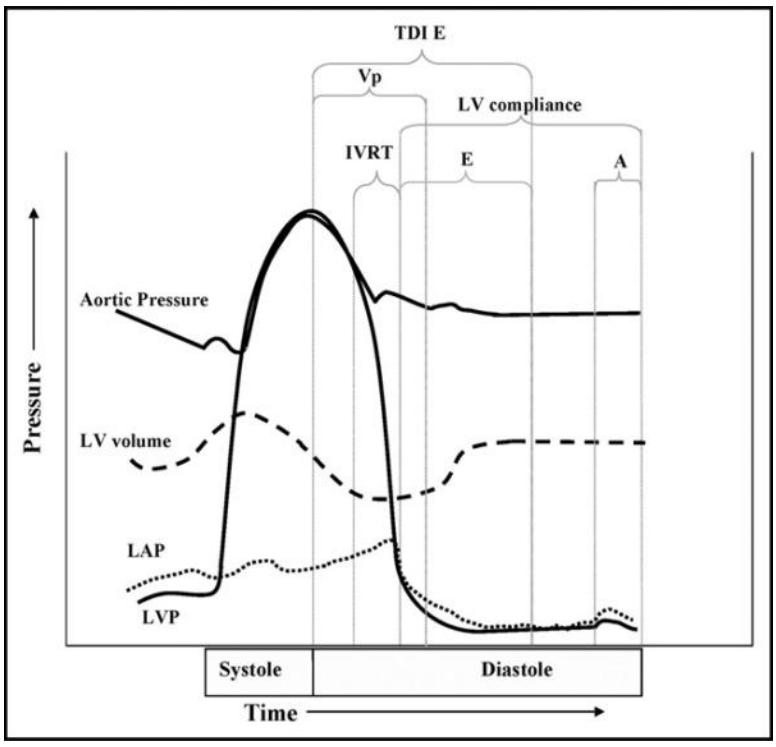


Figure 2.4: Diastolic filling of the left ventricle. LAP = left atrial pressure; LV compliance = left ventricular end-diastolic pressure–volume relation; LVP = left ventricular pressure; Vp = early propagation velocity of mitral inflow (Prasad, Popovic et al. 2007).

Ventricular relaxation represents the rate and duration of the decrease in LV pressure following systolic contraction. The LV undergoes passive relaxation, creating a pressure gradient between the left atrium (LA) and the LV. Blood is then sucked from the LA and is known as LV early filling or in Doppler echocardiography, E wave velocity. Following this, the remaining volume of blood in the LA is then forced in to the LV via atrial contraction, and is known as A wave velocity. In young healthy hearts, a majority of LV filling occurs in the early phase, as the heart is elastic and able to passively relax with ease. During LA contraction, the young compliant LV stretches to accommodate the remaining blood volume. In Doppler echocardiography, this early-to-late filling relationship is known as E/A ratio. A typical healthy young heart will have an E/A ratio of around 2:1, representing a higher rate of

LV filling during passive relaxation. High E/A ratios are associated with healthy LV function. With ageing however, due to the increase in collagen content the LV loses elasticity and compliance. Therefore there is less passive relaxation and a smaller pressure gradient, so early filling of the LV is reduced. Because of this, a larger proportion LV filling occurs in the late phase during atrial contraction. The LA has to contract with more force to push the blood in to the LV, and the reduced compliance of the LV is less able to accommodate the blood volume, creating a higher preload pressure (Arbab-Zadeh, Dijk et al. 2004). In Doppler echocardiography, this produces an augmented A wave (diastole), which decreases the E/A ratio. This is thought to be the most dramatic change in cardiac pump function with ageing (Lakatta 2002), and is usually the earliest manifestation of a disease process (Nishimura and Tajik 1997).

Resting ejection fraction (EF) does not appear to be compromised with healthy ageing. This is partly due to a more vigorous atrial contraction which results in more filling occurring during late diastole, with maintained LV systolic function (Lakatta 2002).

2.6.3 Age-related Regulatory Changes to the Heart

Resting HR does not appear to be altered as a function of age. During aerobic exercise however, the HR_{max} achievable declines by approximately 30% between the ages of 20 and 85 years (Lakatta and Levy 2003). This lower HR_{max} in ageing is in part explained by a reduction in the sensitivity of the β-receptors to sympathetic stimulation. β_1-adrenergic receptors increase HR in response to the neurotransmitter noradrenaline, released by the sympathetic nervous system (Lakatta and Levy 2003). Indeed, Yin et al. showed that the increase in HR in response to the administration of isoproterenol, a β-adrenergic agonist, was

attenuated in the elderly compared to adult dogs (Yin, Spurgeon et al. 1979). De-sensitization of β-adrenergic receptors has also been identified in humans. White et al. observed a profound decrease in cardiac β-adrenergic responsiveness with age, when 26 non-failing donor hearts aged between 1-71 years were subjected to pharmacological investigation (White, Roden et al. 1994). A consequence of reduced sensitivity of β-receptors is chronic elevated sympathetic nerve activity, which is associated with many age-related physiological changes (e.g. hypertension, congestive heart failure, insulin resistance and obesity) (Seals and Esler 2000). With a reduced post-synaptic response of these receptors on the heart, HR will not increase to the same extent in the healthy elderly heart as when compared to a healthy young heart in response to strenuous dynamic exercise. With a lower HR_{max}, maximal cardiac output and therefore aerobic exercise capacity is attenuated with age. It has been shown that exercise capacity has prognostic effects on mortality in elderly individuals, and that augmented exercise capacity is associated with reduced risk of mortality (Nylen, Kokkinos et al. 2010). This again plays a role in the reduced physical functioning observed with ageing, negatively affecting quality of life.

2.6.4 Physical Activity and the Heart

Habitual physical activity pattern could be an important modifier of the development of age-related cardiac stiffening and LV diastolic dysfunction. Arbab-Zadeh et al. (Arbab-Zadeh, Dijk et al. 2004) examined the effects of physical activity on LV compliance in elderly individuals. Twelve healthy sedentary men and women (mean age 69.8 ± 3 years) and 12 age-matched male and female Masters athletes (mean age 67.8 ± 3 years) participated. LV compliance was significantly lower in sedentary elderly individuals compared to Masters

athletes. However, the LV compliance of Masters athletes was similar to a sedentary group of young individuals. Stratton et al. examined cardiac function in healthy young and elderly individuals before and after 6 months endurance training (Stratton 1994). An increase in EF, end-diastolic volume (EDV) and CO were observed. This suggests that physical activity in later life may have beneficial effects on LV function, indicated by a more compliant LV and higher end-diastolic volume and CO. Achieving higher CO would increase exercise capacity in elderly individuals enabling better physical function performance, improving dependency and quality of life.

2.6.5 Ventricular-Arterial Coupling

A concept known as ventricular-arterial coupling describes the relationship between the heart and vasculature (Frenneaux and Williams 2007). Increased large artery stiffness poses an increased afterload on the heart as systolic wall stress is increased. When the heart ejects blood through the aorta, increased aortic stiffness causes increased pressure and stress that the LV has to overcome to force blood out of the ventricle. In response, the heart appears to increase in stiffness, potentially via a fibrosis response. Animal models have shown that cardiac fibrosis is a reactive process in response to LV pressure overload. Robert et al. induced hypertension in rat hearts by continuous infusion of aldosterone for 2 months. They found that this was associated with development of fibrosis (Robert, Besse et al. 1997). This initial fibrosis is accompanied by cardiomyocyte hypertrophy and is a part of an adaptive response aimed at preserving cardiac output while normalizing wall stress (Biernacka and Frangogiannis 2011). Leite-Moreira et al. found that in rabbits, a large acute increase in afterload resulted in a significant slowing of active relaxation and impaired LV diastolic

filling (Leite-Moreira, Correia-Pinto et al. 1999). This suggests that the progressive hypertrophy and stiffening of the ageing heart may be a consequence of peripheral vascular stiffening.

2.7 Ageing and the Vasculature

The vasculature undergoes a multitude of age-related changes that leave us at an increased risk of developing vascular diseases such as hypertension and atherosclerosis. The vasculature may be divided in to two regions, the macro-vasculature that includes the large arteries and veins (> 200 μm), and the microvasculature including arterioles, venules and capillaries (< 200 μm). The conduit arteries of the macro-vasculature carry oxygenated blood from the heart to the organs. Proximal to the target organs, the vascular network divides into smaller arterioles and forms capillaries at the target organs. The capillary network is the site of oxygen and nutrient exchange and is vital to an organs functional ability.

2.7.1 Macro-vascular Ageing

With age, large arteries dilate and their walls become thicker, by a process referred to as intima-medial thickening (IMT) (thickening of the intima and medial artery layers) (Lakatta 2002). Nagai et al. used data from the Baltimore Longitudinal Study of Aging (BLSA) (Shock, Greulich et al. 1984) and found that carotid IMT was significantly increased in 261 men (age 64 ± 15.7 years) and 246 women (age 57.8 ± 15.6 years) compared to data collected when participants were aged 30 years (Nagai, Metter et al. 1998). Najjar et al. states that arteries also widen with age, increasing the size of the lumen (Najjar, Scuteri et al. 2005),

which have been shown by cross-sectional studies (Lakatta 1993). The putative underlying mechanisms for these changes are explained below.

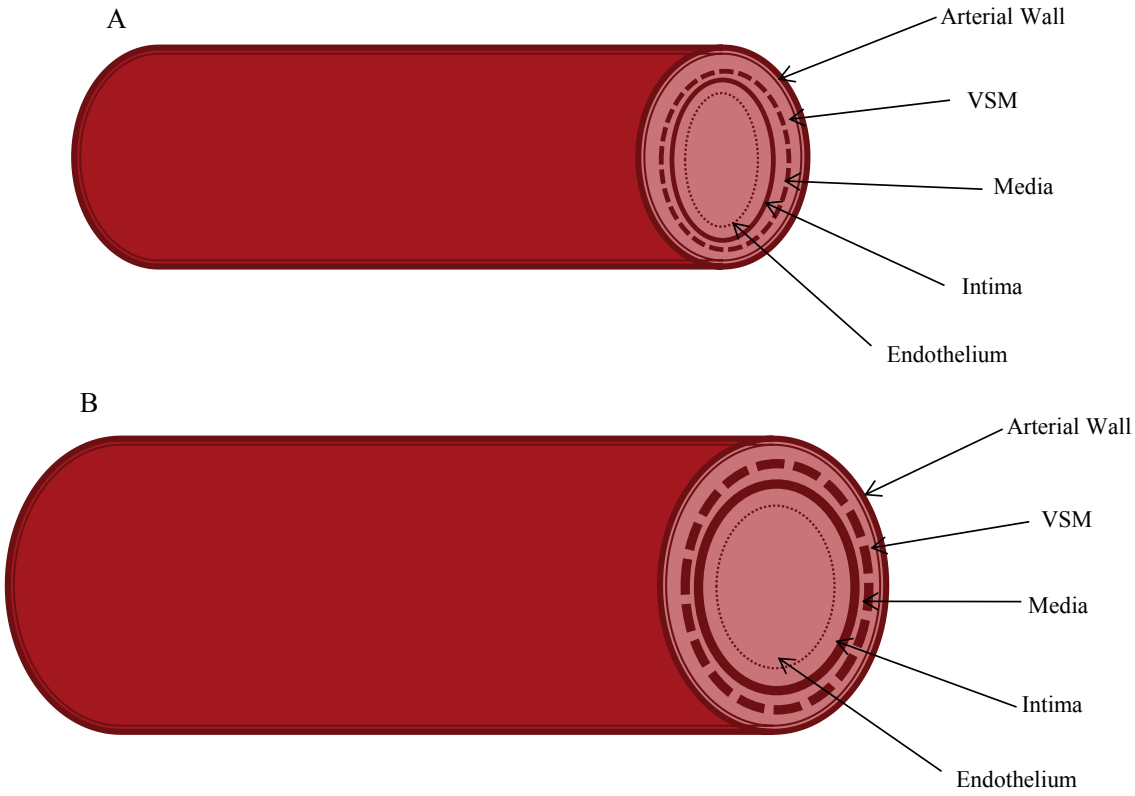


Figure 2.5: Diagram of age-related changes in macro-vasculature (arteries). A: young artery; B: aged artery.

Age-dependent increases in large artery stiffness is also well established (Avolio, Chen et al. 1983, Benetos, Laurent et al. 1993, Frenneaux and Williams 2007). The increase in stiffness results from a depletion of elastin due to the repeated cycles of distensions and elastic recoils of the arterial wall, in addition to deposition of collagen (Najjar, Scuteri et al. 2005). The increased collagen content is due to fibrosis of the vascular smooth muscle layer,

a similar process to fibrosis of the cardiac myocytes. Furthermore, the reduced arterial distensibility in the periphery results in an increase in blood pressure (BP), as the vessels are unable to cushion the increase in blood flow which has been pumped from the LV (Lakatta and Levy 2003). These alterations attenuate the buffering capacity of the large arteries in response to pressure changes. Pressure waves are reflected back from the periphery and form with the forward going wave to produce the characteristic waveform. Normally, in young compliant vessels, the reflected pressure wave arrives in the central arteries following closure of the aortic valve. However, with vascular stiffening the reflected wave arrives earlier and augments central systolic pressure (Asmar, Benetos et al. 1995), a major risk factor for the development of cardiovascular disease. Pulse wave velocity (PWV) increases with ageing, which is an indicator of peripheral and central arterial stiffness (Lakatta and Levy 2003).

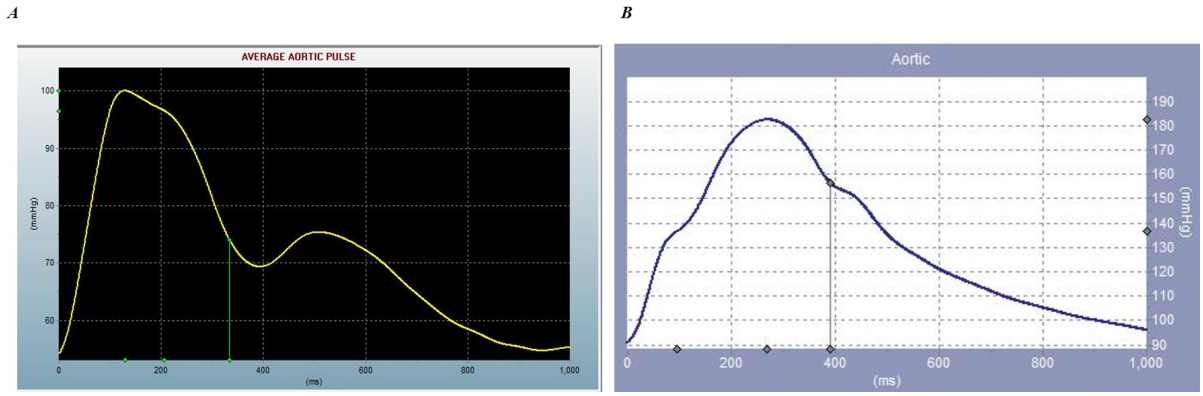


Figure 2.6: Diagram presenting pressure wave and reflected wave from the radial artery in young (A) and elderly (B)

If the aorta was an open-ended tube providing a simple resistance to flow then there would be a single pressure wave present. However, the network of arteries with all the bifurcations and vascular beds produces reflected waves. As the primary pressure wave travels along the arteries, reflected waves are generated from each bifurcation and from the

peripheral vascular beds. These small reflected waves return to the heart, summing to create a reflected wave as shown in Figure 2.6, initiating even before the end of systole. The pressure in the aortic root is the sum of the outgoing and reflected wave, indicated by the green line in Figure 2.6A, presented as the Augmentation Index (AIx). The speed of the outgoing and reflected waves is dependent on the stiffness of the arteries along which they are travelling. Less compliant stiffer arteries cause the waves to travel out and back quicker, arriving back earlier at the heart (Figure 2.6B). The speeds of the waves in the central (aorta) and peripheral (e.g., brachial) arteries are referred to as central and peripheral pulse wave velocity (cPWV and pPWV) and indicate arterial stiffness.

The apparent increase in arterial stiffness observed with ageing is not only attributable to structural changes in the vessel wall, but also to impaired endothelial regulation of the vascular smooth muscle surrounding the arteries (Lakatta and Levy 2003). Endothelial cells were once thought to be 'inert' with no physiological function (Cines, Pollak et al. 1998). However, it is now known that endothelial cells play a very important role in regulating arterial responses to blood flow (via shear stress) (Najjar, Scuteri et al. 2005). Endothelial derived nitric oxide (NO) dilates the arteries in response to an increase in shear stress. A reduction in NO production and bioavailability occurs with ageing, which has been associated with a disruption in the upstream production of endothelial nitric oxide synthase (eNOS), the rate limiting enzyme for endothelial NO production (Seals, Desouza et al. 2008).

ROS and inflammatory cytokines increase with age, as mentioned in Section 2.2.6. Under normal conditions, oxygen radicals are produced endogenously, and levels are increased during oxidative stress. The most common and more reactive radicals are superoxide and hydrogen peroxide (Schnackenberg 2002). Superoxide is produced during

mitochondrial respiration and also by nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase). In ageing, the amount of radicals produced exceeds the resources for metabolism, so oxidative stress occurs which is damaging. Animal studies have shown that superoxide interferes with the production of NO (Versari, Daghini et al. 2009) as NO is susceptible to oxidative scavenging (Beckman and Ames 1998). ROS quench NO after its production and consequently reduces its bioavailability (Versari, Daghini et al. 2009). Superoxide reacts with NO to form peroxynitrite, impairing NO bioavailability, ultimately leading to endothelial dysfunction (Metsios, Stavropoulos-Kalinoglou et al.). A reduction in NO production and bioavailability results in an attenuated vasodilatory response to an increase in blood flow (Celemajer 1994). Endothelial dysfunction is apparent in both the macro- and microcirculations, and is associated with major cardiovascular risk factors, such as the progression of atherosclerotic disease (Versari, Daghini et al. 2009).

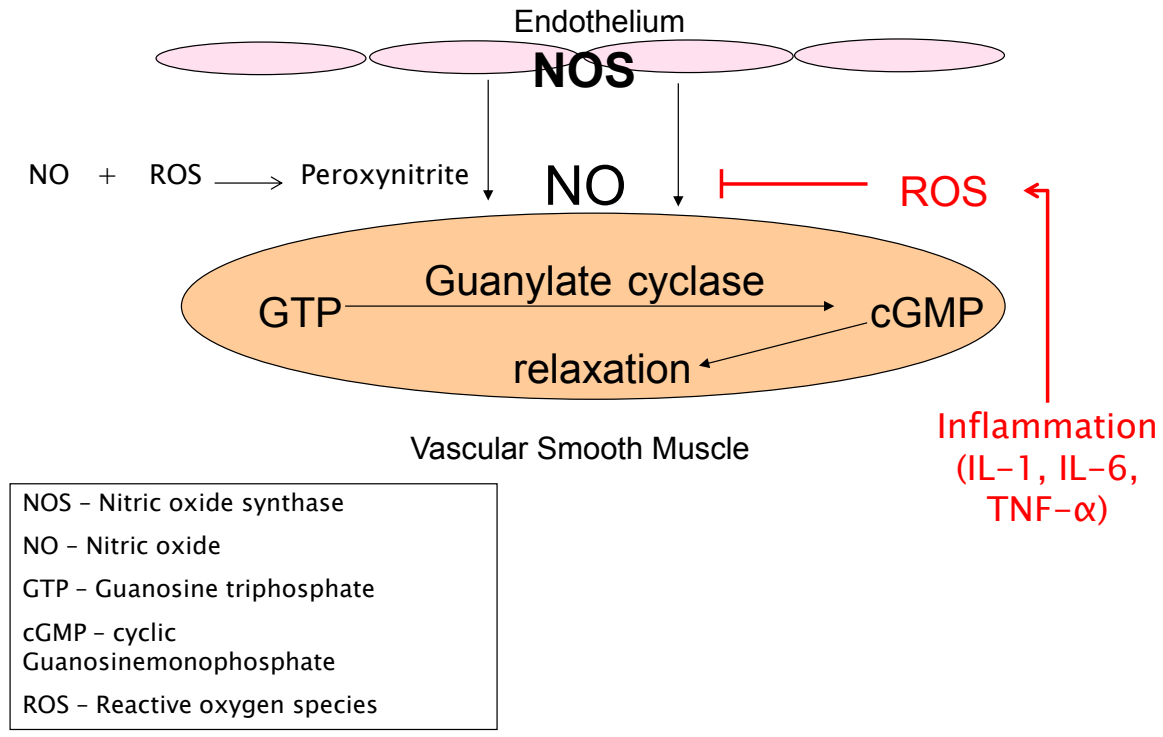


Figure 2.7: Diagram representing the release of NO from the endothelium, and the inhibition of this process via inflammation and ROS.

In addition to impaired vasodilation of the vasculature, the inflammatory cytokine TNF-α upregulates the expression of vascular adhesion molecules, such as vascular adhesion molecule-1 (VCAM-1) (Metsios, Stavropoulos-Kalinoglou et al.). As a result of impaired vasodilation and increased expression of adhesion molecules, low-grade chronic inflammation is involved in the development of atherosclerosis, ultimately leading to cardiovascular disease. Atherosclerosis is an inflammatory disease of the arteries, and involves the development of atherosclerotic lesions which eventually form plaques (Hansson 2001). The lesions impinge on the lumen of the artery, decreasing the blood flow, which results in damage to the tissue that is served by the artery due to oxygen starvation. The lesions of atherosclerosis are responsible for changes in the heart that can lead to myocardial infarction, in the brain to cerebral infarction or stroke, and in the peripheral vasculature to loss of function (Ross 1995).

One of the earliest changes in the endothelium associated with atherosclerosis is the formation of adhesion molecules VCAM-1 and inter-cellular adhesion molecule-1 (ICAM-1), which adhere to T cells (Ross 1995). T cells are a type of leukocyte and form part of the adaptive immune system, which release cytokines in response to threat or damage. Following adhesion, leukocytes transmigrate through the endothelial layer in to the intima of the smooth muscle layer leading to increased IMT (Hansson 2001). The atherosclerotic lesions are developed in these thickened regions, and contain large numbers of immune cells, particularly macrophages and T cells. Most of the T-cells present in the lesions are CD4+ and CD3+ memory cells (Hansson 2001), which increase with ageing. Interactions between

macrophages and T cells lead to replication and migration of vascular smooth muscle cells (VSMCs) in to the lesion, increasing the lesion size (Ross 1995)

Sympathetic nerve activity is chronically elevated, not only in the heart as stated earlier, but also in the vasculature, with advancing age (Seals and Esler 2000). Although a reduction in β-receptor sensitivity to sympathetic firing has been suggested as a potential mechanism, a number of central factors have also been implicated, including NO. Nitric oxide synthase (NOS) isoforms are expressed within the central nervous system, and it is suggested that NO restrains sympathetic outflow (Thomas 2001, Fisher, Young et al. 2009, Young, Fisher et al. 2009). Increased ROS with age may attenuate the production and bioavailability of central NO as in the endothelium. With this reduced NO bioavailability, restraint of sympathetic nerve activity would be suppressed, therefore increasing sympathetic drive. Increased sympathetic nerve activity has also been identified as a contributor, causing increased vasoconstriction (α-adrenergic receptor stimulated) (Dinenno 2001). Dinenno examined the effects of local phentolamine infusion of the femoral artery, which blocks the α-adrenergic receptors, on resting femoral blood flow in healthy young men (mean age = 28 ± 2 years) and older men (mean age = 64 ± 2 years) (Dinenno 2001). Resting femoral artery blood flow and vascular conductance in the older group were significantly lower than the younger group. Local α-adrenergic receptor blockade with phentolamine significantly increased these variables in both groups; however this increase was significantly greater in the old compared with the young men. Importantly blocking sympathetic stimulation of the vascular smooth muscle (VSM) of the femoral artery accounted for approximately 75% of the reduction in resting blood flow in the elderly sample. This observation suggests that other mechanisms are involved with the higher levels of vasoconstriction and lower blood flow in

ageing. The remaining 25% of the difference is likely explained by the impaired NO production and bioavailability and changes in arterial structure.

2.7.2 Micro-vascular Ageing

In addition to the large arteries of the macro-circulation, the micro-circulation also exhibits age-related alterations. Cutaneous (skin) microcirculation is comparable to skeletal muscle microcirculation, and is more accessible than the skeletal muscle vasculature for assessment with non-invasive techniques. Tew et al. investigated the microcirculation of the skin in elderly sedentary and fit, and young sedentary individuals (Tew, Klonizakis et al. 2010). In response to a reactive hyperaemia test, young and elderly fit individuals had a significantly higher peak cutaneous perfusion compared to the elderly sedentary group. Iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP) paired with laser Doppler Flowmetry at the volar aspect of the forearm was also used to assess cutaneous blood flow. In response to ACh (endothelial-dependent dilator) and SNP (endothelial-independent dilator) administration, the young group had significantly higher peak flows compared to the elderly sedentary group also. This study shows that elderly individuals have an attenuated endothelial dependent dilator function in the cutaneous circulation compared to young sedentary counterparts. Schnackenburg et al. examined the renal microvasculature in rabbits, and suggested that ROS, more specifically superoxide caused vasoconstriction (Schnackenberg and Wilcox 2001). It appears that the microvasculature may also be affected by the age-related increase in ROS. It has been suggested that the microcirculation may be even more susceptible to the deleterious effects of ROS and inflammation due to the smaller size of the vessels (Wagenmakers, van Riel et al. 2006).

2.7.3 Consequences of Vascular Ageing

The consequences of macro- and micro-vascular dysfunction with ageing are multiple. Large artery dysfunction not only exposes individuals to an increased risk of hypertension and atherosclerosis, but also negatively impacts delivery of blood towards target organs (Ross 1995, Hansson 2001, Versari, Daghini et al. 2009). Micro-vascular dysfunction causes impairments in the delivery of oxygen and nutrients carried in the blood. In terms of skeletal muscle, a lack of oxygen and nutrient delivery has detrimental effects on exercise capacity. Age-related reductions in skeletal muscle blood flow are apparent, and may lead to reductions in exercise capacity (Proctor, Koch et al. 2004). Glucose and fatty acids are essential for energy metabolism of skeletal muscle. As well as energy metabolism, building of new muscle via protein synthesis is also affected. Amino acids are essential for muscle protein synthesis for repair of damaged muscle and also for building new muscle. Decreased delivery of amino acids results in a decrease of protein synthesis. This, coupled with protein breakdown, progressively results in muscle wasting, muscle weakness, and an increased risk of instability and frailty development (Marzetti, Calvani et al. 2013). Ultimately, age-related sarcopenia occurs, a muscle wasting disease.

2.7.4 Physical Activity and Vascular function

Physical activity in elderly populations has been associated with superior vascular function (Tew, Klonizakis et al. 2010). Tanaka et al. investigated large central artery compliance both cross-sectionally and via an exercise intervention (Tanaka, Dinenno et al.

2000). In the cross-sectional study 151 healthy men aged 18-77 years were examined and separated into groups of sedentary (n = 54), recreationally active (n = 45) and endurance trained (n = 53). Central artery stiffness measurements were acquired via B-mode ultrasound and applanation tonometry. Central arterial compliance was lower in middle-aged and older men compared to young men in all 3 activity groups. There were no significant differences in central arterial compliance between sedentary and recreationally active individuals at any age, however endurance trained middle-aged and elderly individuals had significantly higher arterial compliance compared to the other 2 activity groups of their age-matched counterparts. Following this cross-sectional study, central arterial compliance was examined before and after a 3-month aerobic exercise intervention in the sedentary middle-aged and elderly groups. Results showed that central arterial compliance was significantly increased in both groups following the intervention. Cross-sectional studies have demonstrated that endothelial-dependent dilation, which is dilation via NO release, is improved in older men who regularly perform aerobic exercise compared with their sedentary counterparts. Interventional studies have also shown that endothelial-dependent dilation is improved in previously sedentary older healthy men following a 3-month aerobic training programme of moderate intensity exercise (Seals, Desouza et al. 2008). This enhanced NO bioavailability and endothelial dependent dilation may be due to a reduction in ROS, which has been documented to be a result of aerobic exercise training and regular physical activity (Adams, Linke et al. 2005).

Evidence suggests that physical activity has beneficial effects on micro-vascular function with age as well as in the macro-vasculature. Tew et al. also included a fit elderly group when examining endothelial dilator function in the cutaneous circulation (Tew, Klonizakis et al. 2010). Elderly fit individuals had comparable peak flow in response to ACh

administration to young sedentary individuals, which was significantly higher than their sedentary elderly counterparts. This study not only shows that elderly individuals have an attenuated endothelial dependent dilator function, but also that fit elderly individuals maintain their dilator function in the cutaneous circulation.

Exercise training has been shown to reduce the development of atherosclerosis and endothelial dysfunction by a number of ways. Shear stress resulting from exercise training is known to upregulate endothelial-derived NO bioavailability and improve endothelial-dependent dilation (Tinken, Thijssen et al. 2010). An important long-term adaptation to exercise is the improvement in the resistance to oxidative stress. The enzyme superoxide dismutase (SOD) decomposes ROS and is significantly increased as a result of habitual physical activity (Metsios, Stavropoulos-Kalinoglou et al.). With reduced ROS, NO quenching decreases, allowing the conversion of L-arginine to NO resulting in vasodilation.

2.8 Cardiovascular Responses to Exercise

In order for exercise to be maintained, it is paramount that appropriate cardiovascular adjustments are made to meet the increased metabolic demand of exercising skeletal muscles. This is orchestrated by both central and peripheral neural mechanisms, including feed-forward signals from higher brain centres (i.e. central command) and reflex feedback from group III and IV sensory afferents within the working skeletal muscle in response to mechanical (i.e. muscle mechanoreflex) or metabolic stimuli (i.e. muscle metaboreflex) (Krogh and Lindhard 1913, Alam 1937, Coote, Hilton et al. 1971, McCloskey and Mitchell 1972, Kaufman, Longhurst et al. 1983), resulting in a complex integrated cardiovascular response. Central command represents parasympathetic (vagal) nerve activity responsible for

a reduction in HR, ventricular contractility (inotrophy), stroke volume and therefore cardiac output (Fisher, Young et al. 2015). Feedback reflexes including the muscle mechano- and metaboreflex constitute the exercise pressor reflex, and induce sympathetic nerve activity (SNA), activation of which increases HR (chronotropy) and ventricular contractility (inotropy).

Upon initiation of exercise, cardiac parasympathetic withdrawal occurs and explains the initial increase in HR observed. Sympathetic activation occurs shortly after parasympathetic withdrawal, allowing HR, ventricular contractility, stroke volume and CO to increase further (Fisher, Young et al. 2015). With regards to the vasculature, sympathetic activation causes vasoconstriction of areas of the body with less metabolic demand (e.g., the splanchnic circulation and non-exercising muscles), causing a redistribution of CO toward exercising skeletal muscles. The precise contribution of central command and skeletal muscle afferent feedback to these autonomic alterations is an area of intense research focus, particularly in age and disease (Fisher, Young et al. 2015).

Appropriate autonomic regulation of the cardiovascular system is vital for exercise performance. Populations who have autonomic dysfunction cannot perform even light exercise as failure of increasing central blood volume and cardiac output (CO) occurs, which can lead to exercise intolerance and even syncope (Marshall, Schirger et al. 1961). Conversely, high peripheral SNA can reduce skeletal and cardiac muscle blood flow in disease states and could contribute to exercise intolerance in these populations (Michelini, O'Leary et al. 2015). Ageing is associated with altered resting autonomic regulation, specifically a reduction in parasympathetic nerve activity and vasomotor sympathetic responsiveness (Barnett, Morin et al. 1999, Kuo, Lin et al. 1999). Reduced sensitivity of α-

receptors in the skeletal muscle vasculature and β-receptors in the heart are postulated to lead the higher basal SNA and sympathodominance in the elderly (Lakatta 1993, Smith, Voyles et al. 2007). The reduced sensitivity of cardiac β-receptors is also responsible for the reduced maximum heart rate observed with ageing, contributing to decreased exercise capacity. In the vasculature, an increased muscle vasoconstrictor tone could lead to a reduction in muscle blood flow, an augmented BP response, and again may contribute to the attenuated exercise tolerance (Lakatta 1993, Smith, Voyles et al. 2007).

A large body of scientific interest has been focused on the metaboreflex, due to its key role in cardiovascular regulation during exercise (Crisafulli, Salis et al. 2006, Boushel 2010, Crisafulli 2011). It is well established that the muscle metaboreflex increases SNA and BP (Alam 1937, Mark, Victor et al. 1985), however whether the latter is attributable to an increase in CO (O'Leary 1998, Crisafulli, Scott et al. 2003, Sala-Mercado, Hammond et al. 2006, Crisafulli 2011), total peripheral resistance (TPR) (Bastos, Williamson et al. 2000, Lykidis, White et al. 2008) or both (Bonde-Petersen 1982, Crisafulli, Salis et al. 2006) is equivocal in young populations, and certainly is not currently understood in ageing. To begin to investigate these mechanisms, the importance of understanding what occurs in ‘normal’ physiological functioning under stress is the first key step, (i.e. a healthy young population).

2.9 Summary of literature review

Life expectancy and the average age of the UK population are increasing; however elderly individuals are spending an increased number of years in a frailty-related diseased state. Ageing is coupled with the development of frailty and age-related diseases due to multiple function loss of the immune, musculoskeletal and cardiovascular system, reducing the quality of life of elderly individuals and inflating the costs needed for medical care. There is an age-related fall in physical activity, which may make a significant contribution to the multiple function loss observed with ageing (Rantanen, Guralnik et al. 1999). Conversely, it may be the loss of functioning that reduces our ability to perform physical activity as we age (Rantanen, Guralnik et al. 1999). Figure 2.7 presents a simplified representation of the complex relationship between ageing and cardiovascular dysfunction, and possible mechanistic associations. Ageing is observed with an increase in physical inactivity, inflammation and central and visceral adiposity. Each of these age-related variables has also been shown to be related with one another and may have causal mechanistic links, resulting in cardiovascular dysfunction.

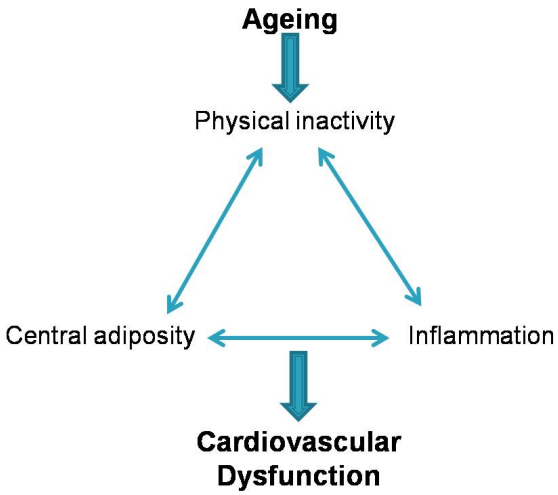


Figure 2.8: Age-related factors contributing to development of cardiovascular dysfunction.

Physical activity participation in the elderly has been associated with improved cardiovascular function both at the cardiac and vascular levels, and may prevail via an inflammatory and central adiposity lowering mechanism. Maintaining or increasing physical activity would be expected to prevent the age-related decline in physiological function. Reduced central adiposity and increased cardiovascular function increases physical functioning of elderly individuals, convalescing quality of life. At present there is limited information regarding the effects of physical activity on the function of multiple body systems in a large cohort of elderly individuals with a wide range of physical activity levels to thus elucidate which of these functions contribute to the development of components of frailty. The majority of research has focussed on separate entities of ageing (e.g. physical limitations, inflammation, immune function, cognition, body composition and cardiovascular function) as individual dependent variables of physical activity. This approach, therefore, fails to elucidate the complete holistic picture of the mechanisms by which gradual decreases in physical activity with ageing lead to the development of components of frailty. It is currently unknown what the associations are by which reduced habitual physical activity

leads to the components of frailty in the ageing population. Therefore, against this background the aims of this thesis are to:

- Determine the physical activity range of a sample of elderly individuals in the Birmingham and Black Country
- Investigate the associations between physical activity, cardiovascular function, inflammation and body composition in this cohort
- Investigate the associations between LV diastolic function, physical activity, inflammation and body composition in this cohort
- Better understand the neural mechanisms underpinning the ‘normal’ cardiovascular responses to exercise in a healthy young population

CHAPTER 3:

GENERAL METHODS

3.1 Ethical Approval

Ethical approval was granted from National Research Ethics Service (NRES) Committee West Midlands - The Black Country.

3.2 Design and Recruitment

A cross-sectional cohort study involving 211 60-80 year olds was performed. Recruitment into the Physical Activity and Healthy Ageing (PAHA) study was through the Primary Care Research Network for Central England (PCRN-CE). The PCRN-CE established first contact between members of the research team at the University of Birmingham and research nurses appointed to the larger general practitioner (GP) Surgery's in the Birmingham and Black Country (BBC) area. The total number of Primary Care Trusts (PCTs) in the BBC area covered by PCRN-CE is 54 and the total number of GP surgeries is 432. GP surgeries complete a search using the inclusion criteria (see below) using the updated former Midland Research Practices Consortium database. Through this route, the PCRN-CE suggested that for every 10 surgeries enrolled around 3000 eligible candidates would be identified. Of this, approximately 10-15% would be interested and 50% of these recruited. Thus for every 10 surgeries enrolled approximately 150 – 200 participants would be recruited. Each PCT was associated with a Townsend score provided to the research team by the PCRN-CE, which is a socio-demographic indication of the local area. This score was used in selection of which GP surgeries to approach in order to recruit approximately equal numbers of individuals from differing socioeconomic backgrounds.

Eligible candidates were approached by their GP surgeries with an invitation letter explaining the aims of the study and a brief summary of the experimental protocol the participants would be involved in. Those with a potential interest were asked to complete a reply slip and return it by freepost to the researchers at the University of Birmingham. Potential participants were then contacted by the research team and the study procedures and aims explained in detail by phone and/or email. Participant information sheets were then sent by post to those that confirmed interest. Candidates were then invited to the Wellcome Trust Clinical Research Facility (WT-CRF) at the Queen Elizabeth Hospital Birmingham. Through this recruitment process a total of 211 participants were recruited into the study (Figure 3.1).

Inclusion Criteria:

1. Aged 60-80 years old
2. Able to walk for 2 minutes or 50 metres without stopping
3. Able to provide written informed consent

Individuals were ruled out of participation if they met any of the exclusion criteria:

Significant medical history in the last 3 years of:

1. Dementia, Parkinson disease, stroke, TIAs, liver disease, cancer
2. Heart Disease, specifically previous myocardial infarct, cardiomyopathy or valvular disease
3. Chest pain (angina pectoris). History of recurrent (no more than 1 or 2 episodes per month over the last 6 months) or sudden chest pain typically radiating to the left arm

or left side of the neck (unless associated with recent cold, cough or episode leading to bruising

4. Blood Pressure >190/120 or tendency to faint
5. Pulmonary disease including asthma/COPD leading to significant lung function loss and prescription of corticosteroids. Other medication is permitted.
6. Rheumatoid or osteoarthritis leading to severe stiffness and exercise intolerance (mild stiffness is allowed if exercise criterium above is met.
7. Current use of corticosteroids
8. Type 1 or 2 diabetes (raised blood sugar when measured on capillary sample is allowed)
9. History of leg pain on exertion, sufficient to limit walking ability to less than 50 metres or <2 mins
10. Any cause to consult the G.P. or report of feeling unwell in the previous 10 days
11. Inability to give informed consent

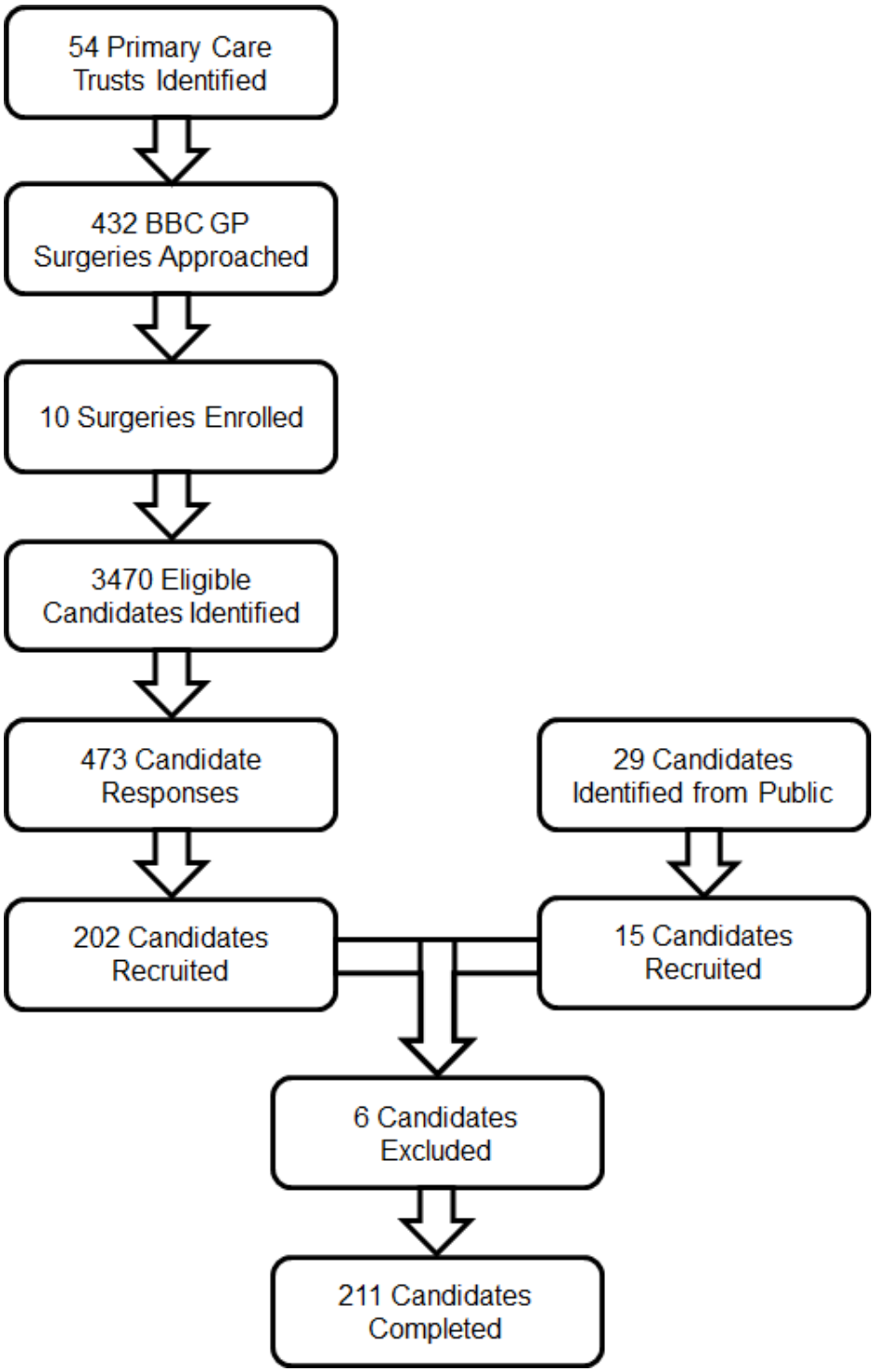


Figure 3.1: Recruitment process of participants into the PAHA study from the Birmingham Black Country (BBC) area.

3.3 Measured Variables

3.3.1 Anthropometric Measurements

Body weight was determined using a calibrated electronic scale, and height was measured with a portable stadiometer, both of which were performed with the participant in the standing position facing backwards during the height measurement and forwards during the weight measurements; and wearing light clothing and no shoes. Values were rounded to the closest centimetre and 100g. From these measurements, BMI (kg/m^2) was calculated using the following equation:

$$BMI = mass\ (kg^2) \div height\ (m)$$

Waist and hip circumference was measured using a flexible graduated measuring tape with the participant in the standing position and the tape placed against the skin.

3.3.2 Fasting Blood Glucose

Participants were asked to not consume any food or drink other than water in the 10-hours prior to arrival at the clinic. Blood glucose was measured using a calibrated monitor (Accu-Check Performa). Following cleansing of the selected finger with a 70% alcohol wipe, the skin was punctured using an Accu-Check Lancet, and the blood allowed to form a small globule. This initial blood sample was cleaned away and a second blood sample allowed to develop, which was used to assess blood glucose. If fasted blood glucose levels were below the General Medical Council (GMC) recommendations of risk of diabetes (7mmol/L), participants were accepted into the study.

3.3.3 'Office' Blood Pressure and Heart Rate

After the participants were seated for at least 15 minutes 'office' BP and HR measurements were taken using a GH Pro200 Dinamap sphygmomanometer, previously validated (O'Brien, Pickering et al. 2002), performed by the WT-CRF research nurses. A BP cuff was wrapped round the upper left arm of each participant, ensuring the artery index was positioned over the brachial artery. Participants were asked to have their feet flat on the floor with their measurement arm in a relaxed, supinated position. A minimum of 3 measurements were taken and the mean calculated.

3.3.4 Blood Sample for Plasma Biomarkers

In the fasted state, participants had approximately 40 ml of venous blood taken from a vein at the antecubital fossa into evacuated vacuum tubes coated with either sodium heparin or EDTA as an anticoagulant or clotting factor for serum (Becton-Dickinson, Oxford, UK). Plasma and serum was frozen at -80°C for later analysis. Peripheral blood mononuclear cells were isolated using density gradient centrifugation and frozen in fetal calf serum (FCS) + 10% dimethyl sulfoxide (DMSO) and stored at -80°C for future analysis of cell compositions. Complete differential counts of EDTA treated whole blood were completed using a calibrated coulter counter within 3 hours after bloods were taken. All blood samples were taken by a phlebotomy trained research nurse.

3.3.5 Assessment of Body Composition

Total and regional bone and body composition assessments were made using dual-energy x-ray absorptiometry (DEXA) and peripheral quantitative computed tomography (pQCT). Both techniques provide information on bone and body composition from minimal radiation exposure and were completed by NHS radiographers. Specifically assessments of total body fat percentage, estimated visceral fat mass, and total lean mass was gained by the DEXA scan. Cross-sectional area, density and muscle mass of the distal radius and tibia was gained by the pQCT. The principle of DEXA is the measurement of transmission of x-rays with high and low energy photons within the body. The different tissues i.e. bone, muscle, fat and skin have different chemical compositions and different densities. X-rays with the same photon energy will pass through the different tissues at different transmissions, so detection of bone, muscle and fat mass that composes the body is enabled (Crabtree, Leonard et al. 2007). Arm and leg muscle cross-sectional area and muscle density were measured using pQCT.

3.4 Resting Cardiovascular Function

3.4.1 Heart Rate and Blood Pressure

Participants were laid in the supine position. A standard 3-lead electrocardiogram (ECG) (Morgan 509, P.K. Morgan Ltd, Rainham, Kent, England) was used to measure HR. BP was measured in the left brachial artery using a standard automated device (Ohmeda 2300 Finapress, Amsterdam, Netherlands) previously validated (Collen, Manhem et al. 2012), whilst beat-to-beat BP was measured using finger photoplethysmography. Participants were

allowed to rest in the supine position for 3 minutes before data collection commenced, and a total of 5 BP measurements were taken during the 5 minute data collection period. HR and beat-to-beat BP were converted from analogue-to-digital signals at 1 kHz and captured for offline analysis (Chart v5.2 and Powerlab, ADInstruments, Bella Vista, NSW, Australia). Participants were asked to relax and breathe normally, and not talk for 5 minutes.

3.4.2 Arterial Stiffness

The most widely used techniques at present due to their good reproducibility and ease of use are pulse wave analysis (PWA) and PWV (Wilkinson, Fuchs et al. 1998). Non-invasive assessment of PWA and PWV were recorded using an applanation tonometer (SphygmoCor Px, ScanMed Medical Instruments, UK). Whilst participants remained in the supine position, brachial BP (mmHg) and distance from the suprasternal notch to recording sites (mm) were measured. Recording sites included the location at which the pulse could be felt from the radial artery at the radial notch, carotid artery at the neck and femoral artery at the groin. Following this the pressure transducer was applied to the desired anatomical locations. Central (cPWV) and peripheral (pPWV) pulse wave velocity and aortic AI_x produced (Hecker, Qiu et al. 2004). For cPWV pressure waveforms at the carotid and femoral arteries were assessed whilst for pPWV the carotid and brachial arteries were assessed. Timing of the produced waveforms were matched to the R wave of the concomitant 3-lead ECG measurements in order to control and normalize to HR, with the arterial path length and values obtained expressed as blood flow in meters per second (m/s). AIx was measured at the radial artery only and is expressed as a percentage (%) of how much the original waveform is augmented from the reflected pressure wave. Several measurements

were taken at each site, and the highest 3-5 measurements in terms of signal quality (i.e. operator index ≥80) were used for analysis, and averaged.

3.5 Left Ventricular Function

Resting LV cardiac function was assessed by Doppler ultrasound echocardiography using an iE33 ultrasound system capable of both 2D and 3-dimensional (3D) images with an X5-1 transducer probe (Philips Medical System, Surrey, UK) (Hascoet, Brierre et al. 2010). All images were obtained and analysed by an experienced sonographer (myself) and stored on an external storage device for offline analysis using the Xcelera and QLab software system. Three standard limb lead spot electrodes were placed on the chest to obtain HR. All 2D measures were collected over 3 consecutive cardiac cycles, using a sample volume of 15mm. Participants laid in a semi-supine position leaning towards the left hand side, with the left arm resting above the head, and supported by cushions to ensure maximum comfort. This position results in the rib cage opening at the apex, widening the echocardiographic window allowing the best quality images. The Doppler probe was placed at 2 different locations on the chest for different images. For apical 4 and 5 chamber images, the probe was placed between the 9^{th}-12^{th} rib spaces. For parasternal long-axis images, the probe was placed just left of the sternum in the mid-chest area. A small amount of transduction gel was placed on the Doppler probe in order for images to be transduced.

3.5.1 Aortic Outflow

To quantify flow through the aortic valve during systole, an apical 5 chamber view was used (Brothers 2008). The velocity profile of the aortic outflow was obtained using pulsed-wave Doppler. Sampling of the flow was taken over the aortic valves at the opening of the aortic orifice. The flow was quantified automatically using the velocity time integral (VTI), which represent the mean distance through which blood travels in the aortic outflow tract during ventricular contraction. Aortic root diameter was measured in the parasternal long-axis view. During post-acquisition analysis SV and CO were calculated using the following formulae (Lewis, Kuo et al. 1984):

$SV\ (ml) = VTI\ (ms) \times \text{aortic root area}\ (cm^2)$

$CO\ (L \cdot min^{-1}) = SV\ (ml) \times HR\ (b \cdot min^{-1})$

3.5.2 Mitral Inflow Velocities

Mitral inflow velocities were assessed from the apical 4 chamber view using pulsed wave Doppler positioned over the mitral valve leaflet tips. From these, data measurements were obtained of the peak inflow velocity during the early phase of LV relaxation (E), which provides an index of LV passive relaxation (Hatle 1993, Cohen 1996, Brothers 2008), and during LA contraction (A), which provides an index of LV compliance and LA systolic function (Cohen 1996, Brothers 2008). These values were subsequently used to further assess diastolic function by calculation of the E/A ratio.

3.5.3 Tissue Doppler Imaging (TDI)

Measurements of peak septal mitral annular early diastolic (E'), late diastolic (A'), and systolic (S') velocities were obtained to provide indices of left ventricular diastolic function (Prasad, Popovic et al. 2007), left atrial systolic function (Nagueh 2001), and left ventricular systolic function (Brothers 2008), via standard TDI techniques (Prasad, Popovic et al. 2007). TDI measurements were obtained from the apical 4 chamber view, with the pulse wave positioned at the junction of the septal mitral annulus and the left ventricular wall. The E' value was subsequently used for a calculation of E/E' ratio, which is an indication of LV filling pressure (Nagueh, Middleton et al. 1997).

3.5.4 Left Ventricular Dimensions

In the parasternal long axis, LV dimensions (Inter-ventricular septum diameter, LV internal diameter during systole and diastole, LV posterior wall diameter) were measured using M-mode Doppler, positioning the Doppler wave just below the mitral valve tips. LV mass was calculated according to Devereux formula (Devereux and Reichek 1977), and EF was also calculated.

3.5.5 3D Doppler Ultrasound Imaging

An apical 4 chamber caption was collected over 4 consecutive cardiac cycles, which are then stitched together to make 1 full cardiac cycle of the whole heart. From this, end diastolic LV volume, end systolic LV volume, ejection fraction, stroke volume, cardiac output, end diastolic LV mass, and end systolic LV mass were measured.

Table 3.1 Reproducibility Study of Echocardiography Measurements

Values are presented as mean ± SD in 5 participants.

Variable	Visit 1	Visit 2	Paired T-Test	Coefficient of Variation (%)
2D				
EF (%)	68.12 ± 3.9	59.66 ± 3.7	0.198	11.8
FS (%)	38.5 ± 3	32 ± 2.6	0.177	16
IVRT (s)	0.078 ± 0.006	0.066 ± 0.01	0.305	33.7
E/A ratio	2.2 ± 0.3	1.9 ± 0.2	0.438	12.3
SV (ml)	51.5 ± 2.7	52.5 ± 3.3	0.85	10.3
3DQ				
EDV (ml)	127.4 ± 16	121 ± 9.7	0.525	8.4
ESV (ml)	56.1 ± 10	54.4 ± 7.1	0.806	14.1
ES Mass (g)	157.6 ± 43	165 ± 37	0.471	11
ED Mass (g)	122 ± 25	112 ± 18	0.427	7.5
3DQ adv				
EDV (ml)	128.2 ± 10.3	126.3 ± 16.5	0.9	10.5
ESV (ml)	56.25 ± 9	52 ± 10.6	0.327	8
EF (%)	56.8 ± 4	59.6 ± 5.5	0.389	4
SV (ml)	72 ± 5	73 ± 26	0.919	15

Reproducibility tests of echocardiography measurements were performed in order to elucidate which measurements would be most reliable. Physiological relevance was also taken in to account when deciding upon which measurements were used for analysis. Generally, a coefficient of variation (CoV) % of ≤5% is considered highly reliable, whereas a CoV ≥10% is considered less reliable. IVRT is particularly challenging to both measure and analyse. IVRT has a CoV of 33.7%, indicating that it should not be used as a measure of LV function.

All Echocardiography data were compared to recommended guidelines from the British Society of Echocardiography (Appendix 3). Appropriate referral was carried out if clinical values were above or below the cut-off points, once technical issues had been ruled out (e.g., questionable image quality). E/A ratio, which is calculated from mitral inflow velocities was the measurement of LV diastolic function used in Chapter 5 of this thesis. Chapter 6 more extensively investigated cardiac mechanics and presents SV, CO, E wave, A wave, E/A ratio, E' wave, A' wave and E/E' ratio.

3.6 Functional |Mobility

Functional mobility was assessed using the 6 metre timed-up-and-go (TUG) test as previously described (Thrane, Joakimsen et al. 2007, Nordin, Lindelof et al. 2008). Briefly, participants were required to stand from a seated position on a chair with arm rests, walk (at normal walk pace) a straight line distance of 3m, turn 180° around, walk back to the chair and successfully sit down. Participants were timed to completion of the protocol and the time was recorded. Functional manoeuvring ability was assessed using the Berg Balance Scale (BBS) (Muir, Berg et al. 2008, Muir, Berg et al. 2010). Briefly, the BBS involves the participant performing a number of balance related tasks and their performance being graded against specific criteria (e.g., amount of time a task takes, amount of distance participant can reach forwards) by the researcher, and recorded. These measurements were carried out by David Bartlett of the School of Immunity and Infection, so as to eliminate inter-observer variability. Selection bias was also eliminated as researchers were not aware of the physical activity status of participants at the time the measurements were taken. Physical activity was recorded in the seven days following the assessment day.

3.7 Assessment of Physical Activity Status

Physical activity (PA) status was determined from seven continuous days of accelerometer wear (GT3X, Actigraph, FL, USA). Participants wore the GT3X around the waist above the non-dominant leg for seven days, except for when bathing (including water based activities). The GT3X was programmed to record data in three orthogonal planes (vertical [VT], antero-posterior [AP] and medio-lateral [ML]) every 60-seconds. Tri-axial vector magnitude (VM) was calculated from the total counts of each of the movement planes using the formula $VM=\sqrt{(VT^2 + AP^2 + ML^2)}$. VM has recently been shown as a reliable measure of PA and is thought to represent a more realistic measure of physical movements (Hazeldine, Hampson et al. 2012). Total time and percentage of time spent in sedentary, light, moderate, vigorous and very vigorous activity levels was assessed using cut-offs previously described (Freedson, Melanson et al. 1998). Other variables collected and computed by the software include energy expenditure, steps and mean metabolic equivalents (METs).

3.8 Plasma Analysis

3.8.1 Inflammatory Cytokines

Detection of serum free cytokines was measured using commercially available XMap multiplex Luminex kits (Bio-Rad, Hemel Hempstead, UK) according to manufacturer's instructions. One kit was specific for type 1 cytokines which contained combinations of the following cytokines (IL-1b, IL-4, IL-6, IL-10, IL-13, IL-17A, TNFα, MCP-1 and vascular endothelial growth factor [VEGF]) whilst the second kit measured macrophage migration inhibitory factor (MIF). Briefly, antibody coupled magnetic beads were added to a 96-well greiner NUNC plate. Serum was first centrifuged at 10,000 x g to remove platelets and cell debris and then diluted 1:4 with assay specific buffer before addition of 50 µL of sample and standards to each well. Plates were then sealed, foil covered and incubated for 30 minutes on an orbital shaker at 350 rpm. Following incubation, washing was performed 3 times before addition of the biotinylated detection antibody and incubated as before. Following incubation, washing was performed and streptavidin-RPE, which binds the detection antibody, was added and incubated as before but for exactly 10 minutes. Following washing and re-suspension plates were read on a Luminex200 instrument (Luminex Corp, Austin, TX, USA). The system identifies and quantitates cytokines based on bead colour and RPE fluorescence. The concentration of the cytokines is determined and automatically calculated by the Bio-Plex ManagerTM (Bio-Rad, Hemel Hempstead, UK) software using a standard curve derived from the recombinant cytokine standard.

3.8.2 C-Reactive Protein

Detection of serum free C-RP was determined by a commercially available high-sensitivity Enzyme-Linked Immunosorbent Assay (ELISA) (IBL International, Hamburg,

Germany). Serum samples were centrifuged at 2,500 xg for 10 minutes to remove platelets before being diluted 1:1000 in kit dilution buffer. Wells pre-coated with monoclonal anti-C-RP antibodies were incubated with 100 µL of either test serum or standards (0 – 10 $mg.L^{-1}$). Plates were covered with adhesive film and incubated for 30 minutes. Following incubation, plates were washed 3 times using kit wash buffer. After patting excess fluid from the plate 100 µL of C-RP conjugated to horseradish peroxidise (HRP) was added and incubated for 30 minutes at RT. Wells were washed as before and 100 µL of chromogen solution containing 3,3',5,5'-tetramethylbenzidine (TMB) was added and incubated for 10 minutes at RT in the dark. Following incubation 50 µl of the stop solution containing 0.5M sulphuric acid (H2SO4) was added to each well.

3.8.3 Adiponectin and Leptin

Detection of plasma adiponectin/Acrp30 and leptin were completed independently using sandwich ELISA kits (R&D Systems, Abingdon, UK). Briefly, mouse anti-human adiponectin or leptin capture antibodies were diluted in sterile PBS to working concentrations of 2, 4 and 4 $\mu g.ml^{-1}$ respectively. Wells were incubated overnight at RT with 100 µL capture antibodies. Following incubation wells were washed 3 times in 400 µL wash buffer (PBS/0.05% Tween® 20). Non-specific binding was reduced by blocking the wells with 300 µl of reagent diluent [PBS + 1% protease free BSA (Fisher Scientific, Loughborough, UK)] for 1 hour at RT. Following this blocking plates were washed 3 times. Leptin (0 – 2000 $pg.ml^{-1}$) and adiponectin (0 – 4000 $pg.ml^{-1}$) standards and test plasma were diluted in reagent diluent before incubating duplicates of 100 µL for 2 hours at RT. Plates were then washed 3 times. Biotinylated mouse anti-human adiponectin, or leptin detection antibodies were diluted

in reagent diluent and 100 µL per well incubated for a further 2 hours at RT. Following incubation the washing was repeated before incubating wells with 100 µL of HRP conjugated Streptavidin for 20 minutes at RT in the dark. Following incubation the washing was repeated before addition of 100 µL TMB substrate solution and incubated for 20 minutes at RT in the dark. Reactions were stopped by addition of 50 µL 2N H2SO4 (Sigma-Aldrich).

3.8.4 Plasminogen Activator Inhibitor-1

Detection of plasma plasminogen activator inhibitor-1 (PAI-1) was completed using a sandwich ELISA kit (Life Technologies). Briefly, wells pre-coated with anti-PAI-1 antibodies were incubated with 100 µL of standards and samples diluted 1:2 in the appropriate buffer provided, covered and incubated for 2 hours at RT. Following incubation, plates were washed 4 times using the kit wash buffer. After patting excess fluid from the plate 100 µL of biotinylated human PAI-1 biotin conjugate solution was added to each well (except the blank) and incubated for a further 2 hours as before. Plates were washed again before 100 µL of streptavidin-HRP working solution was added to each well and plates again covered and incubated at RT for 30 minutes. Following a similar wash, wells were incubated with 100 µL of chromagen solution in the dark for 30 minutes at RT before stopping the reaction with 100 µL of stop buffer.

3.8.5 Macrophage Migration Inhibitory Factor and Endothelin-1

Detection of serum MIF and plasma endothelin-1 were completed independently using sandwich ELISA kits (Abcam®, Cambridge, UK). Briefly, wells pre-coated with anti-

MIF or endothelin-1 antibodies were incubated with 100 µL of standards and samples diluted 1:2 in the appropriate buffer provided, covered and incubated for 1 hour at RT. Following incubation, plates were washed 5 times using the kit wash buffer. After patting excess fluid from the plate 100 µL of HRP conjugated MIF or endothelin-1 working solution was added to each well and plates again covered and incubated at RT for 30 minutes. Following a similar wash, wells were incubated with 100 µL of chromagen solution in the dark for 30 minutes at RT before stopping the reaction with 100 µL of stop buffer.

All plasma biomarkers were analysed by David Bartlett of the School of Immunity and Infection in the research laboratory of the Queen Elizabeth Hospital.

3.9 Candidates contribution to experimental measures obtained

The multi-disciplinary nature of the study meant that data collection was a team effort between myself, my colleague from the School of Immunity and Infection (David Bartlett), and the Wellcome Trust Clinical Research Facility (WT-CRF) nurses (mentioned in the acknowledgements). With respect to my personal contribution to the general methods, I was involved with: logistical planning of the measurements; participant information sheet design; participant recruitment; resting HR via 3-lead ECG, BP and beat-to-beat BP; arterial stiffness via PWA and PWV, LV function via Doppler ultrasound echocardiography; and analysis of these measurements. There was no imputation of missing values throughout the measurements.

CHAPTER 4:

PHYSICAL ACTIVITY AND HEALTHY AGEING (PAHA):

THE INFLUENCE OF PHYSICAL ACTIVITY ON PHYSICAL FUNCTION, BODY COMPOSITION, INFLAMMATORY STATUS AND CARDIOVASCULAR FUNCTION IN AN ELDERLY COHORT

4.1 Abstract

Age-related frailty is a multi-physiological syndrome affecting body composition, the musculoskeletal system, the immune system and the cardiovascular system. A reduction in habitual physical activity with increasing age has been proposed as one of the factors that make a substantial contribution to the development of frailty.

The aim of this study was to test the hypothesis that elderly individuals who have high habitual physical activity levels will have superior physical functioning, lower total body and visceral fat mass, better cardiovascular function, and lower concentrations of plasma biomarkers regarded to play a role in the mechanisms leading to cardiovascular disease.

To test this hypothesis, a cohort of 211 elderly individuals was recruited. Their mean age, height and weight (mean ± standard deviation) were 67 ± 5 years, 167 ± 10 cm and 73 ± 14 kg, respectively. Measures of physical function (BBS, TUG test), body composition (DEXA), plasma proteins and peptides which are known to play a role in the mechanisms that lead to CVD (PAI-1, MIF, endothelin-1, C-RP, IL-1β, IL-4, IL-6, IL-10, IL-13, IL17a, MCP-1, TNF-α, VEGF) and cardiovascular function (HR, BP, central pulse pressure, central and peripheral arterial stiffness) were obtained. Based on a 7-day assessment of physical activity using accelerometry participants were split into quintiles (1 = 20% least active, 5 = 20% most active).

The difference in physical activity level between the lowest and highest quintile was >2 fold ($P<0.05$). Individuals in the second highest quintile had significantly faster walking speeds and TUG times than those in the lowest quintile (2.9 ± 0.6 vs. 2.5 ± 0.5s ; 7.7 ± 1.4 vs. 8.9 ± 1.7s; $P=0.02$. Total body fat, % body fat and estimated visceral fat mass were significantly lower in the highest 3 quintiles compared to the lowest, with 28% lower total

body fat, 8% lower % body fat, and 53% lower estimated visceral fat mass in the highest quintile compared to the lowest quintile. Habitual physical activity did not significantly influence plasma proteins and peptides, with the exception of PAI-1 (a clotting agent) which was lower in 1-3 quintiles compared to quintile 5(9.3 ± 6.6, 9.8 ± 7.3, 9.2 ± 6.6 vs. 13.8 ± 11.4 ng/mL; $P<0.05$). Indices of cardiovascular function were not significantly different between the quintiles.

In this cohort study physical function loss, visceral adiposity and PAI-1 concentration were attenuated by physical activity. However, lean muscle mass, inflammation and oxidative stress, and cardiovascular function were unaltered by physical activity. Despite these null findings, the data in the current study support the hypothesis that high daily physical activity levels is negatively associated with some of the age-related function losses that contribute to frailty in elderly individuals.

4.2 Introduction

It is expected that by the year 2035, one in four individuals worldwide will be aged 65 years or over (Lakatta 2002). With the observed increase in the mean age of the world population, an increase in life expectancy also exists (Branch, Guralnik et al. 1991, Guralnik, Land et al. 1993, Leveille, Guralnik et al. 1999). However, this increase in life expectancy is not coupled with an increase in healthy well-years, so a majority of these years are spent in an unhealthy frail state (Fulop, Larbi et al. 2010). Frailty is loosely described as a general decline in the functional ability of multiple bodily systems (Wilson 2004), and includes dysfunction of the brain (cognition), musculoskeletal system, immune system and cardiovascular system. Fried et al. conducted, defined and validated 5 attributes of reduced physical function that results in frailty onset: unintentional weight loss, muscle weakness, slow walking speed, exhaustion and a low physical activity (Fried, Tangen et al. 2001).

Elderly individuals who lead physically active lifestyles are reported to offset the development of frailty and indeed morbidity and mortality (Gill, Baker et al. 2002, Gill, Baker et al. 2004, Wilson 2004, Chakravarty 2010, Chou, Hwang et al. 2012). For example, Chakravarty et al. conducted a longitudinal, self-administered disability questionnaire-based study in 284 runners and 156 sedentary individuals aged 50 years and older (Chakravarty 2010). Over the 21-year follow up period elderly runners had significantly lower self-declared disability scores and lower mortality rates than the sedentary age-matched controls. The beneficial effects of exercise on frailty have also been shown in interventional studies (Fiatarone, O'Neill et al. 1994). From these studies it is clear that regular physical activity and exercise can reduce and even reverse components of frailty in elderly individuals. Contrary to this, evidence is also available that shows that physical activity can be ineffective and even

harmful in elderly individuals (Latham, Anderson et al. 2003, Moayyeri 2008). Latham et al. failed to observe a positive effect of a 10-week home-based quadriceps resistance intervention on physical health, falls or physical performance in 243 frail individuals, and in addition found the individuals were at an increased risk of musculoskeletal injury (Latham, Anderson et al. 2003). Moayyeri conducted a meta-analysis of 13 cohort studies in elderly individuals with hip-fracture end-points (Moayyeri 2008). It was concluded that there is an increased risk of falling in elderly individuals that are either sedentary or highly physically active compared to those who perform moderate physical activity. Thus the mode and intensity of physical activity in these studies and the increased fall-risk during high intensity exercise may explain the conflicting results, in addition to the health and age of the participants (Keysor 2003). Despite confounding findings, general consensus suggests the balance of evidence tips overwhelmingly towards beneficial effects of physical activity on the onset of age-related frailty. Given the diverse stimulus that physical activity provides and the complex nature of frailty, the exact mechanisms by which physical activity elicits a beneficial effect in the elderly is unclear.

Important factors thought to be key to the development of components of frailty are increased levels of inflammatory cytokines (inflammageing) and presence of inflammatory diseases. Fried et al. reported significantly higher levels of inflammatory cytokines in further healthy frail individuals, particularly IL-6, TNF-α and CRP (Fried, Tangen et al. 2001). Inflammatory cytokines (e.g., IL-1, IL-4, IL-6, TNF-α) are known to contribute to the production of ROS. Evidence suggests age-related increases in concentrations of circulating inflammatory cytokines and ROS may contribute to both cardiovascular and skeletal muscle dysfunction (Pawelec 2002, Panda, Arjona et al. 2009). Several studies have however shown the beneficial effects of physical activity on inflammatory status in elderly individuals

(Mattusch, Dufaux et al. 2000, Taaffe, Harris et al. 2000, Geffken, Cushman et al. 2001, Ford 2002). Physical activity has also been shown to offset the age-related decline in cardiovascular function (Franzoni, Plantinga et al. 2004). Here, the authors concluded that chronic aerobic training is associated with preserved endothelial function in the microcirculation and better antioxidant defences leading to a reduction in oxidative stress (Franzoni, Plantinga et al. 2004). Taddei et al. demonstrated this mechanism when examining endothelial function in whole limb forearm blood flow (Taddei, Galetta et al. 2000). Twelve young and elderly healthy sedentary) and 11 young and 14 elderly matched athletes were studied whereby forearm blood flow measured via strain-gauge plethysmography was examined during infusion of ACh (endothelial-dependent dilator) and N^G-monomethyl-$_L$-arginine (L-NMMA), an NO synthase inhibitor. NO is a potent endothelial dependent vasodilator as explained in Section 2.7.1. In young sedentary subjects and young athletes, the blood flow response to ACh was blunted by L-NMMA. In both elderly subjects, the blood flow response to ACh was significantly lower than in both young groups. However, in elderly sedentary groups the blood flow response to ACh was resistant to L-NMMA. These results suggest that regular physical activity in elderly individuals can at least in part prevent the age-induced development of endothelial dysfunction, probably due to the restoration of NO availability consequent to prevention of production of oxidative stress via increase anti-oxidant defence. It is also well established that with increased age there is a greater proportion of central adiposity (Poehlman, Toth et al. 1995, Song 2004). One of the many detrimental effects of increased central adipose tissue is the production of inflammatory cytokines and plasma proteins and peptides related to cardiovascular risk (IL-6, IL-8, TNF-α, PAI-1, MIF) (Hotamisligil, Arner et al. 1995, Purohit, Ghilchik et al. 1995, Yudkin, Kumari et al. 2000, Fain 2006). Indeed, one potential cause of increased age-related inflammation is

this increase in central adiposity (Wu 2007). As discussed above, physical activity has been shown to reduce age-related inflammation. It could be postulated that the inflammation lowering effects of physical activity in elderly individuals could be due to lowering central adiposity, therefore reducing production of these inflammatory markers. Geffken et al. (2001) conducted an epidemiology study analysing data obtained from the Cardiovascular Healthy Study in 5,888 elderly men and women, investigating the associations between self-reported physical activity levels, inflammatory markers and BMI (Geffken, Cushman et al. 2001). They concluded that, following multivariate analysis, higher levels of physical activity were associated with lower levels of inflammation, which may be mediated by BMI.

Ageing is coupled with a functional decline of the cardiovascular system. The prevalence of cardiovascular disease (e.g., hypertension, atherosclerosis) increases with advancing age (Lakatta 2002). Preceding the development of these age-related diseases is vascular fibrosis and the stiffening of the arteries (Avolio, Chen et al. 1983, Benetos, Laurent et al. 1993, Frenneaux and Williams 2007). Fibrosis describes an increased deposition of connective tissue in the arterial wall (Najjar, Scuteri et al. 2005). This contributes to a reduced arterial distensibility in the periphery, pulse wave amplification and an increase in BP (Lakatta and Levy 2003). Large arteries thus become more prone to shear stress-related endothelial damage. This signals a cascade of inflammatory responses resulting in atherosclerotic plaque formation. The process of fibrosis and atherosclerotic plaque development with age has been associated with age-related increases in inflammation and oxidative stress (Ross 1993, Alexander 1994, Geffken, Cushman et al. 2001). The apparent increase in arterial stiffness observed with ageing is not only due to structural changes in the vessel wall, but also to impaired endothelial regulation of the vascular smooth muscle surrounding the arteries (Lakatta and Levy 2003). NO, an endothelial dependent vasodilator

is quenched by ROS forming peroxynitrite. Decreased availability of NO attenuates vasodilation in response to shear stress (Beckman and Ames 1998, Seals, Desouza et al. 2008, Versari, Daghini et al. 2009). Conversely, shear stress resulting from exercise training is known to upregulate endothelial-derived NO and improve endothelial-dependent dilation (Tinken, Thijssen et al. 2010). A combination of age-associated increases in fibrosis, inflammation and oxidative stress results in vascular dysfunction, increasing the risk of diseases such as hypertension and atherosclerosis.

Regular exercise and physical activity can improve cardiovascular function in elderly individuals. Interventional studies have shown that endothelial-dependent dilation is improved in previously sedentary older healthy men following a three-month aerobic training programme of moderate intensity exercise (Seals, Desouza et al. 2008). Such enhanced endothelial dependent-dilation and NO bioavailability may be due to a reduction in ROS, which has been documented to be a result of aerobic exercise training and regular physical activity (Adams, Linke et al. 2005). To reiterate what was shown by Geffken et al., a reduction of ROS via regular physical activity in elderly individuals may be a consequence of lower central adiposity (Geffken, Cushman et al. 2001). Thus it appears that the positive effects of physical activity on cardiovascular function in elderly individuals results from parallel reductions in central adiposity, inflammation and oxidative stress. Reducing the inflammatory burden of elderly individuals could also be linked to the positive effects physical activity has on other frailty attributes (Fulop, Larbi et al. 2010). Often occurring without diagnosis, the pathology of frailty in parallel leads to many age-related chronic diseases (e.g., metabolic syndrome, type 2 diabetes, many inflammatory diseases, autoimmune diseases, Alzheimer's and cardiovascular disease) thus therapeutic strategies to increase physical activity would be expected to benefit elderly individuals by improving or

maintaining physiological function and a high quality of life (Fried, Tangen et al. 2001, Boyd, Xue et al. 2005, Bergman, Ferrucci et al. 2007, Landi, Abbatecola et al. 2010).

Although the associations between physical activity, central adiposity, inflammation and cardiovascular function in elderly individuals have individually been previously identified as independent variables, there is presently limited information regarding the effects of physical activity on the function of multiple body systems in a large cohort of elderly individuals with a wide range of physical activity levels to thus elucidate which of these functions contribute to the development of components of frailty, as discussed in Section 2.9. This approach, therefore, fails to elucidate the complete holistic picture of the mechanisms by which gradual decreases in physical activity with ageing lead to the development of components of frailty. Therefore, the aim of the physical activity and healthy ageing (PAHA) study is to fill this current gap in knowledge.

4.3 Aims and Hypothesis

The aim of this study was to investigate whether higher levels of physical activity are associated with elderly individuals superiorphysiological functioning. To achieve this, in a large cohort of elderly individuals physical activity, physical function, body composition, inflammatory status, and cardiovascular function including resting HR, BP, central pulse pressure and large artery stiffness was assessed.

It was hypothesized that compared to physically inactive elderly individuals:

1) Elderly individuals that are more physically active would have better physical functioning, such as faster walking speeds and superior balance.

2) More physically active elderly individuals would have lower whole body and visceral fat mass (%) and higher lean mass (%).

3) Elderly individuals who lead physically active lives would have a lower inflammatory burden, indicated by lower plasma concentrations of inflammatory cytokines and inflammation related proteins with vascular effects and markers of oxidative stress.

4) More physically active elderly individuals would have lower resting HR, BP, central pulse pressure and large artery stiffness.

4.4 Methods

4.4.1 Participants

Two hundred and eleven 60-80 year olds (100 males, 111 females) volunteered to participate in the study. Mean age, height and weight (mean ± standard deviation) were 67 ± 5 years, 167 ± 10 cm and 73 ± 14 kg, respectively.

4.4.2 Recruitment

Participants were recruited from the local community through both active recruitment with flyers, and through the Birmingham and Black Country PCT GP Surgeries. Individuals were only approached if they met the inclusion criteria (see Section 3.2).

4.4.3 Consent

Participants were invited to the WT-CRF for a single visit to partake in the study. All the participants arrived in a 10-hour overnight fasted state. First the study details were explained by and written informed consent was obtained by a research nurse. Then a series of functional measurements were made, which together took a maximum of 4 hours. All experimental procedures were performed in accordance with the Declaration of Helsinki and received approval from Birmingham and Black Country PCT Ethical Committee.

4.4.4 Experimental Measurements

Height, weight, BMI, waist and hip circumference, blood glucose and BP were measured upon arrival (see Section 3.3.1, 3.3.2 and 3.3.3 for details). Participants were also asked to provide details of any medications they were currently using. For measurement of inflammatory markers, participants had approximately 40 mL of blood taken from superficial vein at the antecubital fossa (see Sections 3.3.4 and 3.9 for details). Participants were then given a light breakfast.

DEXA was then used to measure total body fat, total lean mass, estimated visceral fat, body fat %, while pQCT was used to determine arm and leg muscle cross-sectional area and muscle density (see Section 3.3.5 for details). Frailty was measured using the TUG test and BBS (see Section 3.7.2 for details) and walk speed was calculated from the TUG test. Arterial stiffness was measured via PWA and PWV, and central pulse pressure calculated (see Section 3.4.2 for details). Physical activity levels were measured using 7 day accelerometry data (see Section 3.8.1 for details).

4.4.5 Physical activity quintiles

To establish the range of physical activity levels in this cohort participants were ranked in to quintiles using SPSS statistical software, controlled for gender. Counts/hour was the physical activity measure chosen to quintile the data. Counts/hour takes in to account all body movements including the upper body, not just steps taken (Sasaki, John et al. 2011). The physical activity levels were divided in 5 groups (1 = 20% of cohort with lowest physical activity levels, 5 = 20% of cohort with highest physical activity levels). This approach was

used following extensive discussions and direction from the study statistician, who advised to split the physical activity levels in to separate groups and investigate differences between them in accordance with previously published data (Thøgersen-Ntoumani, Fox et al. 2005).

4.4.6 Statistical analysis

Multivariate Analysis of Variance (ANOVA) was employed to determine whether there were any significant differences in any of the age-related outcome variables we measured between the PA groups. Correlation analyses were also performed on the key dependent variables presented in this study, using physical activity measured via counts/hour as a continuous independent variable. The statistical tests were adjusted for age, gender and medication use. Distribution tests were performed to decipher normal and non-normally distributed data. Logarithmic transformation was applied to non-normally distributed data. Non-parametric tests were performed for non-normally distributed plasma biomarkers, as typically log transformation fails to ensure normal distribution for these variables. SPSS for Windows (IBM Corporation, Somers, NY, USA) was used for all statistical analyses. Data are presented as mean ± standard deviation (SD).

4.5 Results

4.5.1 Subject Characteristics

Tables 4.1 and 4.2 provide descriptive statistics of the study cohort and separately for men and women. As expected, in our cohort there were significant gender differences with height, weight, waist circumference and hip:waist ratio being significantly greater in men, and hip circumference being significantly greater in women (Table 4.1). Table 4.2 presents frequency statistics for medication use in our sample population. Less than half of our cohort was taking medication (41%). The most common medication used by our participants was anti-hypertensive drugs (28%) and statins (16%).

4.5.2 Physical Activity

Table 4.3 presents the differences in PA levels in the PA quintiles. As expected, PA levels presented as steps/day, steps/hour, counts/day and counts/hour were all significantly different across each PA group. There was a >2-fold increase from Physical Activity Level (PAL) group 1 to PAL group 5 in all of the physical activity variables presented, and each PAL group was significantly different to each other. Steps/ day $F(24, 660) = 51.5$, $p < 0.001$; Steps/hour $F(24, 660) = 62.5$, $p < 0.001$; Counts/day $F(24, 660) = 145.8$, $p < 0.001$; Counts/hour $F(24, 660) = 236.6$, $p < 0.001$.

4.5.3 Measures of Physical Functioning

Results of the physical function tests are presented in Table 4.4. There were no significant differences between the PAL groups for the BBS score. However, post hoc tests indicated that PAL group 4 were significantly faster than PAL group 1 in the TUG and walk speed tests ; TUG test: $F(12, 508) = 2.91$, $P = 0.016$; walk speed test: $F(12, 508) = 2.63$, $p = 0.022$. Results of the correlation analyses are presented in Figures 4.1-4.3. A significant positive association between walk speed and physical activity (r=0.22, p<0.01), and a significant negative association between the TUG and physical activity (r=-0.24, p<0.01) were observed. Interestingly, correlation analyses also indicated a significant positive association between the BBS and physical activity (r=0.20, p<0.01).

4.5.4 Body Composition

Table 4.5 presents body composition data obtained from DEXA and pQCT analysis. Total fat, estimated visceral fat and body fat percentage were significantly lower in PAL groups 3, 4 and 5 compared to PAL group 1; $F(44, 399) = 4.63$, $p = 0.00$; $F(44, 399) = 5.32$, $p = 0.00$; $F(44, 399) = 4.50$, $p = 0.00$. However there were no significant differences in whole body lean mass, single distal radius and tibia muscle density or single arm and leg muscle cross sectional area over the PAL groups. Correlation analyses found significant negative associations between total fat, body fat % and estimated visceral fat mass (r=-0.33, p<0.01; r=-0.23, p<0.01; r=-0.38, p<0.01). Interestingly, a significant positive relationship between leg muscle density and physical activity was also revealed (r=0.20, p<0.001). Leg muscle density and estimated visceral fat mass results are presented in Figures 4.4 and 4.5. No other significant correlations were observed between body composition and physical activity.

4.5.5 Plasma Markers of Inflammation, Oxidative Stress with a Mechanistic Link to Cardiovascular Risk

Table 4.6 presents white blood cell counts, Table 4.7 presents plasma markers of inflammation and Table 4.8 presents markers of oxidative stress and anti-oxidative capacity. There were no significant differences in white blood cell counts including lymphocytes, monocytes and granulocytes between the quintiles in our cohort (Table 4.6). PAI-1 concentration was significantly higher in the lowest PAL group compared to all other PAL groups, $F(68, 611) = 2.45$, $p = 0.04$ (Table 4.7). C-RP, interleukins, MCP-1, TNF-α and VEGF were not significantly different in our cohort across the PAL groups. There were also no significant differences in oxidative stress measured by protein oxidation and lipid peroxide plasma content, or antioxidant capacity in our cohort (Table 4.8). The only significant correlation with physical activity that was observed was PAI-1, where a significant negative relationship was observed (r=-0.19, p<0.01), and presented in Figure 4.6.

4.5.6 Arterial Stiffness

Table 4.9 presents arterial stiffness measurements across the PAL groups including central and peripheral artery stiffness and central pulse pressure. AIx, pPWV and cPWV were not significantly different across the PAL groups. Central systolic, diastolic and mean pulse pressures were also not significantly different across the PAL groups. In addition, no significant correlations were observed between indices of arterial stiffness and PAL

Table 4.1. Subject Characteristics

	All (n=211)	Men (n=100)	Women (n=111)
Age (yrs)	67 ± 5 (60 – 79; 7)	68 ± 5 (60-79; 7)	66 ± 5 (60-79; 6)
Height (cm)	167 ± 10 (146 – 194; 14)	175 ± 8 (155-194)	161 ± 6 (146-180)
Weight (Kg)	73 ± 14 (42 – 125; 19)	80 ± 14 (60-125; 17)	66 ± 11 (42-109; 14)
BMI (Kg/m^2)	26 ± 4 (19 – 40; 4)	26 ± 4 (20-40; 4)	26 ± 4 (18-38; 5)
Hip circumference (cm)	103 ± 11 (81 – 187)	102 ± 9 (83-126)	103 ± 12 (81-187)
Waist circumference (cm)	90 ± 12 (62 – 125)	95 ± 10 (77-125; 15)	85 ± 11 (62-116)
Waist:hip ratio	0.88 ± 0.85 (0.48 – 1.09; 12)	0.93 ± 0.56 (0.76-1.01)	0.83 ± 0.76 (0.48-0.98; 0.1)
Systolic BP (mmHg)	136 ± 17 (96 – 195; 22)	138 ± 16 (96-195)	134 ± 17 (97-191; 23)
Diastolic BP (mmHg)	79 ± 11 (48 – 113; 12)	80 ± 10 (50-113)	78 ± 11 (48-108)
Mean BP (mmHg)	98 ± 11 (68 – 127)	99 ± 10 (68-123)	97 ± 12 (68-127)
Blood Glucose (mmol/L)	5.6 ± 0.6 (3.9 – 7.0)	5.7 ± 0.6 (4.2-6.9)	5.5 ± 0.6 (3.9-7.0)

Values are presented as mean ± SD (range; interquartile range). Interquartile range is presented for non-normally distributed variables.

Table 4.2 Medication use by the study cohort

Medication	Number of participants	Percentage (%)
No medication	86	41
ACE inhibitor	14	6.6
AT1 Blocker	1	0.5
AT2 Blocker	6	2.8
Ca^{2+} Blocker	19	9.0
β-Blocker	5	2.4
Diuretic	14	6.6
Statin	34	16.1
Anti-depressant	9	4.3
Anti-Coagulant	11	5.2

Values are presented as number of participants and percentage (%) of individuals who are on the specified medication.

Table 4.3. Physical activity ranges presented by selected accelerometry variables across PA quintiles

	PA Level (counts/hour)						***F***	***P***	***Partial eta Squared***
	All	1	2	3	4	5			
Steps/day	7515 ± 2760 (1518 – 19161)	4564 ± 1230	6019 ± 1310*	7523 ± 1802*†	8856 ± 2266*†‡	10318 ± 2664*†‡#	51.5	0.00	0.515
Steps/hour	494 ± 170 (115 – 1220)	305 ± 84	405 ± 71*	486 ± 112*†	575 ± 116*†‡	683 ± 163*†‡#	62.5	0.00	0.563
Counts/day	966406 ± 296122 (269122 – 1793054)	599930 ± 105096	766476 ± 147382*	969010 ± 125387*†	1107205 ± 130177*†‡	1354589 ± 214622*†‡#	145.8	0.00	0.750
Counts/hour	63658 ± 18430 (20383 – 114207)	39895 ± 5785	51351 ± 6816*	62581 ± 6674*†	72569 ± 6276*†‡	89743 ± 11469*†‡#	236.6	0.00	0.830

Values are mean ± SD of 211 individuals. *P<0.05 vs. PAL group 1; †P<0.05 vs. PAL group 2; ‡P<0.05 vs. PAL group 3; #P<0.05 vs. PAL group 4.

Table 4.4. Functional frailty measures across PA quintiles

	PA Level						***F***	***P***	***Partial eta Squared***
	All	1	2	3	4	5			
Berg Balance Scale	55.0 ± 1.7 (46.0 – 56.0)	54.6 ± 2.3	54.9 ± 2.0	55.1 ± 1.5	55.1 ± 1.3	55.4 ± 1.1	1.12	0.34	0.023
Timed Up and Go (s)	8.1 ± 1.6 (4.0 – 13.1)	8.9 ± 1.7	8.2 ± 1.7	8.0 ± 1.4	7.7 ± 1.4*	7.9 ± 1.5	2.91	0.02	0.057
Walk Speed (Km/hr)	2.8 ± 0.5 (1.7 – 5.4)	2.5 ± 0.5	2.7 ± 0.5	2.8 ± 0.5	2.9 ± 0.6*	2.8 ± 0.5	2.63	0.03	0.052

Values are mean ± SD of 211 individuals *P<0.05 vs. PAL group 1

Table 4.5. Body composition variables across PA quintiles

	PA Level						***F***	***P***	***Partial eta Squared***
	All	1	2	3	4	5			
Total Fat (g)	23314 ± 7854	28090 ± 9289	24958 ± 6454	22341 ± 7997*	21406 ± 6058*	20131 ± 6304*	4.63	0.00	0.140
Total Lean (g)	44817 ± 9917	46171 ± 11459	45701 ± 9667	42847 ± 9896	44785 ± 9612	45014 ± 8966	0.21	0.94	0.007
Estimated Visceral Fat Mass (g)	977 ± 791	1456 ± 1002	1072 ± 762	786 ± 641*	884 ± 790*	687 ± 359*	5.32	0.00	0.157
Body Fat %	34 ± 8	37 ± 6	36 ± 8	34 ± 9*	33 ± 8*	31 ± 7*	4.50	0.00	0.136
Arm Muscle Density (g/cm^3)	74.9 ± 1.8	74.8 ± 1.9	75.6 ± 1.8	74.4 ± 1.9	74.6 ± 1.9	75.4 ± 1.7	1.36	0.25	0.045
Arm Muscle Area (mm^2)	3059 ± 870	3178 ± 859	3114 ± 952	2886 ± 845	3037 ± 943	3120 ± 799	0.13	0.97	0.005
Leg Muscle Density (g/cm^3)	72.8 ± 2.8	72.0 ± 3.2	74.0 ± 1.9	72.7 ± 2.0	72.8 ± 2.4	73.0 ± 3.7	1.22	0.30	0.041
Leg Muscle Area (mm^2)	6098 ± 1302	6343 ± 1417	6034 ± 1009	6154 ± 1108	5942 ± 1633	5983 ± 1094	0.69	0.60	0.024

Values are mean ± SD of 211 individuals. *P<0.05 vs. PAL group 1. The first 4 values were obtained with DEXA and reflect whole body measurements; while the last 4 were obtained with pQCT and reflects values in one single arm muscle (distal radius) and one single leg muscle (distal tibia)

Table 4.6. White blood cell (WBC) counts across PA quintiles

	PA Level						***F***	***P***	***Partial eta Squared***
	All	1	2	3	4	5			
WBC count (x10^9/L)	5.22 ± 1.41	5.63 ± 1.54	5.15 ± 1.18	5.19 ± 1.33	5.05 ± 1.67	5.13 ± 1.32	0.86	0.49	0.019
Lymphocytes (x10^9/L)	1.56 ± 0.58	1.69 ± 0.95	1.51 ± 0.41	1.51 ± 0.43	1.51 ± 0.59	1.59 ± 0.46	0.60	0.67	0.013
Monocytes (x10^9/L)	0.40 ± 0.15	0.43 ± 0.18	0.38 ± 0.14	0.40 ± 0.12	0.42 ± 0.18	0.39 ± 0.11	0.61	0.65	0.014
Granulocytes (x10^9/L)	3.27 ± 1.07	3.54 ± 1.05	3.26 ± 0.95	3.29 ± 1.09	3.12 ± 1.12	3.16 ± 1.14	0.75	0.56	0.016
Lymphocytes (%)	30.1 ± 7.4	29.7 ± 9.3	29.7 ± 7.2	29.5 ± 6.6	30.1 ± 6.4	31.8 ± 7.7	0.57	0.68	0.013
Monocytes (%)	7.9 ± 2.5	7.7 ± 2.6	7.4 ± 2.3	8.0 ± 2.4	8.5 ± 2.5	7.9 ± 2.7	0.75	0.56	0.017
Granulocytes (%)	62.1 ± 7.9	63.1 ± 9.5	62.8 ± 7.4	62.7 ± 7.3	61.6 ± 6.9	60.4 ± 8.4	0.60	0.66	0.013

Values are mean ± SD of 211 individuals

Table 4.7. Plasma markers of cardiovascular risk factor levels across PA quintiles

	PA Level						***F***	***P***	***Partial eta Squared***
	All	1	2	3	4	5			
PAI-1 (ng/mL)	1.1 ± 0.8	1.4 ± 1.1	1.2 ± 0.8	0.9 ± 0.7*	1.0 ± 0.7*	0.9 ± 0.7*	2.45	0.04	0.054
MIF (ng/mL)	1.5 ± 2.2	1.6 ± 2.1	1.7 ± 2.2	1.1 ± 1.5	1.7 ± 2.9	1.6 ± 2.2	0.38	0.82	0.009
Endothelin-1 pg/mL)	2.1 ± 5.31	2.3 ± 6.7	1.6 ± 4.5	1.2 ± 3.1	3.6 ± 7.7	1.7 ± 3.7	1.45	0.19	0.052
CRP (mg/L)	1.4 ± 1.4	1.6 ± 1.4	1.3 ± 0.9	1.3 ± 1.3	1.8 ± 2.0	1.1 ± 1.0	1.41	0.23	0.032
IL-1β (pg/mL)	0.04 ± 0.21	0.00 ± 0.02	0.05 ± 0.25	0.04 ± 0.17	0.06 ± 0.32	0.03 ± 0.14	0.36	0.84	0.008
IL-4 (pg/mL)	0.03 ± 0.28	0.12 ± 0.64	0.02 ± 0.10	0.01 ± 0.07	0.00 ± 0.00	0.02 ± 0.1	0.961	0.43	0.022
IL-6 (pg/mL)	1.12 ± 4.3	1.0 ± 3.2	1.6 ± 6.8	1.4 ± 5.0	0.8 ± 2.0	0.7 ± 1.4	0.31	0.87	0.007
IL-10 (pg/mL0	1.7 ± 5.2	2.6 ± 6.6	1.0 ± 3.2	2.5 ± 6.0	0.8 ± 3.0	1.9 ± 6.3	0.94	0.44	0.022
IL-13 (pg/mL)	0.97± 1.13	1.20 ± 1.19	0.95 ± 1.16	0.94 ± 1.28	0.97 ± 1.15	0.81 ± 0.81	0.50	0.74	0.011
IL-17a (pg/mL)	6.1 ± 15.2	10.3 ± 19.2	3.5 ± 8.4	8.3 ± 19.6	3.6 ± 10.9	5.4 ± 15.0	1.39	0.24	0.032
MCP-1 (pg/mL)	24.1 ± 9.9	22.8 ± 8.6	24.9 ± 9.4	22.0 ± 8.0	26.7 ± 12.5	23.9 ± 10.2	1.31	0.27	0.030
TNF-α (pg/mL)	0.5 ± 1.5	0.1 ± 0.3	0.2 ± 0.7	0.6 ± 1.6	0.6 ± 2.1	0.9 ± 1.7	1.01	0.39	0.031
VEGF (pg/mL)	69.4 ± 57.1	80.0 ± 70.5	61.4 ± 50.6	69.6 ± 50.6	65.6 ± 48.3	72.8 ± 68.0	0.53	0.71	0.012

Values are mean ± SD of 211 individuals *P<0.05 vs. PAL group 1

Table 4.8. Plasma oxidative stress markers and antioxidant capacity across PA quintiles

	PA Level						***F***	***P***	***Partial eta Squared***
	All	1	2	3	4	5			
Protein oxidation	5.1 ± 1.6	4.7 ± 1.2	5.3 ± 1.8	5.2 ± 1.4	4.6 ± 1.2	5.4 ± 2.0	1.89	0.12	0.041
Total antioxidant capacity	465.8 ± 94.5	496.1 ± 88.8	481.6 ± 114.2	449.3 ± 86.7	450.2 ± 78.9	453.2 ± 93.9	1.81	0.13	0.041
Lipid peroxides/mL plasma	337.1 ± 33.4	338.1 ± 35.3	334.0 ± 33.6	343.1 ± 32.5	341.2 ± 39.9	327.6 ± 20.5	1.14	0.34	0.026

Values are mean ± SD of 211 individuals.

Table 4.9. PWA (AIx), PWV and central pulse pressure across PA quintiles

	PA Level							
	All	1	2	3	4	5	***F***	***P***	***Partial eta Squared***
Arterial Stiffness									
AIx @ 75bpm	29.8 ± 9.5 (3.7 – 56.0)	32.6 ± 6.9	28.7 ± 10.0	29.9 ± 10.1	29.6 ± 9.5	28.9 ± 10.2	0.56	0.69	0.018
pPWV	8.7 ± 1.3 (5.4 – 12.3)	8.8 ± 1.6	8.6 ± 1.3	8.9 ± 1.4	8.6 ± 1.3	8.7 ± 1.1	0.33	0.86	0.010
cPWV	8.7 ± 2.0 (4.9 – 14.2)	8.5 ± 2.0	8.8 ± 1.8	8.8 ± 1.8	9.0 ± 2.3	8.3 ± 1.8	0.56	0.69	0.018
Central BP (mmHg)									
Systolic	123.0 ± 17.3 (87.7 – 184.0)	124.5 ± 20.0	117.0 ± 12.4	128.1 ± 21.3	120.7 ± 12.1	123.5 ± 17.1	1.87	0.12	0.042
Diastolic	76.0 ± 9.1 (46.3 – 105.0)	76.1 ± 12.2	73.3 ± 7.1	78.5 ± 10.1	75.4 ± 6.8	76.0 ± 8.5	0.84	0.50	0.019
Mean	95.5 ± 11.3 (67.0 – 133.7)	96.2 ± 14.0	91.4 ± 7.9	98.8 ± 14.7	94.0 ± 8.5	95.5 ± 11.3	1.33	0.26	0.030
Heart Rate (bpm)									
Seated	67.2 ± 11.0 (42.0 – 111.0)	67.8 ± 10.8	70.1 ± 11.6	68.2 ± 11.4	65.1 ± 8.2	64.4 ± 11.8	1.87	0.11	0.040
Supine	62.4 ± 9.8 (37.6 – 109.5)	63.3 ± 8.4	63.1 ± 9.8	62.9 ± 9.8	61.1 ± 7.7	61.9 ± 13.0	0.18	0.95	0.004

Values are mean ± SD of 211 individuals.

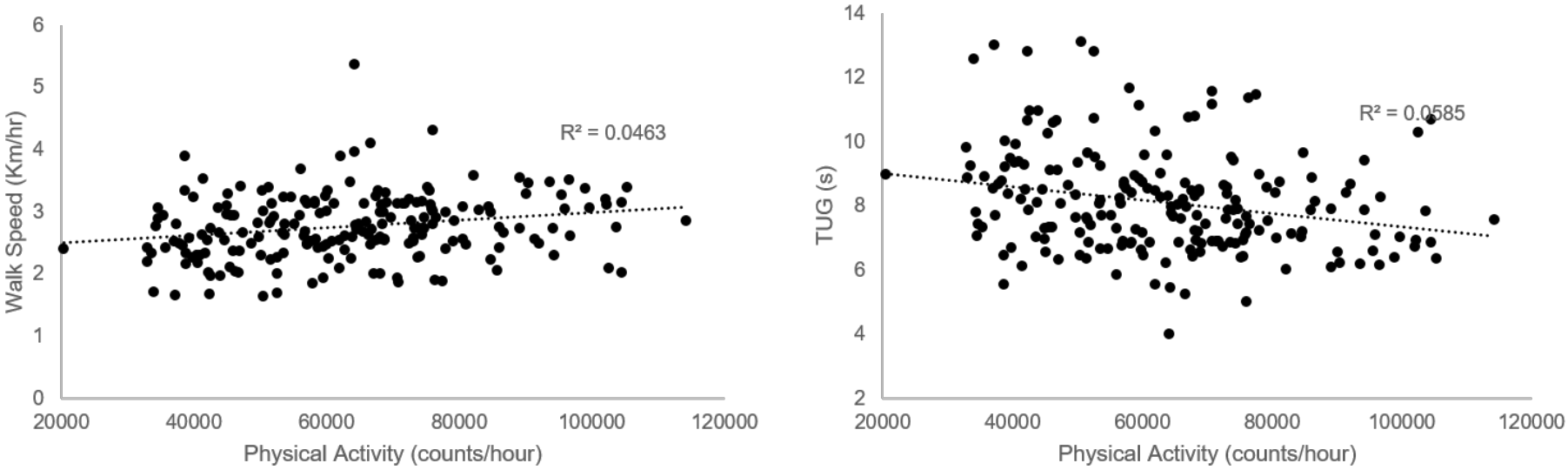
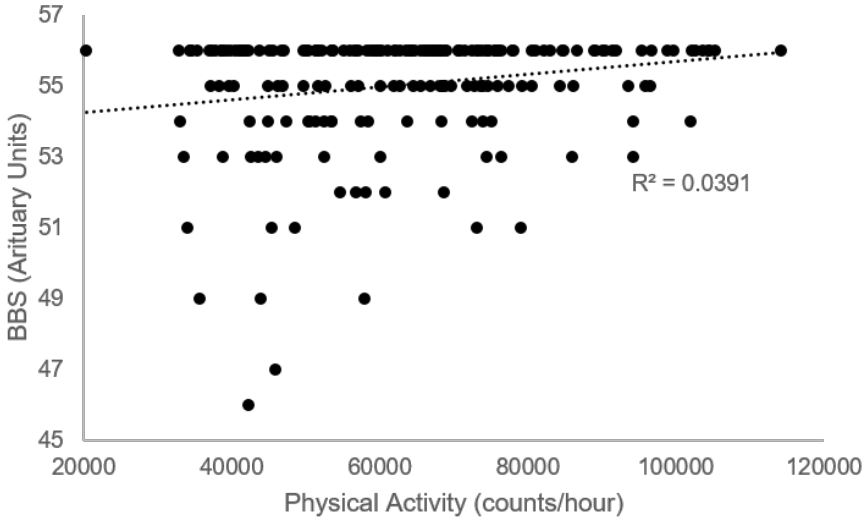


Figure 4.1 Presents the significant positive association between physical activity measured via counts/hour and walk speed (r=0.22, p<0.01; left panel); Figure 4.2 Presents the significant negative association between physical activity and TUG (r=-0.24, p<0.01; right).



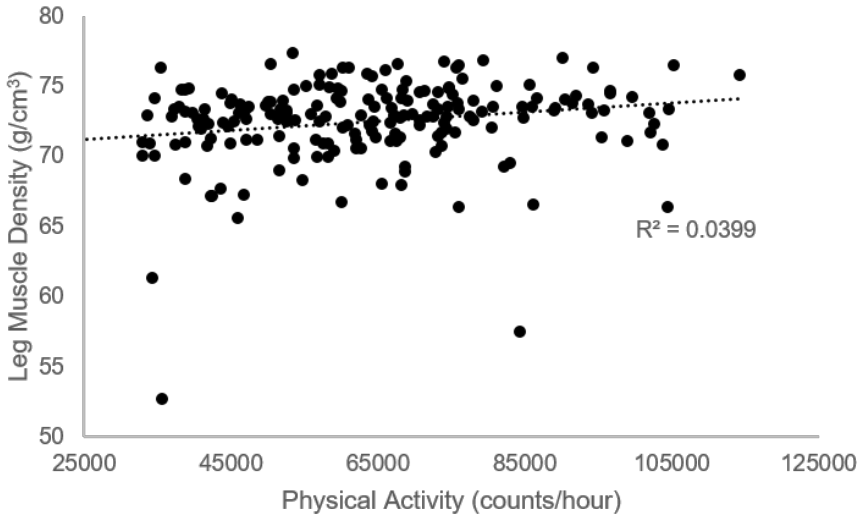
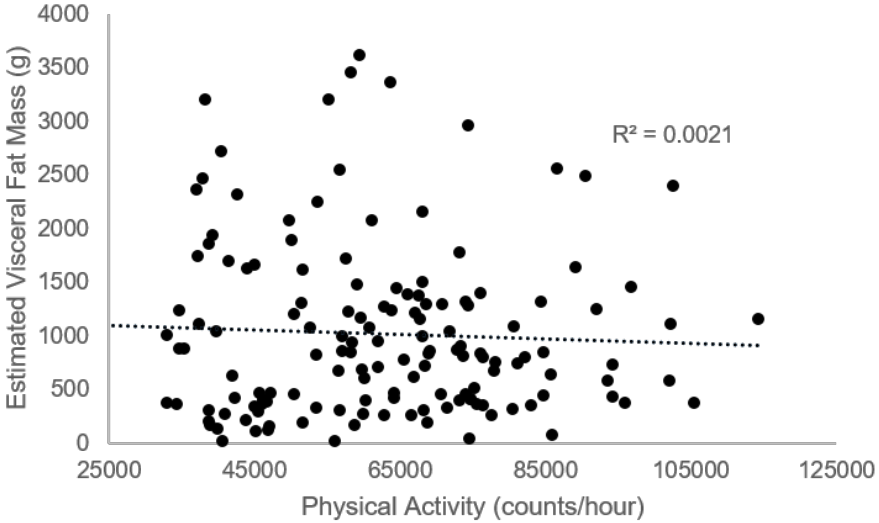


Figure 4.3 Presents the significant positive association between physical activity measured via counts/hour and the BBS (r=0.20, p<0.01; left).
Figure 4.4 Presents the significant positive association between physical activity and leg muscle density (r=0.20, p<0.01; right).



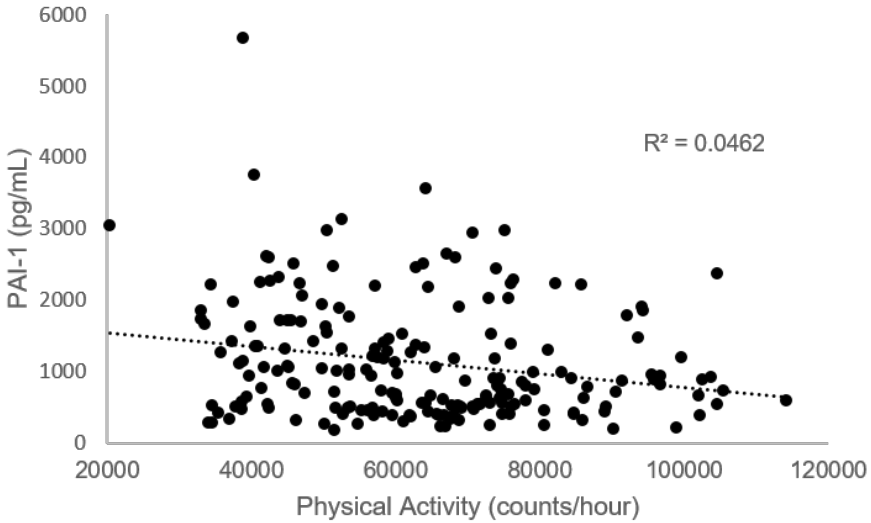


Figure 4.5 Presents a significant negative association between physical activity measured via counts/hour and estimated visceral fat mass (r=-0.38, p<0.01; left); Figure 4.6 Presents a significant negative association between physical activity and plasma PAI-1 concentration (r=-0.19, p<0.01; right).

4.6 Discussion

The purpose of the present study was to investigate whether higher levels of physical activity were associated with superior physiological functioning in elderly individuals. The main findings were: individuals with higher physical activity levels demonstrated faster walking speeds and a faster completion of the TUG test indicative of better physical functioning. Higher levels of physical activity were associated with a lower total body fat, body fat % and estimated visceral fat mass. However, lean body mass measured with DEXA and the mass of the distal tibia muscle (leg) and distal radius muscle (arm) measured with pQCT was not significantly different across the PAL quintiles. High physical activity represented by PAL groups 3-5 presented significantly lower plasma levels of the cardiovascular risk factor PAI-1 compared to the lowest PAL groups, which is in agreement with the hypothesis. However, in contrary to the hypothesis the majority of plasma markers of inflammation with mechanistic links to the future development of cardiovascular disease (MIF, endothelin-1, C-RP, IL-1β, IL-4, IL-6, IL-10, IL-13, IL-17a) and markers of oxidative stress were not significantly different across the PAL quintiles, refuting our hypothesis that higher levels of physical activity in our cohort would result in significantly lower plasma levels of these markers. Contrary to our hypothesis, cardiovascular function represented by resting HR, BP, central pulse pressure and large artery stiffness were not significantly different across the physical activity quintiles.

4.6.1. Age, Physical Activity and Physical Function

The development of age-related frailty is associated with a decline in physical function (Buchner and Wagner 1992, Rockwood, Fox et al. 1994, Fried, Tangen et al. 2001). One of the attributes of the inferior physical function identified by Fried et al. was that maintenance of a low PAL reduced habitual walking speed (Fried, Tangen et al. 2001). Other studies have shown that participation of elderly individuals in exercise training interventions attenuated the physical function loss (Fiatarone, O'Neill et al. 1994, Campbell, Robertson et al. 1997, Gill, Baker et al. 2002, Chakravarty, Hubert et al. 2008). In agreement with previous research and our hypothesis, results from this cohort illustrate that maintenance of a higher physical activity level was associated with faster walking speeds and faster completion of the TUG test in PAL group 4 compared to PAL group 1. Interestingly, although PAL group 5 was numerically faster than PAL group 1, the difference was not significant. Elderly individuals who are able to perform activities of daily living with ease for longer are more able to live independently and maintaining physical function as we age is therefore important to prevent disability onset and improve quality of life (Fries 1980, Fries 2002, Chakravarty, Hubert et al. 2008).

Balance measured by the BBS was not different among the PAL quintiles in this cohort. Fiatarone et al. who found an improvement in stair climbing ability following a 10-week resistance training intervention in elderly individuals, also identified an increase in muscle strength and cross-sectional area (Fiatarone, O'Neill et al. 1994). They suggested that this may have aided advances in physical function. Differences in leg and arm muscle cross-sectional area between the physical activity quintiles were not observed in this cohort . A cross-sectional study from the Cardiovascular Health Study showed that elderly individuals who had higher levels of fat mass reported higher levels of disability based on a time

administered 0.5 mile walk, and walking up 10 stairs, however no effect of fat-free mass was observed (Visser, Langlois et al. 1998). It is tempting to speculate that findings in this study of higher walking speeds with higher physical activity levels could be explained by lower levels of body fat, as carrying less body weight will enable faster walking speeds, but not necessarily affect balance. Correlation analysis however did expose a significant positive association between physical activity and leg muscle density in this cohort, which supports the positive association observed between physical activity and the BBS, and agrees with the findings of Fiatarone et al. (1994).

4.6.2. Age, Physical Activity and Body Composition

A noticeable change with age is the development of increased central adiposity. Previous research that has examined body composition in elderly individuals concluded that older individuals possessed significantly higher body fatness and central adiposity (Poehlman, Toth et al. 1995), particularly in women (Song 2004). It is well known that physical activity reduces body fat (DiPietro 1995). Slentz et al. conducted an exercise intervention study on 120 overweight men and women aged 40-65 years (Slentz, Duscha et al. 2004). Compared with controls, exercise groups significantly decreased abdominal skinfold fat, and hip and waist circumference measurements. Our findings support both previous research and our hypothesis, that elderly individuals who have higher physical activity will have lower body fat and estimated visceral fat mass compared to those who are more sedentary. In this cohort, elderly individuals in the highest 3 physical activity quintiles had significantly less total fat, % body fat and estimated visceral fat mass compared to individuals in the lowest physical activity quintile, controlled for gender. Central adiposity

poses the highest risk to cardiovascular disease development in both young and elderly individuals compared to other areas of adiposity (Prineas, Folsom et al. 1993). Visceral adipose tissue located in the central region secretes inflammatory cytokines that are associated with cardiovascular disease development (Wisse 2004, Fain 2006). The significantly lower levels of estimated visceral fat in this cohort in the 3 highest physical activity quintiles may in part explain the significantly lower circulating plasma levels of PAI-1 in the 3 highest physical activity quintiles vs. the lowest.

4.6.3. Age, Physical Activity and Inflammation

High levels of physical activity have been shown to counter-act the age-associated chronic increase in circulating inflammatory cytokines, which are mechanistically linked to future development of cardiovascular disease (Geffken, Cushman et al. 2001, Reuben, Judd-Hamilton et al. 2003, Colbert, Visser et al. 2004). Results from this cohort do not exhibit a significant effect of physical activity on circulating inflammatory cytokines. Some studies that have reported lower inflammatory cytokines in cross-sectional studies of elderly individuals have relied on self-assessment physical activity questionnaires (Geffken, Cushman et al. 2001, Colbert, Visser et al. 2004). However, self-report may not be an accurate representation of actual physical activity levels as participants have a tendency to over-estimate the amount of physical activity performed (Troiano, Berrigan et al. 2008). Reasons for the conflicting findings may therefore be due to the physical activity range in this cohort. A recent review by Chen et al. explaining the effects of physical activity on inflammatory and thrombotic status in both young and elderly individuals, highlighted that

the lowest amount of weekly exercise training volume needed to reduce inflammation may need to be individualized (Chen, Apostolakis et al. 2014).

Significantly lower concentrations of plasma PAI-1 were detected in the higher PAL groups (3-5) compared to the lowest PAL group. PAI-1 is the major physiological inhibitor of plasminogen activator in plasma. An increase in PAI-1 is associated with an increased risk of thrombosis and atherosclerotic plaque growth and via this mechanism may lead to cardiovascular dysfunction and disease (Vaughan 2005). PAI-1 levels increase with age, however elderly individuals who live physically active lifestyles present significantly higher levels of tPA (tissue-type plasminogen activator) the antagonist compound of PAI-1 (Vaughan 2005). Stratton et al. demonstrated that following a 6 month intensive endurance exercise intervention in elderly men PAI-1 concentration was significantly reduced (Stratton, Chandler et al. 1991). Inflammatory cytokines IL-1, IL-6, CRP and TNF-α stimulate PAI-1 and promote vascular inflammation and atherosclerosis. However, in the present study we did not identify any increases in these inflammatory markers with higher physical activity. Chen et al. note however that there are other pathways that could account for the beneficial effects of physical activity on thrombotic agents such as PAI-1 and tPA which up-regulate fibrinolysis (Mazzeo 1991, El-Sayed, Lin et al. 1995, el-Sayed 1996, Wang, Jen et al. 1997, Wang and Chow 2004, Jahangard, Torkaman et al. 2009, Chen, Apostolakis et al. 2014). For example, another potential source of PAI-1 in elderly individuals is visceral adipose tissue (Vaughan 2005). The higher physically active elderly individuals in this cohort were observed to have significantly lower estimated visceral adipose tissue and PAI-1 concentrations (See section 4.6.2).

4.6.4. Age, Physical Activity and Cardiovascular Function

Physical inactivity is an independent risk factor for cardiovascular dysfunction and disease progression (Blair, Kohl et al. 1995, Thijssen, Maiorana et al. 2010). A reduction in the compliance of the large arteries is also an independent risk factor for the development of cardiovascular disease in well functioning older adults (Tanaka, Dinenno et al. 2000, Sutton-Tyrrell, Najjar et al. 2005). Research has shown that partaking in regular physical activity is associated with reducing vascular dysfunction and vascular events in elderly men and women (Paffenbarger, Hyde et al. 1986, Hakim, Curb et al. 1999, Sesso, Paffenbarger et al. 2000, Tanaka, Dinenno et al. 2000, Myers, Prakash et al. 2002). However, the results of this cohort contradict previous research as central and peripheral arterial stiffness was not significantly associated with habitual physical activity levels between the groups. One possible explanation for the conflicting findings is that the difference in physical activity level between group 1 and 5 was too small to maintain cardiovascular function. Tanaka et al. examined central arterial compliance in healthy young and older males (18-77 years) of different physical activity levels for at least the previous 2 years (sedentary, recreationally active and endurance trained) (Tanaka, Dinenno et al. 2000). They found no difference in central arterial compliance between sedentary and recreationally active men at any age. However, arterial compliance was significantly higher in the middle-aged and older endurance trained men compared to the less active groups. This group also performed a 3-month aerobic exercise intervention study on 20 middle-aged men (53 ± 2 years), and found that central arterial compliance increased to levels similar to the endurance trained middle-aged and older men. Although in our cohort there is a >2 fold increase from PAL group 1 to 5, the range may not be great enough to detect alterations in arterial structure and function.

4.6.5. Methodological Considerations

This study examined physical activity and a range of physical functions thought to be altered with age in a cohort of 211 elderly individuals. Although physical activity did have significant beneficial effects on walking speed, whole body and visceral adiposity, and thrombotic activity (PAI-1 concentration), no identifiable differences were observed for the remaining plasma peptides and proteins associated with increased cardiovascular risk, cardiovascular function or lean muscle mass. In this cohort 5.2% of individuals were taking anticoagulant medication, which is known to reduce PAI-1 concentrations (Hadigan, Meigs et al. 2001). However, medication use was selected as a covariate in the analysis, eradicating any confounding effects on PAI-1 concentration. A chi-square test was performed to test for differences in anticoagulant medication, and confirmed no significant difference between quintiles, X^2 (4, N = 202) = 3, p = 0.54.There are a number of methodological considerations that may partly give rise to the lack of effect of physical activity on these variables in this particular cohort.

To note is the characteristics of the cohort and a possible selection bias. All participants were healthy elderly individuals (60-80 years old) with no apparent pathophysiology or disease. Although participants were deemed healthy, many were on anti-hypertensive medication (28%). These medicated individuals could have fallen in to the less physically active groups, potentially masking the effect of inactivity on blood pressure and arterial stiffness. Chi-square tests were performed to test for differences between quintiles for all medication use (ACE inhibitor, AT2 blocker, Ca^{2+} blocker, β-blocker, Diuretic, Statin, Anti-depressant), and confirmed no significant difference; X2 (4, N = 202) 2.4, p = 0.67; X2 (4, N = 202) 3.9, p = 0.42; X2 (4, N = 202) 0.34, p = 0.99; X2 (4, N = 202) 6.4, p= 0.17; X2 (4, N = 202) 1.7, p = 0.80; X2 (4, N = 202) 1.8, P = 0.77; X2 (4 = 202) 1.7, p = 0.79. The

inclusion criteria for this cohort however allowed individuals with blood pressure less than 190/120 mmHg (see section 4.4.2 for details), which would include hypertensive individuals. In many clinical trials comorbidities have been the main reason to exclude elderly individuals. However, if exclusion criteria are too restricted, it may compromise the generalizability of the results (Ferrucci, Guralnik et al. 2004). In addition it should be noted that the demographic of this cohort is taken from the Harborne area of the West Midlands, which are deemed a very healthy, active sample group. Therefore care should be taken when generalising these findings to the whole UK population.

The physical activity range of this cohort is also a possible confounder. Although the physical activity levels between the least and most active individuals were significantly different (>2 fold increase), none of the cohort fell in to an extreme case of sedentary behaviour (0-159 counts/min) (Carr and Mahar 2012) or highly trained athletes. In the general population, physical activity levels in elderly individuals vary from completely sedentary to highly trained Masters athletes. Masters athletes present a unique sub-group of the over 70 population and demonstrate "exceptionally successful ageing"(Tanaka and Seals 2008). As well as outstanding athletic performance, endurance-trained ageing runners show reduced disability and mortality compared to age-matched sedentary individuals (Chakravarty, Hubert et al. 2008). In contrast, at the other end of the physical activity spectrum many extremely sedentary elderly individuals are in diseased states. This feeds back in to the selection bias limitation of this study, in that the cohort was reasonably active. The physical activity range in this cohort may not be broad enough to detect minor perturbations of physiological homeostasis in healthy elderly individuals, despite total time and percentage of time spent in sedentary, light, moderate, vigorous and very vigorous activity levels being assessed using cut-offs previously described (Freedson and Miller 2000). However, the fact

that differences in physical functioning and body composition were present in the lowest and highest physical activity groups, suggests firstly that these age-related changes precede and possibly predict an increased risk of other functional loss such as cardiovascular dysfunction, and secondly that physical activity can attenuate these detrimental changes. An alternative approach to assess physical activity status in this elderly cohort would have been using a submaximal aerobic capacity test (Noonan and Dean 2000). Maximal aerobic capacity tests are the gold standard assessment for cardiorespiratory fitness, but do acutely increase the risk of an adverse cardiovascular event. Submaximal tests allow the prediction of cardiorespiratory fitness but with a reduced risk of complications (Noonan and Dean 2000). However, the study aims did not necessitate the quantification of cardiorespiratory fitness levels per sé, but to examine the range of habitual daily activity levels of an elderly cohort, warranting the utilization of 7-day accelerometry rather than an aerobic exercise test.

The cross-sectional design of this cohort study is also a limitation. Cross-sectional studies provide only a snap shot of individuals' lifestyle and physiological functioning. Longitudinal studies provide a superior indication of lifestyle on physiological functioning. Exercise intervention studies in elderly individuals have also provided positive findings on physical functioning, body composition and cardiovascular function (Fiatarone, O'Neill et al. 1994, Tanaka, Dinenno et al. 2000, Slentz, Duscha et al. 2004). Ford (2002) conducted a cross-sectional study in elderly individuals and observed significant beneficial effects of physical activity on inflammatory status in elderly individuals, with a far greater sample size of 13,748. This cohort study may therefore be underpowered to identify effects of physical activity on certain age-related outcome measures. Post-hoc calculations revealed that the achieved statistical power was relatively low for a number of inflammatory markers and indices of cardiovascular function and thus the potential for a type II error is acknowledged.

For example, the observed partial eta squared for some of the key inflammatory (CRP=0.03) and cardiovascular variables (arterial stiffness=0.02) suggests that much larger sample sizes (13270 and 29845, respectively) would have been necessary order to identify PAL-associated differences in these variables in a cohort of elderly individuals. However, due to recruitment practicalities the cohort size was limited to 211.

4.6.6. Conclusion

In this cohort study physical function loss, visceral adiposity and PAI-1 concentrations were associated with physical activity. However, lean muscle mass, inflammation and oxidative stress, and cardiovascular function including resting HR, BP, central pulse pressure and arterial stiffness were not associated with physical activity. Despite these null findings, the current study provides some information regarding the effects of daily physical activity on age-related function loss in a cohort of elderly individuals.

CHAPTER 5:

THE INFLUENCE OF BODY COMPOSITION, INFLAMMATORY STATUS AND CARDIOVASCULAR RISK FACTORS ON LEFT VENTRICULAR DIASTOLIC FUNCTION IN AN ELDERLY COHORT

5.1 Abstract

Chronic heart failure (CHF) is a major health concern for the elderly population in many elderly people. In a significant proportion of CHF cases, systolic function is preserved and is entirely attributable to a diastolic abnormality (i.e., diastolic heart failure) with preservation of systolic function under resting conditions. Age-related diastolic function loss is associated with the future development of diastolic heart failure.

The aim of this study was to test the hypothesis that in elderly individuals LV diastolic function loss would be associated with increased visceral adiposity, inflammation, oxidative stress, arterial stiffness, and cardiovascular risk factors and reduced physical activity.

To test this, 175 elderly individuals from the PAHA cohort were selected (age, height and weight 60 ± 5 years, 167 ± 10 cm, and 72 ± 14 kg, respectively [mean ± SD]). Measures of LV diastolic function (E/A ratio), body composition (DEXA, BMI, Waist:Hip ratio), inflammation (plasma C-RP concentration), oxidative stress (plasma lipid peroxide concentration), central arterial stiffness (cPWV), cardiovascular risk factors (resting MAP, HR, TPR), and physical activity (counts/hour via accelerometry) were obtained. Multiple Hierarchical Regression analysis was employed to statistically test the associations between these variables and LV diastolic function.

When age, gender and anti-hypertensive medication were statistically controlled for there was a significant negative association between LV diastolic function and visceral adipose tissue mass ($P<0.05$, t = -2.14), and LV diastolic function and MAP ($P<0.05$, t = -2.44). No association was observed between LV diastolic function and the following

variables: BMI, Waist:Hip ratio, C-RP, lipid peroxides, central arterial stiffness, HR, TPR and physical activity..

In this cohort MAP and visceral adiposity revealed a significant negative association with LV diastolic function (measured as E/A ratio). It could be possible that a number of predictor associations have been missed in this cohort as the sample size may be too small, and underlying pathologies such as renal dysfunction and variety of inflammatory diseases may have been missed.

5.2 Introduction

The prevalence of cardiovascular disease increases with advancing age, including diseases such as hypertension, stroke, atherosclerosis and CHF (Lakatta 2002). In 2010, the National Statistics for Death in the US reported that the leading cause of mortality was diseases of the heart, where 24.2% of all individuals who died during that year did so as a result of heart disease (Murphy, Xu et al. 2013). There is growing evidence that a significant proportion of CHF is caused predominantly by an abnormality in diastolic function (i.e. diastolic heart failure),(Vasan, Benjamin et al. 1995, Aurigemma, Gottdiener et al. 2001, Zile and Brutsaert 2002). Studies suggest that 30% of elderly patients with CHF have diastolic heart failure, whereby ejection fraction (systolic function) is maintained (Senni, Tribouilloy et al. 1998, Gottdiener, Arnold et al. 2000, Baxter and Gray 2002). In the absence of cardiovascular disease in apparently healthy ageing, impairments in LV diastolic function can still prevail and may be a risk factor for the future development of diastolic heart failure (Prasad, Popovic et al. 2007, Biernacka and Frangogiannis 2011).

Abnormalities of the LV include increased stiffness which may or may not be coupled with a reduction in relaxation (Zile and Brutsaert 2002). Classically in ageing, early LV diastolic filling or E wave velocity is reduced, and late diastolic filling or A wave velocity is increased (Lakatta 2003). Therefore, the ratio of E wave to A wave (E/A ratio – measure of LV diastolic function) decreases. Reduced E/A ratio corresponds to a majority of left ventricular filling occurring in the late phase during atrial contraction, as opposed to the early phase during LV active relaxation. It is speculated that this is due to LV stiffening with ageing, resulting in an attenuated ability to actively relax and draw blood from the LA via the Frank Starling mechanism (Lakatta 2003, Frenneaux and Williams 2007, Prasad, Popovic et

al. 2007). Enhanced atrial contribution to LV filling corresponds to greater cardiac work required to force the larger remaining LA volume in to the LV and is associated with age-related LA enlargement (Lakatta 2003). Manifestation of LA enlargement in elderly individuals is harmful and a risk factor for the future development of atrial fibrillation and stroke (Vaziri, Larson et al. 1995).

Mechanisms of LV stiffening with advancing age are thought to be due to a multitude of physiological changes that occur in the cardiovascular system (Arbab-Zadeh, Dijk et al. 2004, Kass 2005). These often develop prior to cardiovascular disease onset and result in the development of LV diastolic dysfunction. A hallmark of ageing is the progressive deposition of collagen in both the vascular walls and cardiac tissue. This process is known as fibrosis (Song, Yao et al. 1999, Lakatta 2003, Orlandi 2004, Burkauskiene, Mackiewicz et al. 2006, Biernacka and Frangogiannis 2011). Cardiac fibrosis is a major cause of increased myocardial stiffness (Biernacka and Frangogiannis 2011) and major contributor to the observed LV hypertrophy. Despite a reduction in the number of cardiomyocytes there is an increase in LV mass (Olivetti, Melissari et al. 1991) due to the increased collagen deposition. Initially, fibrosis is accompanied by cardiomyocyte hypertrophy as part of an adaptive response aimed at preserving CO while normalizing wall stress (Biernacka and Frangogiannis 2011). In combination with a reduction in relaxation this leads to impaired LV filling, limiting the exercise tolerance in healthy elderly individuals and reducing quality of life (Vanoverschelde, Essamri et al. 1993, Kitzman, Gardin et al. 2001, Lakatta 2003).

The onset of cardiac fibrosis may be a reactive process in response to LV pressure overload (i.e. hypertenstion) (Robert, Besse et al. 1997). Robert et al. induced hypertension in rats by a chronic 1 month infusion of aldosterone-salt and this was associated with the development of fibrosis. It could be hypothesised that the progressive fibrosis leading to

hypertrophy and stiffening of the ageing heart may also (at least in part) be a consequence of peripheral vascular stiffening.

Age-dependent increases in vascular stiffening are well established (Avolio, Chen et al. 1983, Benetos, Laurent et al. 1993, Frenneaux and Williams 2007). Structurally, the diameter of the lumen increases and the walls become thicker, known as intima-medial thickening (thickening of the intima and medial artery layers) (Lakatta 2002). Fibrosis of the vascular smooth muscle layer occurs also, as it does in the cardiac myocytes, resulting in stiffening of the arteries. Arterial stiffness, measured in this study as cPWV, causes an increased cardiac afterload and consequently an increase in systolic wall stress (Chen, Nakayama et al. 1998, Frenneaux and Williams 2007). The left ventricle must therefore contract with more force to eject the LV EDV into the less compliant aorta. In addition to increasing cardiac afterload, arterial stiffening also leads to hypertension, a major risk factor for the development of cardiovascular disease (O'Rourke 1990, Laurent, Boutouyrie et al. 2001, Boutouyrie, Tropeano et al. 2002).

Another age-related factor that could in the long term lead to impairments in vascular and cardiac function is chronic systemic inflammation, which occurs in many elderly individuals and therefore has been given the name “inflammaging”. Inflammaging describes a chronic increase in inflammatory cytokines such as C-Reactive Protein (C-RP), interleukin-6 (IL-6), IL-1, tumour necrosis factor-alpha (TNF-α) and reactive oxygen species (ROS) (Fubini and Hubbard 2003). Inflammatory cytokines and ROS have direct pathological effects on the vasculature to include impaired vasodilation in response to physiological stimuli (insulin and exercise) and increased basal levels of vasoconstriction (Lakatta 2002). Bioavailability of the potent vasodilator NO derived from the endothelial layer of the vasculature is reduced as chronic inflammation activates NADPH oxidase and increases ROS

production. ROS react with NO to produce peroxynitrite which is one of the mechanisms leading to vasodilation (Kojda and Harrison 1999). A combination of impaired NO bioavailability and reduced vasodilation with increased vasoconstriction and arterial stiffness, increases the afterload and TPR and is thought to play an important role in the development of chronic hypertension and LV diastolic dysfunction (Panza, Casino et al. 1994, Perticone, Ceravolo et al. 2001). Age-related chronic inflammation and the development of fibrosis may be causally linked. Increased age-related systemic inflammation leads to the local delivery of cytokines and growth factors that stimulate collagen synthesis (Wagenmakers, van Riel et al. 2006). Orlandi et al. induced atherosclerosis (a vascular inflammatory disease) in rabbits by causing hypercholesterolemia via a 9-month hyperlipemic diet. VCAM-1 (an adhesion molecule expressed in response to cytokine activation) increased and there was a dramatic reduction of coronary endothelial cell eNOS activity leading to reduced NO bioavailability (Orlandi 2004). Furthermore, in addition to the onset of atherosclerosis, cardiac fibrosis was also noted. This implies that vascular and cardiac tissue may have a common starting point of leukocyte adhesion and penetration in to both the deeper macrovascular layers and interstitium of the cardiac tissue.

A potential mechanism for age-related chronic inflammation is an increase in visceral adiposity. It is well established that in later life body fat becomes centralized (Song 2004). This may be due to the reduction in physical activity with age (Jae, Heffernan et al. 2009), although it is debated whether total adipose tissue mass changes, or is merely re-distributed to central areas. Visceral adipose tissue produces pro-inflammatory cytokines which as discussed above can have detrimental effects on the cardiovascular system both at the vascular and cardiac level. Libhaber et al. examined the independent contribution of indexes of adiposity on LV diastolic function (E/A ratio) (Libhaber, Norton et al. 2009). Waist

circumference (an indicative measure of central adiposity) was a significant negative predictor of E/A ratio. Palmeiri et al. investigated LV mass in 1,577 overweight and non-overweight men (mean age 53 ± 12 years) (Palmieri, de Simone et al. 2001). Investigators found that overweight individuals with LV hypertrophy also presented hypertension. Although the causative links between these factors may be debated, these results show that excess adipose tissue is associated with development of hypertension and LV hypertrophy in older men.

A habitual physical activity pattern could be an important modifier of the development of age-related cardiac and vascular stiffening, LV diastolic dysfunction, inflammation and central adiposity. Arbab-Zadeh et al. examined the effects of physical activity on LV compliance in elderly individuals (Arbab-Zadeh, Dijk et al. 2004). Twelve healthy sedentary men and women (mean age 69.8 ± 3 years) and 12 age-matched male and female Masters athletes (mean age 67.8 ± 3 years) participated. LV compliance was significantly lower in sedentary elderly individuals compared to Masters athletes. However, the LV compliance of Masters athletes was similar to a group of young sedentary individuals. This suggests that physical activity in later life may have beneficial effects on LV function, indicated by a more compliant LV. Physical in activity has also been shown to be related to the age-associated increase in inflammatory status (Mattusch, Dufaux et al. 2000, Taaffe, Harris et al. 2000, Geffken, Cushman et al. 2001). A large cross-sectional study in 13,748 US citizens (age ≥ 20 years old) was conducted examining physical activity status and plasma C-RP levels (Ford 2002). Those participants who were more physically active had significantly lower C-RP levels across all age ranges. The inflammation lowering effects of physical activity in elderly individuals could be a consequence of decreased central adiposity. Geffken et al. conducted an epidemiology study analysing data obtained from the Cardiovascular

Healthy Study in 5,888 men and women aged >65 years old, investigating the associations between self-reported physical activity levels, inflammatory markers and BMI (Geffken, Cushman et al. 2001). Following multivariate analysis, higher levels of physical activity were associated with lower levels of inflammation, which may be mediated by BMI. Elderly individuals who appear healthy may have underlying dysfunction of the heart (i.e., LV diastolic dysfunction); that may eventually result in development of heart disease such as congestive heart failure and coronary artery disease. LV diastolic dysfunction has been shown to be predictive of diastolic heart failure (Aurigemma, Gottdiener et al. 2001). Given the high mortality rates associated with heart disease (Murphy, Xu et al. 2013) the detection of LV diastolic dysfunction and development of effective interventional strategies is critical. Although tentative evidence suggests the positive effects of physical activity in elderly individuals on LV diastolic function, inflammatory status and visceral adiposity, there is limited information regarding the associations between these variables in a single cohort, and secondarily it is unclear which (if any) of these variables is the main driving factor for LV diastolic dysfunction in the elderly population.

5.3 Aims and Hypothesis

The aim of this study was to investigate the associations between LV diastolic function and the following variables: central adiposity, inflammation, resting HR, MAP and TPR, and physical activity in an elderly cohort.

It was hypothesized that in this elderly cohort:

1) LV diastolic function would be negatively associated with body composition measured by estimated visceral fat mass, waist:hip ratio and BMI.

2) LV diastolic function would be negatively associated with inflammatory status and oxidative stress, as indicated by plasma C-RP and leptin:adiponectin ratio, and lipid peroxide concentration.

3) LV diastolic function would be negatively associated with arterial stiffness, TPR, resting HR and MAP.

4) LV diastolic function would be positively associated with high physical activity measured as accelerometer counts/hour.

5.4. Methodology

5.4.1. Participants

One hundred and seventy five healthy elderly individuals from the cohort of 211 had valid left ventricular diastolic function measures of E/A ratio, which were included in this analysis. Their mean age, height and weight (mean ± standard deviation) were 67 ± 5 years, 167 ± 10 cm and 73 ± 14 kg, respectively.

5.4.2. Recruitment

Participants were recruited from the local community through both active recruitment with flyers, and through the Birmingham and Black Country Primary Care Trust (PCT) General Practice (GP) Surgeries. Individuals were only approached if they met the inclusion criteria (see Section 3.2).

5.4.3. Consent

Participants were invited to the Wellcome Trust Clinical Research Facility (WT-CRF) for a single visit to partake in the study. Testing took a maximum of 4 hours. All the participants arrived in a 10-hour overnight fasted state. The study details were explained by research nurses and written informed consent was obtained. All experimental procedures were performed in accordance with the Declaration of Helsinki and received approval from Birmingham and Black Country PCT Ethical Committee.

5.4.4. Experimental Measurements

Height, weight, BMI, waist and hip circumference, blood glucose and BP were measured upon arrival (see Section 3.3.1, 3.3.2 and 3.3.3 for details). Participants were also asked to provide details of any medications they were currently using. For inflammatory marker measurements, participants had approximately 40 mL of blood taken from superficial vein at the antecubital fossa (see Sections 3.3.4 and 3.9 for details). Participants were then given a light breakfast. Visceral adiposity was measured using DEXA, and presented as estimated visceral fat mass, (see Section 3.3.5 for details). Plasma CRP was used as the main marker of systemic inflammation, as it has been identified as a strong predictor of cardiovascular risk in previous research (Koenig, Sund et al. 1999), (see section 3.9.2 for details). Arterial stiffness was measured via PWV (see Section 3.4.2 for details). Central pulse wave velocity (cPWV) was selected in this analysis as a measure of vascular function due to the predictive effects on cardiovascular mortality (Laurent, Boutouyrie et al. 2001). LV diastolic function was measured using 2D Doppler ultrasound echocardiography (see Section 3.5 for details), and presented as E/A ratio (see Section 3.5.2 for details). Physical activity was determined from the acceloremeter recordings on seven continuous days (see Section 3.8.1 for details). Counts/hour was used to represent physical activity as it takes in to account all significant movements of the whole body, not solely steps (Sasaki, John et al. 2011).

5.4.5. Statistical Analyses

Multiple Hierarchical Regression was employed to test the main hypotheses and determine the relative importance of physical activity, inflammation, visceral fat mass and

resting HR, MAP and TPR in predicting the variance in E/A ratio in the study cohort. Distribution tests were performed to test for normal and non-normally distributed data. Logarithmic transformation was applied to non-normally distributed data. Non-parametric tests were performed for non-normally distributed plasma biomarkers, as typically log transformation fails to ensure normal distribution for these variables. Initially, correlation analysis was performed on the selected predictor variables to ensure collinearity assumptions were met. Univariate analysis was then performed on each predictor variable against the dependent variable (E/A ratio) to highlight any significant predictors. The significant predictors were then included in the Multiple Hierarchical Regression models. SPSS for Windows (IBM Corporation, Somers, NY, USA) was used for all statistical analyses. Data are presented as mean ± standard deviation (SD).

5.5 Results

5.5.1. Subject Characteristics

Table 5.1 provides descriptive statistics of the study cohort separately for men and women. As expected, in this cohort there were significant sex differences in height, weight and waist circumference being significantly greater in men. Table 5.2 presents frequency statistics for anti-hypertensive medication use in our sample population that could affect LV diastolic function. More than half of this cohort was taking anti-hypertensive medication (59%). The most common anti-hypertensive used by our participants was calcium blockers (19%).

5.5.2. Preliminary Analyses

Descriptive statistics for the dependent variable E/A ratio, and predictor variables (Resting HR, MAP, TPR, cPWV, BMI, Waist:Hip ratio, Visceral fat mass, Counts/hour, plasma C-RP and lipid peroxide concentrations and the plasma leptin:adiponectin ratio are presented in Table 5.3 for the whole sample and for men and women separately.

5.5.3. Predicting Left Ventricular Diastolic Function

Univariate and multivariate hierarchical analyses were employed to examine whether potential cardiovascular risk factors could predict the variance in LV diastolic function (E/A ratio), presented in Table 5.4. In Model 1 (unadjusted) univariate analysis indicates that MAP was a significant negative predictor of 28% of the variance in E/A ratio, and physical activity

(counts/hour) was a significant positive predictor of 2% of the variance in E/A ratio over and above all other measured variables (i.e. resting HR, TPR, cPWV, BMI, Waist:Hip ratio, visceral fat mass, plasma C-RP and lipid peroxide concentration, Leptin:Adiponectin ratio). Although a 2% predictor was statistically significant, the physiological and clinical relevance is questionable. This means that a higher MAP and a lower physical activity level (PAL), predict a lower E/A ratio, demonstrating a stiffer LV and worse diastolic function. The multivariate analysis shows that MAP and PAL both predict 5% of the variance in E/A ratio in our cohort. MAP is a stronger predictor of E/A ratio, as MAP has a higher *t* value compared to PAL (-2.31 vs. 2.14).

In Model 2 (adjusted for age and gender), univariate analysis indicates that MAP, BMI and visceral fat mass are significant negative predictors of the variance in E/A ratio (6%, 5 % and 7% respectively), over and above all the other variables. This means that the higher the MAP, BMI and visceral fat mass, the lower the E/A ratio and therefore diastolic function. BMI and visceral fat mass are collinearly related, so are not independently predicting E/A ratio. Visceral fat has a higher R^2 value than BMI (0.07 vs. 0.06), so was included in the mutually adjusted multivariate model instead of BMI. The multivariate analysis shows that MAP and visceral fat mass are significant negative predictors of 11% of the variance in E/A ratio in our cohort. MAP has a higher *t* value than visceral fat indicating that this is a stronger predictor.

In Model 3 (adjusting for age, gender, and anti-hypertensive medication) univariate analysis shows that MAP significantly negatively predicts 7% of the variance in E/A ratio, and visceral fat mass significantly negatively predicts 9% of the variance in E/A ratio, over and above all other predictor variables. This means that the higher MAP and visceral fat mass, the lower the E/A ratio and therefore diastolic function. The mutually adjusted

multivariate analysis shows MAP and visceral fat mass significantly negatively predict 12% of the variance in E/A ratio in our cohort. MAP has a higher *t* value than visceral fat indicating that this is a stronger predictor. The ultimate predictor in our cohort for the variance in E/A ratio, when taking in to account age, gender and anti-hypertensive medication, is MAP. MAP significantly predicts the variance in E/A ratio in our cohort, over and above other cardiovascular risk factors.

Table 5.1 Subject characteristics

	All (n=168)	Male (n=75)	Female (n=93)
Age (yr)	60 ± 5	67 ± 5	66 ± 5
	(60 – 79)	(60-79)	(60-79)
Height (cm)	167 ± 10	175 ± 8	161 ± 6
	(146 – 194)	(156 – 194)	(146-180)
Weight (Kg)	72 ± 14	80 ± 13	66 ± 11
	(42 – 121)	(61-121)	(42-109)
Hip circumference (cm)	103 ± 11	102 ± 9	103 ± 12
	(81 – 187)	(83-126)	(81-187)
Waist circumference (cm)	90 ± 11	95 ± 10	86 ± 11
	(62 – 118)	(77 – 118)	(62 – 116)
Systolic BP (mmHg)	136 ± 17	137 ± 16	135 ± 18
	(96 – 195)	(96-195)	(97-191)
Diastolic BP (mmHg)	78 ± 10	77 ± 9	78 ± 11
	(48 – 108)	(50-99)	(48 – 108)
Blood Glucose (mmol/L)	5.6 ± 0.6	5.6 ± 0.6	5.6 ± 0.6
	(3.9 – 7.0)	(4.2 – 6.9)	(3.9 – 7.0)

Values are presented as mean ± SD

Table 5.2 Anti-hypertensive medication use by the study cohort

Drug	All (Absolute,%)	Males (Absolute,%)	Females (Absolute,%)
Calcium Blocker	19, 9	8, 8	11, 9.9
ACE inhibitor	14, 6.6	8, 8	6, 5.4
AT1 Blocker	1, 0.5	1, 1	0, 0
ATII blocker	6, 2.8	2, 2	4, 3.6
Beta Blocker	5, 2.4	1, 1	4, 3.6
Diuretics	14, 6.6	6, 6	8, 7.2

Values are presented as frequency and percentage (%) of individuals who are on the specified medication

Table 5.3 Descriptive statistics of the dependent variable (E/A ratio) and predictor variables in this Cohort

Variables	All (Mean ± SD, Range)	Males (Mean ± SD, Range)	Females (Mean ± SD, Range)
E/A ratio	**1.10 ± 0.29 (0.59-2.50)**	**1.13 ± 0.28 (0.67-2.30)**	**1.08 ± 0.30 (0.59-2.50)**
Resting HR (bpm)	68 ± 12 (42-111)	66 ± 13 (42-111)	70 ± 10 (50-93)
MAP (mmHg)	98 ± 11 (68-127)	99 ± 10 (68-123)	97 ± 12 (68-127)
TPR (mmHg/L/min)	486 ± 189 (175-1302)	539 ± 215 (175-1302)	441 ± 150 (208-979)
cPWV (m/s)	8.72 ± 1.96 (4.90-14.17)	9.43 ± 2.12 (4.90-14.17)	8.38 ± 1.78 (5.05-13.77)
BMI (kg/m^2)	25.80 ± 3.74 (18.46-39.55)	26.10 ± 3.52 (20.24-39.55)	25.54 ± 3.92 (18.46-37.65)
Waist:hip ratio	0.87 ± 0.09 (0.48-1.09)	0.93 ± 0.06 (0.76-1.09)	0.83 ± 0.08 (0.48-0.98)
Visceral fat (g)	1009 ± 780 (19-3620)	1401 ± 866 (188-3620)	679 ± 506 (19-2492)
Counts/hour	63292 ± 18557 (20383-114207)	61388 ± 18719 (20383-114207)	65018 ± 18326 (32910-104528)
Plasma C-RP (mg/L)	1.41 ± 1.39 (0.06-8.33)	1.33 ± 1.38 (0.08-8.33)	1.48 ± 1.40 (0.06-6.64)
Plasma leptin:adiponectin ratio	4.04 ± 3.41 (0.06-18.48)	3.03 ± 3.16 (0.06-15.65)	4.94 ± 3.39 (0.21-18.48)
Plasma lipid peroxides (units per ml)	3345 ± 34 (260-439)	334 ± 30 (266-414)	336 ± 36 (260-439)

Values are presented as mean ± SD

Table 5.4 Univariate and Multivariate hierarchical analyses for predicting LV diastolic function

Variables E/A ratio	Model 1				Model 2				Model 3			
	β	*t*	*P*	R^2	β	*t*	*P*	R^2	β	*t*	*P*	R^2
Univariate												
Resting HR	-0.14	-1.90	.059	0.01	-0.12	-1.63	.105	0.04	-0.12	-1.63	.106	0.05
MAP	**-0.18**	**-2.50**	**.013***	**0.28**	**-0.18**	**-2.51**	**.013***	**0.06**	**-0.18**	**-2.44**	**.016***	**0.07**
TPR	0.06	0.68	.500	-0.00	0.04	0.42	.677	-0.00	0.05	0.53	.599	0.01
cPWV	-0.07	-0.73	.465	-0.00	-0.04	-0.48	.634	0.01	-0.06	-0.65	.520	0.02
BMI	-0.12	-1.64	.104	0.01	**-0.14**	**-1.98**	**.049***	**0.05**	-0.11	-1.45	.149	0.05
Waist:Hip	0.00	0.01	.992	-0.01	-0.13	-1.43	.154	0.04	-0.13	-1.45	.150	0.05
Visceral fat	-0.17	-1.89	.061	0.02	**-0.24**	**-2.49**	**.014***	**0.07**	**-0.21**	**-2.14**	**.034***	**0.09**
Counts/hour	**0.17**	**2.20**	**.029***	**0.02**	0.14	1.86	.065	0.05	0.14	1.79	.075	0.05
C-RP	-0.13	-1.68	.095	0.01	-0.11	-1.56	.122	0.05	-0.08	-1.10	.272	0.05
Leptin:Adiponectin	-0.10	-1.32	.188	-0.14	-0.09	-1.20	.233	0.04	-0.05	-0.59	.558	0.04
Oxidative Stress	-0.10	-1.30	.196	0.00	-0.09	-1.18	.241	0.04	-0.10	-1.30	.197	0.05
Mutually Adjusted												
MAP	-0.17	-2.31	.022*		-0.21	-2.37	.019*		-0.20	-2.33	.021*	
Counts/hour	0.16	2.14	.034*	0.05	-	-	-		-	-	-	
Visceral Fat	-	-	-		-0.21	-2.22	.028*	0.11	-0.18	-1.86	.065	0.12

Note: Gender was coded as 1 = males and 2 = females. *$P<0.05$

5.6. Discussion

The aim of this study was to examine the relationships between LV diastolic function, physical activity, inflammation, visceral adiposity, resting arterial stiffness, HR, MAP and TPR. The main findings of this study were when controlling for age, gender and anti-hypertensive medication use, LV diastolic function was negatively associated with estimated visceral fat mass, while waist:hip ratio and BMI were not significantly associated with LV diastolic function. LV diastolic function was not associated with inflammatory status or oxidative stress, as index using plasma C-RP and lipid peroxide concentration, and plasma leptin:adiponectin ratio. LV diastolic function was not significantly associated with arterial stiffness, TPR or resting HR, however it was significantly and negatively associated with MAP. LV diastolic function was not significantly associated with physical activity.

The prominent finding resulting from this study was that MAP was the major negative predictor of LV diastolic function, above all other variables examined including waist:hip ratio, BMI, plasma C-RP and lipid peroxide concentration, plasma leptin:adiponectin ratio, physical activity, resting HR, TPR and arterial stiffness. When controlling for age, gender and anti-hypertensive medication, estimated visceral adipose tissue mass also has a significant negative association with LV diastolic function, second only in importance to MAP.

5.6.1 Age, LV Diastolic Function and Body Composition

It is well known that central and visceral adiposity increases as we age. Poehlman et al. (Poehlman, Toth et al. 1995) conducted a large cross-sectional study on men and women

aged 18-88 years, and measured total body fat mass using hydrostatic weighing and central adiposity using waist circumference. It was concluded that older individuals possessed a significantly higher body fat mass and central adiposity, particularly in women. Song et al. conducted a 2 year follow-up study examining body composition in elderly African American women (age at baseline 75.5 ± 5.1 years) using DEXA and found that with advancing age, their total body fat mass increased and became centralized, as previously discussed (Song 2004). Visceral adiposity produces more inflammatory cytokines due to the abundance of macrophages compared to other adipose tissue stores (Berg and Scherer 2005), and has been shown to support a causal role for the development of LV hypertrophy (LVH) (MacMahon, Wilcken et al. 1986, Devereux and Alderman 1993). Hense et al. investigated the relationship between body size, body composition, and LV mass (LVM) in 653 men and 718 women aged 25 to 74 years, and observed that total fat mass had a positive relationship with LVM in both men and women (Hense, Gneiting et al. 1998). It could therefore be expected that, as adiposity increases with age, it would be related to changes in the LV, potentially via an increase in inflammation and promotion of cardiac fibrosis.

To the best of my knowledge, this study is the first to measure visceral adipose tissue mass and LV diastolic function via E/A ratio in a single elderly cohort, and is also the first to show that visceral adipose tissue mass is an independent predictor of the variance in LV diastolic dysfunction. Where previous studies have observed a negative association between whole body fat mass and LV mass, this cohort study suggests a negative association particularly of the visceral adipose tissue mass on LV function (MacMahon, Wilcken et al. 1986, Devereux and Alderman 1993, Hense, Gneiting et al. 1998). This observation is important as there is convincing published evidence that up to 60% of the cells in visceral adipose tissue are macrophages and that both the macrophages and the adipose tissue cells in

this compartment are an important source of inflammatory cytokines, PAI-1 and MCP-1 (Berg & Scherer (2005), Gustaffson et al 2007, Andersen et al 2008). It is probably for this reason that central adiposity poses the highest risk to cardiovascular disease development in both young and elderly individuals compared to other areas of adiposity (Prineas, Folsom et al. 1993). Visceral adipose tissue located in the central region secretes inflammatory cytokines that are associated with cardiovascular disease development (Wisse 2004, Fain 2006).

5.6.2 Age, LV Diastolic Function, Inflammation and ROS

Inflammation is known to increase with age and has detrimental effects on the cardiovascular system (Pawelec 2002, Berg and Scherer 2005, Panda, Arjona et al. 2009). In ageing, inflammation has been proposed to result from increased visceral adiposity. Given that in this cohort, estimated visceral fat mass had a significant negative association with LV diastolic function, it might be expected that a similar relationship between plasma markers of inflammation and LV diastolic function would be observed. Plasma C-RP was determined as it is the gold standard inflammatory risk factor associated with cardiovascular disease onset (Ballou and Kushner 1992, Sowers, Jannausch et al. 2002). However, there was no significant association between plasma C-RP concentration and LV diastolic function in this cohort.

The lack of an association between these variables is difficult to understand given that this contradicts earlier studies in large cohorts. For example, Schrager et al. used data from a large cross-sectional study on elderly individuals in Italy (InCHIANTI study), to examine the effects of central adiposity measured via anthropometry, on inflammatory markers (Schrager, Metter et al. 2006). From the analysis it was found that elderly individuals with higher waist

circumference (as an index marker of central adiposity), had higher levels of inflammation, specifically plasma C-RP and IL-6. A potential reason for the current study failing to observe plasma C-RP as a significant predictor of LV diastolic dysfunction could be the population studied. In this cohort the mean waist circumference was 90 cm, compared to 96 cm in the study by Schrager et al. In the study of Schrager et al individuals who had a significantly higher plasma C-RP and IL-6 had a mean waist circumference of 103 cm. This raises the possibility that there was a smaller number of individuals in this cohort with a large enough visceral adipose tissue mass to produce a significant increase in plasma C-RP concentrations.

Increased oxidative stress has been related to diseases observed with ageing such as cardiovascular disease (CVD) (Heitzer, Schlinzig et al. 2001), and is associated with vascular dysfunction (further explained in section 5.6.3). Oxidative stress describes a state at which production of ROS overloads the intrinsic ability to quench them, for example a reduction in anti-oxidants and anti-oxidative capacity (Monaghan, Metcalfe et al. 2009). It therefore was expected that in this cohort ROS would be negatively associated with LV diastolic function. However, ROS production measured as the plasma concentration of lipid peroxides, was not significantly associated with LV diastolic function. As a substantial amount of the ROS production may have occurred in endothelial cells and the vascular smooth muscle layer (see section 5.2) plasma lipid peroxides may not have been the best biomarker. Another possible explanation is the measurement of ROS in this study (plasma concentration of lipid peroxides). ROS have short half-lives meaning the appearance of them is labile in nature, and consequently difficult to measure (Monaghan, Metcalfe et al. 2009). Therefore, it is more common to measure the damage caused by ROS, rather than ROS per se (Monaghan, Metcalfe et al. 2009). Lipid peroxide concentration is not a direct measure of ROS, but a consequence of its actions. Although this may be seen as a limiting factor, ultimately the

damage being caused by ROS is detrimental to the cardiovascular system rather than the ROS themselves, giving this method some justification. The chosen method however only measures the damaging effects of ROS systemically, rather than directly at the affected site (e.g. the heart, vascular wall). Utilizing plasma concentrations of ROS damage however is less invasive and easily applicable compared to tissue sampling.

5.6.3 Ageing, LV Diastolic Function and Cardiovascular Risk Factors

Several cardiovascular variables/risk factors were examined and their potential effect on LV diastolic function in this cohort (i.e., HR, MAP, TPR and large central artery stiffness). Resting HR is not observed to increase as a function of age per se, but research suggests that it increases as a result of poor physical fitness (Erikssen, Liestol et al. 1998, Lakatta 2003). MAP, TPR and arterial stiffness however are observed to increase during the ageing process. In this cohort MAP was significantly and negatively associated with LV diastolic function, whereas no association with HR, TPR and large central artery stiffness was observed. The finding that MAP was negatively associated with LV diastolic dysfunction, but TPR and arterial stiffness were not was somewhat unexpected. Indeed, an increase in blood pressure has been found to be a result of increased arterial stiffness in elderly individuals (O'Rourke 1990). Arterial stiffness is associated with an increase in inflammatory cytokines and oxidative stress. Kuwahara et al. performed a study in hypertensive rats, measuring MAP, E/A ratio, inflammation and fibrosis over 28 days in aortic constricted rats and sham controls (Kuwahara, Kai et al. 2004). Aortic constriction was employed to provide a pressure overload stimulus in order to mimic hypertension. Aortic constriction caused an increase in MAP and perivascular macrophage accumulation after 1 day and fibrosis was observed at day

3. Accumulation of macrophages in the vascular wall leads to the local production of inflammatory cytokines such as C-RP, TNF-α and IL-6. In addition to causing this inflammatory response, E/A ratio also decreased in rats with aortic constriction. It was suggested that myocardial fibrosis is also triggered by a macrophage-mediated inflammatory process that is generated by the arterial wall in response to pressure overload. This suggests that increases in MAP may precede significant increases in inflammation, oxidative stress, fibrosis and TPR, potentially explaining the null findings of this study in terms of the links between inflammatory cytokines, oxidative damage and central arterial stiffness.

Arterial hemodynamic stress is associated with production of proinflammatory cytokines around the arterial wall (Nicoletti and Michel 1999). Indeed an increase in arterial pressure itself is potentially a strong signal for activation of macrophage infiltration, and activates NADPH oxidase, which produces superoxide anions (ROS) (Wagenmakers, van Riel et al. 2006). In hypertensive states, production of ROS and induction of inflammatory cytokines have been shown in the arterial wall (Hisashi, Fumitaka et al. 2005). Figure 5.1 details the effects of inflammation on vasodilatation. Inflammatory cytokines are associated with an increase in ROS (Nagai, Anzai et al. 2011), which quench endothelial NO before vasodilatation occurs. In the reaction between superoxide anions (produced by NADPHoxidase) and NO peroxynitrate is produced as a bi-product (Figure 5.1). A reduction of NO synthesis and arterial fibrosis reduces compliance of the arteries leading to arterial stiffness. Decreased vascular compliance is associated with ageing and hypertension (McVeigh, Burns et al. 1991). Given the evidence it would be expected that in this cohort, inflammation, oxidative stress, arterial stiffness and TPR would be significantly associated with LV diastolic function, however these associations were not significant. This potentially suggests an alternative mechanism for the association between MAP and LV diastolic

function. It has been postulated that renal mechanisms may give rise to the onset of hypertension, in elderly individuals (Sarnak, Levey et al. 2003, Vanholder, Massy et al. 2005). Most cardiovascular disease risk factors, such as older age, diabetes mellitus, hypertension, LVH, and low high-density lipoprotein (HDL) cholesterol, are highly prevalent in chronic kidney disease (CKD). In CKD patients, hypertension results in pressure overload and leads to LVH, which may lead to diastolic dysfunction (Sarnak, Levey et al. 2003). Dysfunction of the renal system results in reduced removal of extra fluid, potentially increasing blood pressure (Sarnak, Levey et al. 2003, Vanholder, Massy et al. 2005). Renal dysfunction in this elderly cohort may therefore play a role in the association between MAP and LV diastolic function.

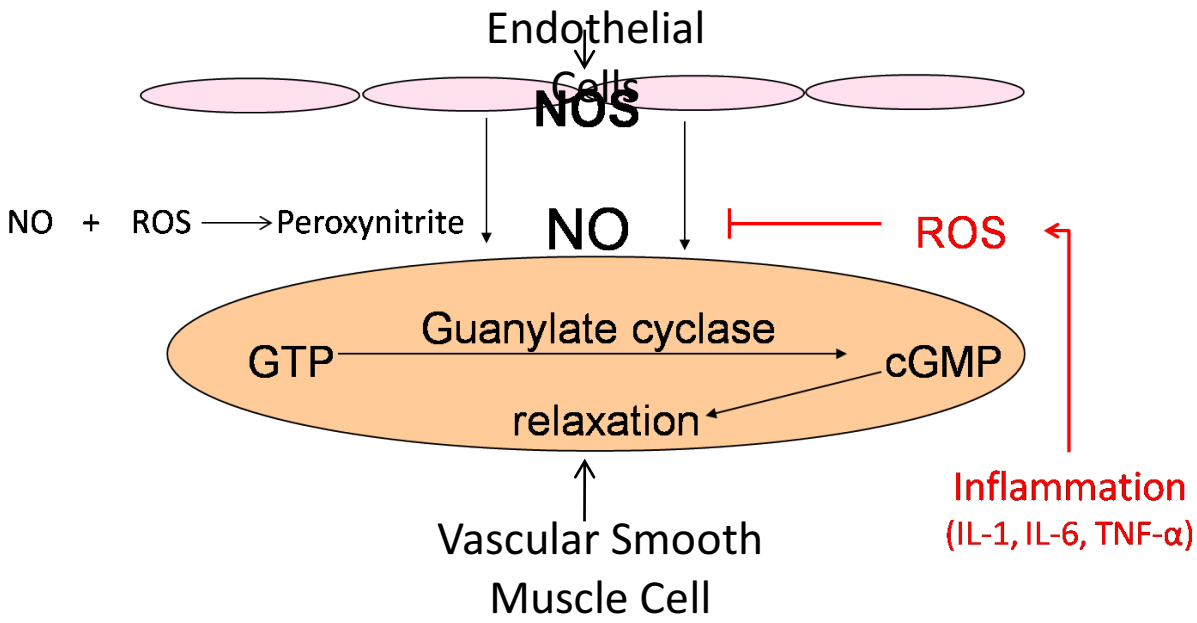


Figure 5.1: Diagram of the effects of inflammation and ROS on NO bioavailability and consequentially vasodilation. Endothelial Cells (ECs); Vascular Smooth Muscle Cell (VSMcs). TNF-α and IL-6 bind to receptors on the luminal site of the ECs and lead to induction of NADPHoxidase in EC's and production of superoxide anions (most reactive

species of ROS) which quenches NO in the ECs. Peroxynitrite is a bi-product of ROS quenching NO. NO activates Gunanylate cyclase, converting GTP to cGMP resulting in VSMc relaxation. The attenuated bioavailability of NO reduces this activation cascade, so vasdilation is decreased.

5.6.4 Ageing, LV Diastolic Function and Physical Activity

In this cohort study it was hypothesized that physical activity would be positively associated with LV diastolic function, possibly via a central adiposity lowering mechanism. Given previous evidence (Arbab-Zadeh, Dijk et al. 2004), it would be expected that physical activity be positively associated with LV diastolic function, therefore the lack of association between physical activity and LV diastolic function in this cohort was unexpected. The reason for conflicting findings of Arbab-Zadeh et al. and the present study could be due to the extreme conditioned state of masters athletes, compared to our recreationally active healthy elderly individuals. Masters athletes are considered to be a model of "exceptionally successful ageing" and strive to maintain their physiological performance (Tanaka and Seals 2008). Gates et al. examined LV diastolic function represented by E/A ratio in 138 healthy young (18-37 years), middle-aged (38-57 years) and older (58-77) men (Gates, Tanaka et al. 2003). Participants were classed as either sedentary, recreationally active or endurance trained. An ageing effect of LV diastolic function was present in the participants, and physical activity status did not prevent this ageing effect. This study concurs with the findings of this cohort, although this cohort is larger (175 vs. 138 participants), in that recreational and even endurance physical activity had no significant effect on LV diastolic function, potentially supporting the concept that the positive effects of exercise on LV

diastolic function are only detected in extremely highly trained masters athletes, as in the study by Arbab-Zadeh et al. The same group examined the effects of central fat mass on LV diastolic function, and found that increases in total adiposity have an important contribution to the age-related development of LV diastolic dysfunction (Gates, Gentile et al. 2003). This relays to the findings of this cohort study in that estimated visceral fat mass was significantly negatively associated with LV diastolic function, second only to MAP, suggesting that the age-related increase in visceral adiposity may a contributing mechanism to age-related LV diastolic dysfunction, rather than physical activity per se, contrary to the study hypothesis.

5.6.5 Methodological Considerations

The first limitation to note in this study is the characteristics of the cohort and a possible selection bias. All participants were healthy elderly individuals (60-80 years old) with no apparent pathophysiology or disease. Although participants were deemed healthy, many were on anti-hypertensive medication (28%). These medicated individuals could naturally have high blood pressure due to inactivity, and could potentially mask the effect of physical activity on LV diastolic function. However, anti-hypertensive medication use was selected as a covariate in the analysis presented in Model 3, eradicating any confounding effects on LV diastolic function. The inclusion criteria for this cohort allowed individuals with blood pressure less than 190/120 mmHg (see section 3.2 for details), which would include hypertensive individuals. In many clinical trials comorbidities have been the main reason to exclude elderly individuals. However, as explained in Chapter 4 section 4.6.5, if exclusion criteria are too restricted, it may compromise the generalizability of the results (Ferrucci, Guralnik et al. 2004).

As in Chapter 4, the physical activity range of this cohort is also a possible confounder. None of the cohort fell in to an extreme case of sedentary behaviour or highly trained athletes, despite the physical activity levels between the least and most active individuals being significantly different (5-fold increase), Tanaka and Seals revealed the vast range of physical activity levels in elderly individuals from completely sedentary to highly trained masters athletes (Tanaka and Seals 2008). Endurance-trained ageing runners show reduced disability and mortality compared to age-matched sedentary individuals (Chakravarty, Hubert et al. 2008). In contrast, many extremely sedentary elderly individuals are in diseased states. This feeds back in to the selection bias limitation of this study, in that the cohort was reasonably active. As explained in Section 4.6.5 of Chapter 4, again the physical activity range in this cohort may not be broad enough to detect minor differences of physiological function in healthy elderly individuals or LV diastolic impairments.

Another limitation may be the technique used to examine LV function. Two-dimensional echocardiography was utilized; however an alternative technique would be Magnetic Resonance Imaging (MRI) scanning. Arbab-Zadeh et al. validated their echocardiography measurements with cardiac magnetic resonance imaging (MRI) measurements (Arbab-Zadeh, Dijk et al. 2004) and yielded an excellent correlation between the methods (r=0.9; slope 0.9; P<0.001; coefficient of variation, 12.5%). So although the use of MRI for LV diastolic function measures would be an alternative method, echocardiography is more than adequate and has been robustly used in many studies of LV diastolic function (Spirito and Maron 1988, Prasad, Popovic et al. 2007, Libhaber, Norton et al. 2009). Reliability of the measurement of E/A ratio could also be a limiting factor. Reproducibilty tests for the measurement of E/A ratio were performed before commencement of the study, and produced a CoV of 12.3%. As mentioned previously in Section 3.5.5, a CoV

of >10% is regarded as having reduced reliability. However, E/A ratio was chosen as the measurement of choice for LV diastolic function as it is thought to be the most dramatic change in cardiac pump function with ageing and is usually the earliest manifestation of a disease process, as discussed in Section 2.6.2 (Nishimura and Tajik 1997, Lakatta 2002). Additionally, if the mean value is typically low, such as is the case for E/A ratio which generally ranges from 1.2-2.1, the CoV tends to be higher, which should be taken in to account when interpreting this CoV value.

5.6.6 Conclusion

In this cohort MAP and visceral adiposity had a significant negative association with LV diastolic function (measured as E/A/ratio). However, no significant associations were observed between LV diastolic function and BMI, Waist:Hip ratio, plasma C-RP and lipid peroxide concentration, central arterial stiffness, resting HR and TPR and physical activity levels measured with accelerometers. In this same cohort, as presented in Chapter 4 higher visceral adiposity has been shown to have a negative association with physical activity. It could be possible that a number of predictor associations have been missed in this cohort as the sample size was too small and as we may have missed underlying pathologies such as renal dysfunction and variety of inflammatory diseases

CHAPTER 6:

EFFECT OF MUSCLE METABOREFLEX ACTIVATION ON CENTRAL HEMODYNAMICS AND CARDIAC FUNCTION IN HUMANS

6.1 Abstract

We sought to determine how the mode of muscle metaboreflex activation influences the central hemodynamic response and cardiac inotropic and lusotropic function in healthy humans.

Ten healthy males performed isometric handgrip (IHG) with and without post exercise ischemia (PEI) to examine the influence of isolated muscle metaboreflex activation, and rhythmic handgrip (RHG) with and without ischemia to examine the influence of enhanced muscle metaboreflex activation. HR and BP were continuously monitored. Stroke volume (SV, Doppler echocardiography) was measured, cardiac output (CO = HR×SV) and total peripheral resistance (TPR = mean BP/CO) calculated, and indices of LV systolic and diastolic function were obtained (TDI).

During isolated muscle metaboreflex activation with PEI following IHG, mean BP (+23±3 mmHg) and TPR were elevated from baseline resting (P<0.05), while HR, SV and CO were unchanged. Enhanced muscle metaboreceptor activation during ischemic RHG augmented the increase in mean BP, CO and HR (P<0.05 ischemic vs. free-flow RHG), while SV and TPR were unchanged from baseline. Neither isolated (PEI) nor enhanced muscle metaboreflex activation altered left ventricular systolic function (systolic myocardial velocity, S'), but left atrial systolic function (late diastolic myocardial velocity, A') was enhanced.

These findings indicate that the mode of muscle metaboreceptor activation (during vs. post handgrip) determines whether the resultant pressor response is flow (CO) or vasoconstriction (TPR) mediated, and that while left ventricular systolic function is unchanged, enhanced left atrial systolic function likely aids the preservation of SV during muscle metaboreflex engagement.

6.2 Introduction

The increased metabolic demand of the exercising skeletal muscles is accompanied by a complex integrated cardiovascular response. This is orchestrated by both central and peripheral neural mechanisms, including feed-forward signals from higher brain centres (i.e. central command) and reflex feedback from group III and IV sensory afferents within the working skeletal muscle in response to mechanical (i.e. muscle mechanoreflex) or metabolic stimuli (i.e. muscle metaboreflex) (Krogh and Lindhard 1913, Alam 1937, Coote, Hilton et al. 1971, McCloskey and Mitchell 1972, Kaufman, Longhurst et al. 1983). Although it is well established that the muscle metaboreflex increases sympathetic nerve activity and BP (Alam 1937, Mark, Victor et al. 1985), whether the latter is attributable to an increase in CO (O'Leary 1998, Crisafulli, Scott et al. 2003, Sala-Mercado, Hammond et al. 2006, Crisafulli 2011), TPR (Bastos, Williamson et al. 2000, Lykidis, White et al. 2008) or both (Bonde-Petersen 1982, Crisafulli, Salis et al. 2006) is equivocal. Germane to this debate is the issue of how the mode of muscle metaboreflex activation (i.e. during vs. following exercise) effects SV and cardiac chronotropic, inotropic and lusitropic function in humans.

The majority of studies that have examined the hemodynamic effects of muscle metaboreflex activation in humans have used a PEI manoeuvre, whereby a cuff is inflated around the exercising limb to a supra-systolic level just before the end of exercise, trapping the metabolites within the muscle (Alam 1937). Maintaining cuff inflation following exercise isolates the metaboreflex, in the absence of mechanoreflex or central command activation. During this manoeuvre the exercise-induced elevation in sympathetic nerve activity and BP remain high, whereas HR typically returns towards resting levels (Alam 1937, Mark, Victor et al. 1985). This may be explained by the reactivation of cardiac parasympathetic tone, due

to baroreflex activation and/or loss of the inhibitory effect of central command and the muscle mechanoreflex, which masks any potential sympathetic chronotropic effect (O'Leary 1993, Nishiyasu, Tan et al. 1994, Iellamo, Massaro et al. 1999, Fisher, Seifert et al. 2010). The observation that HR rapidly returns towards resting levels during PEI has led some to suggestion that the muscle metaboreflex has little influence on the heart (Freund, Hobbs et al. 1978, Mark, Victor et al. 1985, Victor, Seals et al. 1987, Rowell and O'Leary 1990). Similarly, several studies have observed that SV is also unchanged during PEI (Pawelczyk, Pawelczyk et al. 1997, Bastos, Williamson et al. 2000, Crisafulli, Salis et al. 2006, Lykidis, White et al. 2008), suggesting that the elevated BP during PEI is not attributable to an increase in CO, but is secondary to an increase in TPR. However, this has not been a consistent finding and several investigations utilizing thoracic impedance cardiography have reported that the BP elevation during PEI occurs secondary to an increase in CO due to an elevation in SV in humans (Crisafulli, Scott et al. 2003, Crisafulli, Salis et al. 2006, Crisafulli 2011). This elevation in SV during PEI has been attributed in part to a muscle metaboreflex mediated increase in myocardial contractility and relative increase in diastolic filling time coincident with a reduction in HR below baseline levels (Crisafulli, Scott et al. 2003, Crisafulli, Salis et al. 2006, Crisafulli 2011). Although PEI is an effective and established means of isolating the actions of the muscle metaboreflex the accompanying autonomic alterations, and the absence of central command and muscle mechanoreflex activation, means that any observed effects of PEI on SV may not be representative of the effects of metabolically sensitive muscle afferents during exercise.

An alternative means of studying the actions of the metaboreflex is to engage it during exercise by partially or completely restricting perfusion to the exercising skeletal muscle (O'Leary 1998, Shoemaker, Mattar et al. 2007, Coutsos, Sala-Mercado et al. 2010, Crisafulli

2011). This enhances the metaboreflex, and perhaps because central command and the muscle mechanoreflex are also activated, both BP and HR increase due to heightened sympathetic activity and to a lesser extent the inhibition of cardiac parasympathetic tone (O'Leary 1993, Hartwich, Dear et al. 2011, Fisher, Adlan et al. 2013). In a recent canine study, partial restriction of hindlimb perfusion during treadmill exercise evoked a significant elevation in BP attributable to an increase in CO secondary to an increase in HR, while SV and peripheral vascular conductance remained unchanged (Spranger, Sala-Mercado et al. 2013). Notably, the chronotropic response was accompanied by an increase in LV contractility and rate of relaxation, suggesting that the muscle metaboreflex affects multiple facets of myocardial performance. In contrast, the effect of enhancing metaboreflex during exercise on SV and myocardial function remains incompletely understood in humans. Doppler echocardiography provides an attractive means of assessing how the mode of muscle activation (i.e. during vs. following exercise) affects cardiac inotropic and lusitropic function in humans, and thus may help explain the resultant changes in SV. Quantification of mitral inflow velocities and the velocity of left ventricle contraction and relaxation along its longitudinal axis using pulse-wave TDI have been used to assess cardiac systolic and diastolic function during isometric handgrip exercise (Muller 2013), but the specific contribution of the metaboreflex has not been determined.

Much scientific interest has been focused on the metaboreflex, due to its role in cardiovascular regulation during exercise (Crisafulli, Salis et al. 2006, Boushel 2010, Crisafulli 2011), but also its over-activation in heart failure patients, which consequentially limits exercise capacity (O'Leary, Sala-Mercado et al. 2004, Ansorge, Augustyniak et al. 2005, Crisafulli, Salis et al. 2007, Piepoli, Dimopoulos et al. 2008). As well as in heart failure patients, exercise capacity in elderly individuals is limited, and has prognostic effects on

cardiovascular mortality (Nylen, Kokkinos et al. 2010). As discussed in Section 2.8 of this thesis, ageing is associated with altered resting autonomic regulation, specifically a reduction of parasympathetic nerve activity and vasomotor sympathetic responsiveness (Barnett, Morin et al. 1999, Kuo, Lin et al. 1999). Reduced sensitivity of α-receptors in the skeletal muscle vasculature and β-receptors in the heart occurs, and are believed to lead the higher basal SNA and sympathodominance in the elderly (Lakatta 1993, Smith, Voyles et al. 2007).The reduced sensitivity of cardiac β-receptors is also responsible for the reduced HR_{max} observed with ageing, contributing to decreased exercise capacity. In the vasculature, increased muscle vasoconstrictor tone could lead to a reduction in muscle blood flow, an augmented BP response, and again may contribute to attenuated exercise tolerance (Lakatta 1993, Smith, Voyles et al. 2007). The metaboreflex increases sympathetic nerve activity and BP, which are characteristics of ageing. Mechanisms by how the metaboreflex increases BP during exercise in ageing may be different than in young due to increased arterial stiffness, reduced LV diastolic function and HR_{max} achievable. Therefore, understanding the mechanisms of metaboreflex over-activity on the BP response to exercise in young individuals is the first key step to understanding potential mechanisms of reduced exercise capacity in elderly individuals.

6.3 Aims and Hypothesis

Our primary aim was to investigate how the mode of muscle metaboreflex activation (i.e. during vs. following handgrip) affects the central hemodynamic response and cardiac systolic and diastolic function in healthy humans determined using echocardiographic imaging. We hypothesised that SV would only be increased by the muscle metaboreflex when it was activated in isolation during PEI and not when it was augmented during ischemic exercise.

6.4 Methodology

6.4.1 Ethical Approval

The study protocol and procedures conformed to the Declaration of Helsinki and were approved by the local ethics review committee (University of Birmingham). Subjects were informed of the study purpose and potential risks before providing written informed consent for participation.

6.4.2 Subject Recruitment

Ten male participants recruited from the undergraduate population of the University of Birmingham, UK took part in the study (age 20 ± 1 yr; height 181 ± 7 cm; weight 78 ± 10 kg; mean ± SD). The sample size was determined in accordance with recently published studies in the area (Muller, Mast et al. 2013). No subjects were taking any medications and all were free from cardiovascular, pulmonary, renal, metabolic and neurological conditions. An initial visit to the laboratory served to accustom subjects to the laboratory environment and procedures. Prior to testing subjects refrained from strenuous exercise and alcohol intake for 24 hours, caffeine intake for 12 hours, and food intake for 2 hours. All experiments were conducted in the same room with a mean ambient temperature of 23 ± 1 °C.

6.4.3 Experimental Measurements

Heart rate (HR) was measured using an ECG and beat-to-beat BP was measured non-invasively from the left hand via finger plethysmography (Portapress Finapres Medical Systems, Amsterdam, The Netherlands). The left forearm and hand were supported on an arm

rest raised to the level of the heart. The arterial pressure waveform was integrated on a beat-to-beat basis to obtain MAP. A handgrip dynamometer was held in the right hand, and force was displayed on a computer screen to provide visual feedback to the subject relating to the grip force generated. HR, BP and force were converted from analogue-to-digital signals at 1 kHz (1401, Cambridge Electric Design, Cambridge, UK) and captured for offline analysis (Spike 2, Cambridge Electric Design).

6.4.4 Echocardiography

Echocardiographic images were acquired using ultrasound equipment (Sonos 5500, Philips Medical Systems Andover, MA, USA), using an S3 two-dimensional transducer (1-3 MHz). All images were obtained and analysed by an experienced sonographer (CM) and stored on an external storage device for offline analysis.

Aortic Outflow: To quantify flow through the aortic valve during systole, an apical 5 chamber view was used. The velocity profile of the aortic outflow was obtained using pulsed-wave Doppler with a sample volume of 2.0 mm placed in the LV outflow tract at the opening of the aortic orifice. The LV outflow tract diameter was measured in the parasternal long axis view. Trans-aortic flow, representing SV was calculated from the cross sectional area and VTI, which represents the mean distance through which blood travels in the aortic outflow tract during ventricular contraction.

Mitral Inflow Velocities: Mitral inflow velocities were assessed from the apical 4 chamber view using pulsed wave Doppler with a sample volume of 2.0 mm positioned over the mitral valve leaflet tips. From these, data measurements were obtained of the peak inflow velocity during the early phase of LV relaxation (E), which provides an index of LV diastolic

function, and during LA contraction (A), which provides an index of LA systolic function (Hatle 1993, Cohen 1996). These values were subsequently used to further assess diastolic function by calculation of the E/A ratio, and left atrial systolic function by calculation of the relative contribution of A to left ventricular filling during diastole, using the following formula: [A/(E + A)] ×100 (Hatle 1993, Prasad, Popovic et al. 2007, Brothers 2008).

TDI: Measurements of peak septal mitral annular early diastolic (E'), late diastolic (A'), and systolic (S') velocities were obtained to provide indices of LV diastolic function, LA systolic function, and left ventricular systolic function, via standard TDI techniques (Nagueh, Middleton et al. 1997, Prasad, Popovic et al. 2007). TDI measurements were obtained from the apical 4 chamber view with a 2.0 mm sample volume positioned at the junction of the septal mitral annulus and the LV wall. The mitral annulus at the septum was examined because, while the cardiac apex remains relatively fixed, it moves in a direction approximately parallel to the ultrasound beam and is relatively unaffected by movement of the heart within the chest cavity (Sohn, Chai et al. 1997). The E' value was subsequently used for a calculation of E/E' ratio, which is a strong correlate of LVfilling pressure measured invasively (Nagueh, Middleton et al. 1997). S' velocity is representative of the peak velocity of mitral annular movement during systole, and has been validated as a measure of cardiac systolic function (Gorcsan 1998, Shimizu 1998).

6.4.5 Experimental Protocol

All examinations were performed whilst participants were in the semi-recumbent position. A cuff was placed around the right upper arm, which was set to rapidly inflate to supra-systolic pressure (220 mmHg) to occlude both vascular inflow and outflow using an automated cuff inflator and air source (AG101, Hokanson Inc., Bellevue, WA, USA) as

required. Following instrumentation, maximal voluntary contraction (MVC) was determined. They were given 3-5 attempts with a 1 min rest period between each. Participants performed 4 bouts of handgrip exercise in total, performed at 25% of their MVC:

Isolated Metaboreflex Activation: Participants rested for 3 min, then performed 3 min of IHG. Ten seconds before the end of contraction, the cuff on the upper arm was rapidly inflated to a supra-systolic level (>200 mmHg), and remained inflated for a further 3 min (PEI) before being deflated. An IHG control trial was also performed, which consisted of 3 min rest, 3 min IHG, and 3 min of recovery under free-flow conditions. Trials were performed in a randomized order.

Enhanced Metaboreflex Activation: Participants rested for 3 min and then performed RHG contraction guided by a metronome (1 s contraction, 1 s relaxation). Prior to the start of the RHG trial the upper arm cuff was rapidly inflated to a supra-systolic level. The purpose of this ischemic RHG trial was to enhance the muscle metaboreflex during exercise, to better represent the effects of activating metabolically sensitive muscle afferents during exercise.

Participants continued performing the RHG contractions until volitional fatigue. Following this the upper arm cuff remained elevated for a further 3 min (PEI). A control bout of RHG was also performed without cuff inflation, so that contractions were performed under free-flow conditions. The duration of the free-flow RHG period was time-matched to the fatiguing ischemic RHG bout. As such the free-flow RHG trial was always performed after the ischemic rhythmic handgrip trial. Ratings of perceived exertion (RPE) during handgrip were obtained using the Borg Scale (6-20) (Borg 1982).

6.4.6 Data Analysis

Mean HR and BP data were obtained on a beat-to-beat basis and averages calculated at baseline (3 min), end-exercise (last 1 min) and recovery (last 2 min of PEI or free-flow recovery). Echocardiographic images for calculation of aortic outflow, mitral inflow and mitral annular velocities were obtained from three cardiac cycles at, or around, end expiration. This acquisition procedure was undertaken in triplicate at baseline, once during the last minute of exercise, and in duplicate during the last 2 min of PEI or free-flow recovery. Measurements were then pooled to provide a mean value for each experimental phase. Aortic root diameter was obtained in triplicate prior to the beginning of the first handgrip trial. During post-acquisition analysis SV (ml) was calculated as VTI (cm) × aortic root area (cm^2), CO (L/min) was calculated as SV (ml) × HR (b/min) / 1000, and TPR (mmHg/L/min) calculated as MAP (mmHg) / CO (L/min). Post-acquisition analysis was performed blinded to the condition to reduce selection bias.

6.4.7 Statistical Analysis

Comparisons of physiological variables were made using a two-way repeated-measures ANOVA, in which experimental phase (baseline, handgrip, recovery [PEI, free-flow]) and trial (metaboreflex isolation [PEI] vs. free-flow recovery; enhanced metaboreflex activation [ischemic rhythmic handgrip] vs. free-flow exercise) were the main factors. *Post hoc* analysis was employed using Student Newman Keuls tests to evaluate significant main effects and interactions. Paired t-tests were used to compare RPE during handgrip bouts. Statistical significance was set at $P < 0.05$. Analyses were conducted using SigmaStat (Jandel Scientific Software, SPSS, Chicago, IL) for Windows.

6.5 Results

6.5.1 Isolated Metaboreflex Activation

As expected, isometric handgrip exercise significantly increased MAP, CO and HR from baseline levels ($P<0.05$), whereas TPR and SV remained unchanged (Figure 6.1). During isolated muscle metaboreflex activation with PEI, MAP and TPR were elevated ($P<0.05$ vs. baseline), while CO and HR returned to baseline. During recovery under free-flow conditions neither MAP, TPR, CO nor HR were significantly different from baseline ($P>0.05$; Figure 6.1).

Peak E velocity remained unchanged from baseline during isometric handgrip, while peak A velocity increased ($P<0.05$ vs. baseline). E/A ratio was significantly decreased during IHG, and the relative contribution of A to LV filling significantly increased ($P<0.05$ vs. baseline; Figure 6.2). Peak A velocity, peak E velocity, E/A ratio and the relative contribution of A to LV filling were not significantly different from baseline during either PEI or free-flow recovery ($P>0.05$).

IHG significantly decreased TDI determined peak E' velocity, and increased peak A' velocity and E/E' ratio ($P<0.05$ vs. baseline), whereas S' velocity remained unchanged ($P>0.05$ vs. baseline; Table 6.1). During PEI, peak A' velocity remained elevated above baseline ($P<0.05$), whereas during free-flow recovery A' returned to baseline. PEI following isometric exercise had no significant effect on either peak E' velocity, peak S' velocity, or E/E' ($P>0.05$ vs. baseline; Table 6.1). RPE was not significantly different between IHG trials (14.6 ± 0.5 vs. 14.4 ± 0.5 au, isometric handgrip with PEI vs., isometric handgrip with free-flow recovery: $P>0.05$).

6.5.2 Enhanced Metaboreflex Activation

RHG under free-flow conditions did not significantly alter MAP, CO, TPR, HR or SV. However, enhancement of the metaboreflex during ischemic RHG caused a significant increase in MAP, CO and HR ($P<0.05$ vs. baseline and free-flow rhythmic handgrip; Figure 3), whereas TPR and SV remained unchanged. During PEI following ischemic RHG, MAP and TPR were elevated above baseline ($P<0.05$) while CO, HR and SV were at baseline levels ($P>0.05$; Figure 6.3).

Peak E velocity was unchanged from baseline during RHG under either free-flow or ischemic conditions ($P>0.05$; Figure 6.4). During free-flow RHG exercise, peak A velocity remained unchanged ($P>0.05$ vs. baseline), but increased during ischemic exercise ($P<0.05$ vs. baseline). E/A ratio was reduced during free-flow and ischemic RHG ($P<0.05$ vs. baseline), and the relative contribution of A to LV filling increased ($P<0.05$). All peak transmitral valve flow velocities were unchanged from baseline during PEI and free-flow recovery (P>0.05).

TDI derived E' and S' velocities were unchanged from baseline during free-flow and ischemic RHG ($P>0.05$; Table 6.2). A' was increased during both free-flow and ischemic RHG ($P<0.05$ vs. baseline), while E/E' was increased during ischemic RHG and remained elevated during PEI ($P<0.05$ vs. baseline). All other TDI derived velocities of LV contraction (S') or relaxation (E', A') were not significantly different from baseline during PEI ($P>0.05$; Table 2). RPE was significantly greater during ischemic RHG (19.1 ± 0.3 au) than RHG under free-flow conditions (10.7 ± 0.5 au: $P<0.05$).

Table 6.1 Tissue Doppler data during isometric handgrip (IHG) and either free-flow recovery or post exercise ischemia (PEI).

	Rest	IHG	Free-flow recovery	Rest	IHG	PEI	Phase	Trial	Interaction
E' (cm/s)	12.5 ± 0.5	11.2 ± 0.6	12.6 ± 0.4	13.0 ± 0.5	11.7 ± 0.7	12.9 ± 0.7	< 0.05	0.196	0.915
A' (cm/s)	5.2 ± 0.4	7.2 ± 0.7*	5.8 ± 0.5	5.4 ± 0.4	7.4 ± 0.5*	7.2 ± 0.6*†	< 0.05	< 0.05	< 0.05
E/E'	6.6 ± 0.3	7.5 ± 0.5	6.7 ± 0.2	6.4 ± 0.3	7.9 ± 0.6	7.0 ± 0.4	< 0.05	0.589	0.328
S' (cm/s)	8.7 ± 0.6	8.3 ± 0.5	9.0 ± 0.5	8.6 ± 0.4	8.4 ± 0.4	8.5 ± 0.4	0.225	0.438	0.096

E', peak septal mitral annular early diastolic velocity; A', peak septal mitral annular late diastolic velocity; E/E', ratio between the peak mitral inflow velocity during the early phase of LV relaxation and peak septal mitral annular early diastolic velocity; S', peak septal mitral annular systolic velocity. *P* values represent results of ANOVA. Data are expressed as mean ± SE. **P*<0.05 *versus* baseline of the corresponding trial; †*P*<0.05 *versus* the corresponding time point of the opposing trial.

Table 6.2 Tissue Doppler measurements during rhythmic handgrip (RHG) and PEI

	Rest	RHG	Free-flow recovery	Rest	Ischemic RHG	PEI	Phase	Trial	Interaction
E' (cm/s)	12.5 ± 0.5	12.2 ± 0.7	12.9 ± 0.8	12.9 ± 0.8	11.9 ± 0.8	12.4 ± 0.7	0.214	0.689	0.326
A' (cm/s)	5.1 ± 0.4	6.6 ± 0.6	5.6 ± 0.5	6.0 ± 0.4	8.0 ± 0.6	8.0 ± 0.7	< 0.05	< 0.05	0.148
E/E'	6.8 ± 0.4	7.1 ± 0.4	6.7 ± 0.4	7.0 ± 0.5	8.2 ± 0.6*†	7.4 ± 0.4†	< 0.05	< 0.05	< 0.05
S' (cm/s)	8.3 ± 0.4	8.3 ± 0.3	8.4 ± 0.4	9.0 ± 0.5	9.0 ± 0.3	8.3 ± 0.3	0.468	< 0.05	0.131

E', peak septal mitral annular early diastolic velocity; A', peak septal mitral annular late diastolic velocity; E/E', ratio between the peak inflow velocity during the early phase of LV relaxation and peak septal mitral annular early diastolic velocity ; S', peak septal mitral annular systolic

velocity. *P* values represent results of ANOVA. Data are expressed as mean ± SE. **P*<0.05 *versus* baseline of the corresponding trial; †*P*<0.05 *versus* the corresponding time point of the opposing trial.

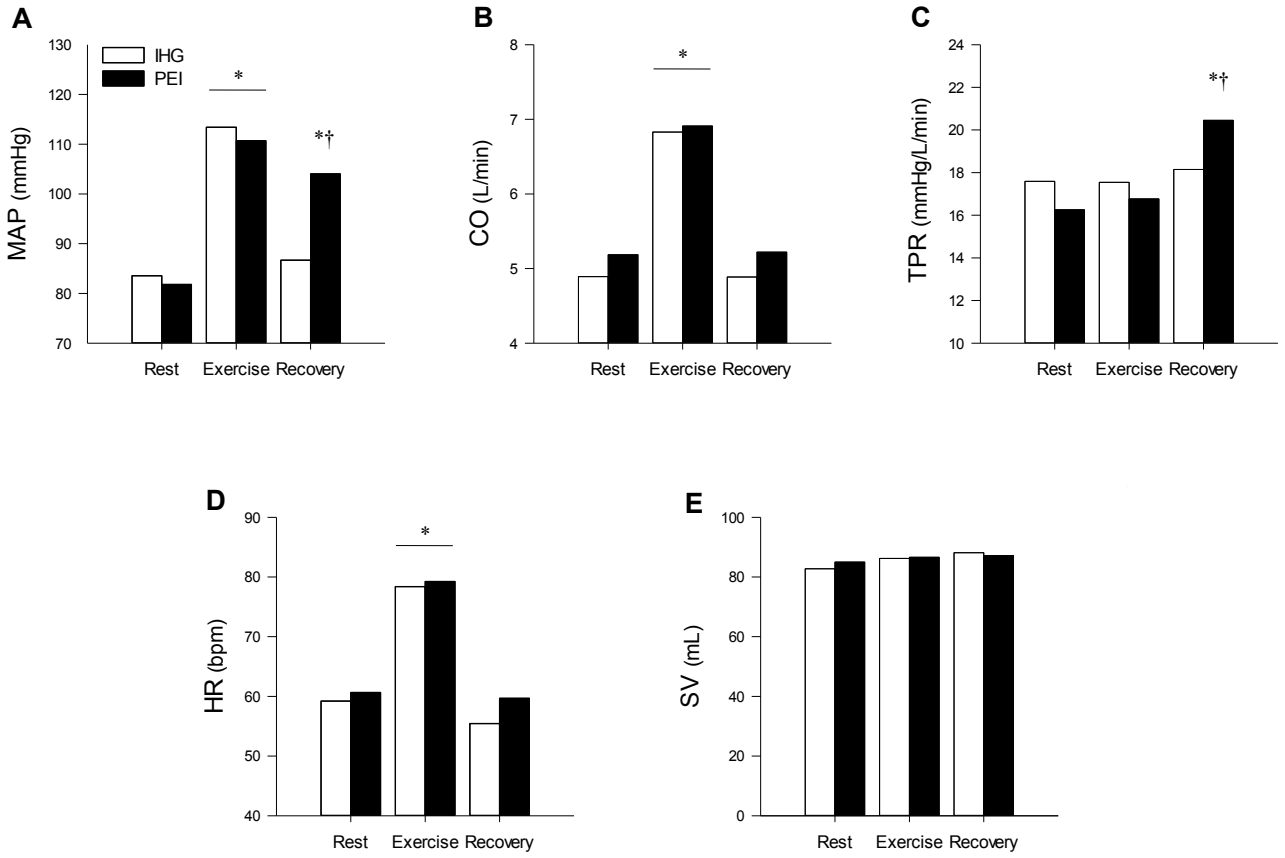


Fig. 6.1 Hemodynamic data during isometric handgrip (IHG) and either free-flow recovery (FF) or post exercise ischemia (PEI). *Mean arterial pressure (MAP; panel A), cardiac output (CO; panel B), total peripheral resistance (TPR; panel C), heart rate (HR; panel D), and stroke volume (SV; panel E). MAP, CO and HR were significantly increased during IHG, while TPR and SV were unchanged from baseline. During PEI, MAP and TPR were elevated above baseline, while CO, HR and SV were at baseline levels. *P<0.05 versus baseline of the corresponding trial; †P<0.05 versus the corresponding time point of the opposing trial.*

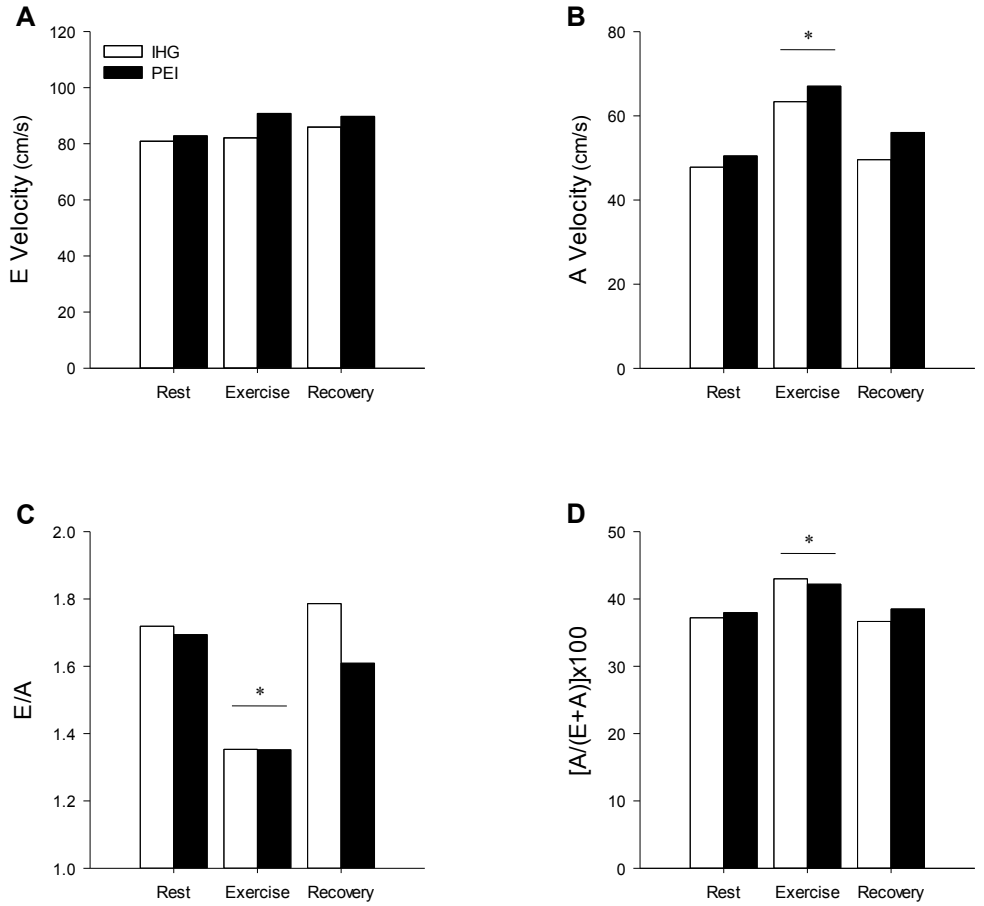


Fig. 6.2 Mitral inflow velocities during isometric handgrip (IHG) and either free-flow recovery (FF) or post exercise ischemia (PEI). *Peak E velocity (panel A), peak A velocity (panel B), E/A ratio (panel C), and the relative contribution of A to LV filling (panel D). During IHG, peak A velocity and the relative contribution of A to LV filling were increased, peak E velocity was unchanged, and E/A ratio decreased. During PEI, all mitral inflow velocities were at baseline levels. *P<0.05 versus baseline of the corresponding trial.*

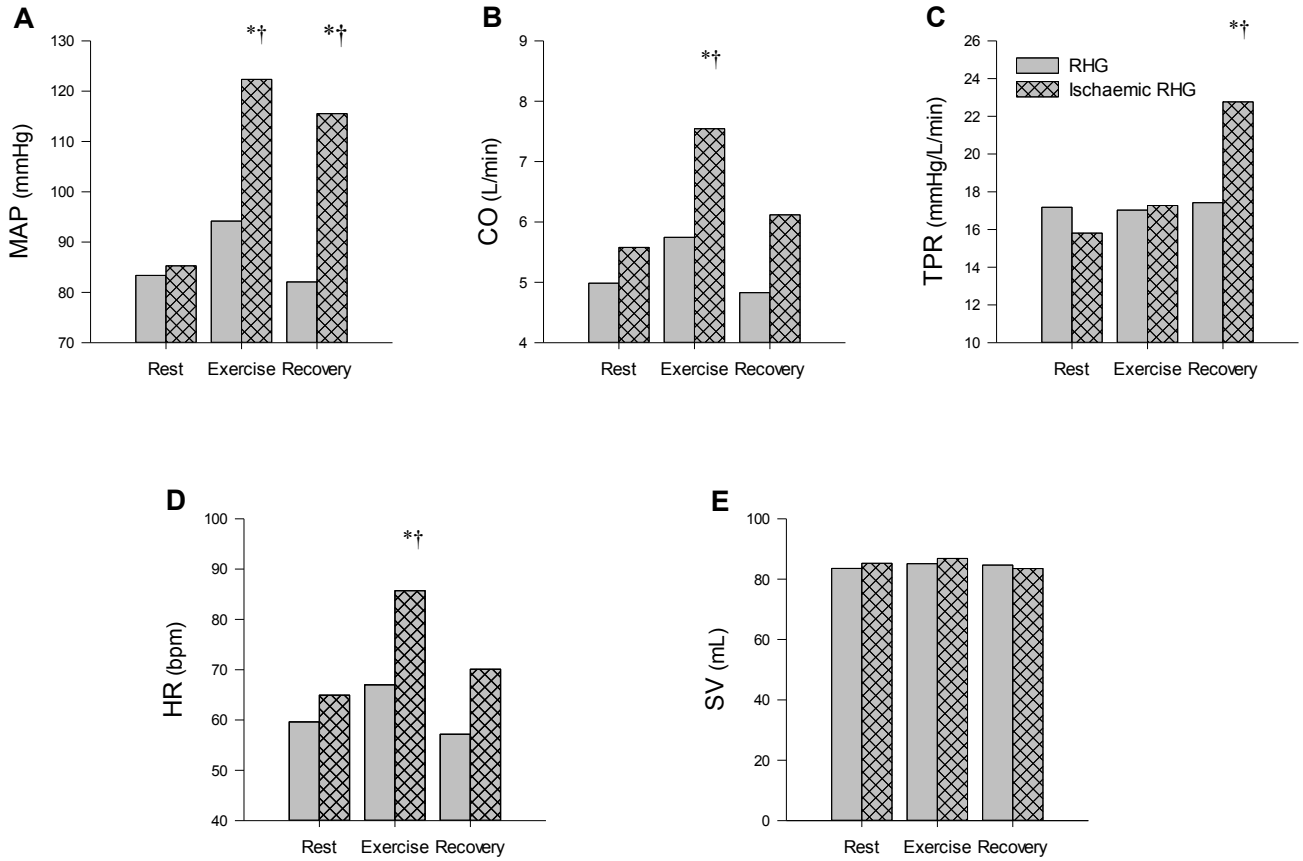


Fig.6. 3 Hemodynamic data during rhythmic handgrip (RHG) under free-flow (FF) conditions, and rhythmic handgrip with ischemia followed by post exercise ischemia (PEI). *Mean arterial pressure (MAP; panel A), cardiac output (CO; panel B), total peripheral resistance (TPR; panel C), heart rate (HR; panel D), and stroke volume (SV; panel E). Ischemia augmented the RHG mediated increase in MAP, CO and HR, while TPR and SV were unchanged from baseline. During PEI, MAP and TPR were elevated above baseline, while CO, HR and SV remained at baseline levels. *P<0.05 versus baseline of the corresponding trial; †P<0.05 versus the corresponding time point of the opposing trial.*

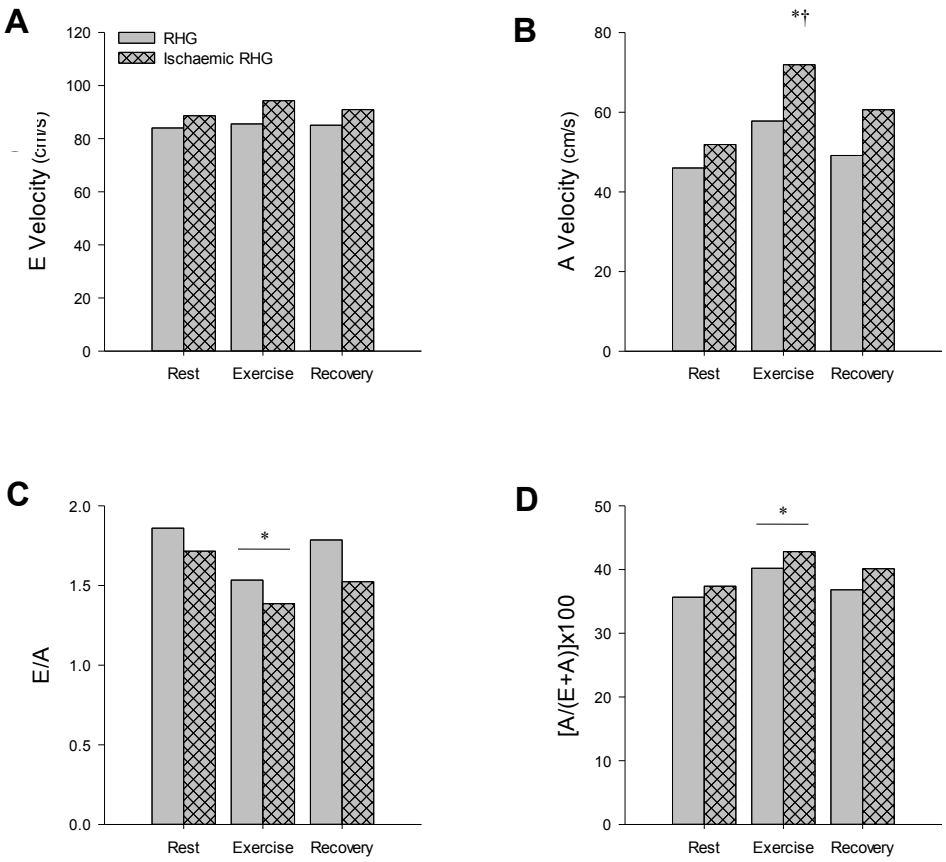


Fig. 6.4 Mitral inflow velocity data during rhythmic handgrip (RHG) under free-flow (FF) conditions and rhythmic handgrip with ischemia followed by post exercise ischemia (PEI). *Peak E velocity (panel A), peak A velocity (panel B), E/A ratio (panel C), and the relative contribution of A to LV filling (panel D). During RHG and RHG with ischemia, the relative contribution of A to LV filling increased, peak E velocity was unchanged, and E/A ratio decreased. Peak A velocity was*

*increased during ischemic RHG. During PEI, all mitral inflow velocities were at baseline levels. *P<0.05 versus baseline of the corresponding trial; †P<0.05 versus the corresponding time point of the opposing trial.*

6.6 Discussion

There are a number of salient findings resulting from this study. First, while the mode of metaboreflex activation determines the mechanism by which BP is elevated, neither was observed to alter SV as determined using Doppler echocardiography. During isolated metaboreflex activation (PEI) following handgrip the increase in BP is secondary to an elevation in TPR, whereas the increase in BP during enhanced metaboreflex activation (ischemic handgrip) is secondary to an elevation in CO, driven by increased HR. Second, LV contractility, assessed from left ventricle systolic wall velocity (S'), remained unchanged during either isolated or enhanced metaboreflex activation. Finally, LA systolic function, assessed from peak velocity of the mitral annulus at late diastolic filling (A'), was augmented by the enhanced metaboreflex activation and during PEI following IHG. Collectively, these findings indicate that the mode of muscle metaboreflex activation (during vs. post handgrip) determines whether the resultant pressor response is flow (CO) or vasoconstriction (TPR) mediated, and that enhanced LA systolic function can serve as a valuable means of preserving SV during enhanced and isolated muscle metaboreflex activation.

6.6.1 Isolated Metaboreflex Activation

It is well established that BP is increased during metaboreflex activation, but whether this response is due to an increase in CO or TPR is equivocal. We observed that during isolated metaboreflex activation with PEI following handgrip, HR and SV, and thus CO remained at baseline levels. Therefore, in line with the studies of Lykidis et al. and Bastos et al., the metaboreflex mediated increases in BP could be explained by increased TPR (Bastos, Williamson et al. 2000, Lykidis, White et al. 2008). In contrast, Spranger et al. reported a HR mediated elevation in CO during PEI following treadmill exercise in dogs (Spranger, Sala-

Mercado et al. 2013), while Crisafulli and colleagues observed that the BP elevation during PEI was caused by an increase in CO, secondary to an elevation in SV (Crisafulli, Scott et al. 2003, Crisafulli, Salis et al. 2006, Crisafulli 2011). Aside from potential species differences (dogs, humans), these heterogeneous findings may in part be explained by variations in the methods used to determine SV (Doppler echocardiography, thoracic impedance cardiography), and the exercise modality and intensity utilized. HR is typically elevated during PEI following dynamic exercise of a large muscle mass (Freund, Hobbs et al. 1978, Spranger, Sala-Mercado et al. 2013), due to muscle metaboreflex mediated increase in cardiac sympathetic nerve activity and withdrawal of cardiac parasympathetic activity (Fisher, Adlan et al. 2013), and consequently a flow-mediated pressor response is evoked by muscle metaboreflex activation (Spranger, Sala-Mercado et al. 2013). In contrast, when increases in CO are experimentally prevented (Sheriff, Augustyniak et al. 1998) or are attenuated as a consequence of congestive heart failure (Hammond, Augustyniak et al. 2000), the resultant pressor response to metaboreflex activation in treadmill exercising dogs is mediated by vasoconstriction. Similarly, we observed that during PEI following exercise of a relatively small muscle mass (e.g. handgrip), where an enhancement of cardiac parasympathetic tone likely restricts HR and CO from increasing (O'Leary 1993, Fisher, Seifert et al. 2010) the resultant pressor response is also mediated by vasoconstriction.

Crisafulli and colleagues (Crisafulli, Scott et al. 2003, Crisafulli, Salis et al. 2006, Crisafulli 2011) have indicated that isolated muscle metaboreflex activation may augment LV contractility and thus augment SV during PEI particularly when accompanied by a relative increase in diastolic filling time due to a fall in HR below baseline levels. In these investigations contractility was determined on the basis of changes in pre-ejection period (PEP), LV ejection time (VET) and the PEP/VET ratio using impedance cardiography. To

further understand how the muscle metaboreflex activation affects myocardial systolic function and help explain the potential changes in SV during PEI, Doppler echocardiography velocity measurements were made of the left ventricle along its longitudinal axis during contraction and relaxation (TDI). Interestingly, LV systolic function as determined using TDI (S') was unaltered from rest during PEI. Methodological differences in the assessment of cardiac function may partly explain the discrepant findings regarding the influence of isolated metaboreflex activation on myocardial contractility. However, given that systolic time intervals are reduced during PEI following dynamic knee exercise at 70% MVC, but not 30% MVC (Crisafulli, Salis et al. 2006), it is likely that exercise intensity and modality are important modulators of muscle metaboreflex mediated changes in systolic function.

Although we did not observe an increase in LV systolic function during PEI following handgrip, the fact that SV was preserved despite the elevated afterload implies that cardiac work was enhanced. Notably, Bastos et al. reported that the increase in afterload during PEI following isometric handgrip was accompanied by an increase in end-systolic volume, however as end-diastolic volume was also concomitantly increased (i.e. greater preload) SV was preserved (Bastos, Williamson et al. 2000). As such, they suggested that an enhanced Frank-Starling effect made an important contribution to the maintenance of SV during PEI following handgrip, and that an increase in myocardial contractility was not requisite (Bastos, Williamson et al. 2000). In accordance with this suggestion, we observed that the peak velocity of the mitral annulus at late diastolic filling (A') was increased during PEI following IHG. This is suggestive of an enhanced LA systolic function, and likely represents a valuable means of enhancing LV filling and thus preserving SV during isolated muscle metaboreflex activation with PEI. In addition, the muscle metaboreflex likely contributes to the

augmentation of cardiac preload via the redistribution of blood from the viscera to the central circulation (Bastos, Williamson et al. 2000).

6.6.2 Enhanced Metaboreflex Activation

In the present study, the enhancement of the metaboreflex by IHG augmented BP secondary to an increase in CO (i.e., not TPR). This augmented CO response was driven by an increase in HR while SV was unchanged. This concurs with studies in dogs where enhanced metaboreflex activation by partial occlusion of the terminal aorta during treadmill exercise increased BP and CO, secondary to an elevation in HR (O'Leary 1998, Spranger, Sala-Mercado et al. 2013). One possible explanation for the differential effects of PEI (isolated metaboreflex) and IHG (enhanced metaboreflex) on CO and HR relates to differences in cardiac autonomic neural activity. As mentioned above, during PEI following handgrip HR returns to resting levels as cardiac parasympathetic tone is reactivated (O'Leary 1993, Fisher, Seifert et al. 2010), and seemingly as a consequence the elevation of BP during this isolated metaboreflex activation results from an increase in TPR. In contrast, ischemic exercise augments sympathetic activity and inhibits cardiac parasympathetic activity (Victor and Seals 1989, Hartwich, Dear et al. 2011, Fisher, Adlan et al. 2013, Hartwich, Aldred et al. 2013), at a time when central command and the mechanoreflex activation also reduce cardiac parasympathetic activity (Mitchell, Reeves et al. 1989, Gladwell, Fletcher et al. 2005). As a result HR, and thus CO and BP are increased.

It is notable that SV was preserved during IHG despite the increase in afterload (increased BP) and reduction in diastolic filling time (increased HR). Indeed, pacing studies in dogs (White, Patrick et al. 1971) and recently humans (Munch, Svendsen et al. 2013) have demonstrated that increases in HR can decrease SV, thus implying that cardiac systolic and/or

diastolic function is altered during enhanced muscle metaboreflex activation. Indeed, muscle metaboreflex activation during treadmill running in dogs elicits substantial increases in ventricular contractility as determined using invasive approaches (e.g. dP/dt max) (Sala-Mercado, Hammond et al. 2006). However, we observed that S' velocity (peak mitral annular systolic velocity) was unchanged from rest during RHG or RHG with ischemia. This in part agrees with the findings of Muller et al., who reported that S' velocity was unchanged from rest during fatiguing IHG in young individuals (Muller 2013), and Krzeminski who showed no change in systolic time intervals during IHG (Krzeminski 1990).

Spranger et al. reported that enhanced muscle metaboreflex activation during treadmill exercise in dogs augmented LV relaxation (Spranger, Sala-Mercado et al. 2013). To investigate this in humans LV diastolic function was assessed by determination of the peak transmitral flow velocity during early (E) and late (A) filling of the left ventricle. E is associated with relaxation and suction of the left ventricle, while A represents blood flow in to the left ventricle secondary to the contraction of the left atrium and is associated with LV compliance (Cohen 1996). The ratio of E/A is commonly used as a measure of LV diastolic function (George, Naylor et al. 2010), and at baseline E/A ratio was >1.0 as a majority of LV filling occurs during the early phase (George, Naylor et al. 2010). During IHG E/A ratio was markedly decreased, as the relative contribution of A to overall LV filling increased. During ischemic RHG both A and A' (the peak velocity of the mitral annulus during late diastolic filling determined with TDI) were greater than under free-flow conditions, suggestive of enhanced LA systolic function with augmented muscle metaboreflex activation. It is speculated that increases in HR caused by either IHG or ischemic RHG to enhance the muscle metaboreflex, increase in the relative contribution of LA contraction to LV filling to compensate for the reduction in LV filling time, and represent an important means of aiding

the preservation of SV under these conditions by maintaining or enhancing EDV (Bastos, Williamson et al. 2000).

Similar to the findings of Muller et al., a decrease in E' velocity during isometric handgrip was observed, however this measure of peak early diastolic mitral annular velocity was not different from baseline during PEI or during RHG with or without ischemia. Of note, E/E' ratio was significantly increased during IHG, RHG with ischemia and PEI following ischemic RHG (Muller 2013). This index of left ventricle active relaxation and compliance has been shown to correlate well with LV filling pressure both at rest and during exercise (Burgess, Jenkins et al. 2006), but it is difficult to discern the precise underlying mechanisms for the apparent exercise-induced increase in E/E' ratio. However, the fact that it is exacerbated by robust muscle metaboreflex activation is noteworthy. The activation of the metaboreflex produces a robust increase in sympathetic nerve activity to the heart and peripheral vasculature. An increase in cardiac sympathetic nerve activity may not only induce coronary vasodilatation via β-adrenergic mediated dilatation and an increase in cardiac work, but has the capacity to induce coronary vasoconstriction via an effect on α_1-adrenergic receptors (O'Leary, Sala-Mercado et al. 2007, Coutsos, Sala-Mercado et al. 2010). Studies in exercising canines have indicated that muscle metaboreflex mediated α-adrenergic vasoconstriction limits increases in coronary blood flow thus impairing cardiac function (O'Leary, Sala-Mercado et al. 2007, Coutsos, Sala-Mercado et al. 2010). These observations may have particular importance for patient populations in which heightened skeletal muscle afferent sensitivity has been identified, such as heart failure (Piepoli, Clark et al. 1996, Coutsos, Sala-Mercado et al. 2013). Furthermore, an enhanced muscle metaboreflex mediated increase in peripheral sympathetic vasoconstrictor tone has the potential to impair cardiac function via an acute change in cardiac afterload. Indeed, recent research by Weiner et al.

examining LV twist mechanics, found that the increase in afterload during isometric exercise can impair diastolic function (Weiner, Weyman et al. 2012). In the present study we observed that enhanced muscle metaboreflex activation was associated with an increase in E/E'. Interestingly, patients with an exaggerated exercise-induced increase in the ratio of early diastolic transmitral velocity to early diastolic mitral annular velocity have reduced exercise capacity (Burgess, Jenkins et al. 2006). However, it is acknowledged that the present investigations were all undertaken in young healthy individuals and additional studies are required examining the links between muscle afferent sensitivity, cardiac function and exercise capacity in patient populations and elderly.

6.6.3 Muscle Metaboreflex Control of the Heart: Implications for Ageing

Resting SNA is elevated in aging (Ng, Callister et al. 1993), in addition to other cardiovascular alterations. Muller et al. examined cardiac mechanics in both young (mean age = 26 ± 1 year) and older adults (mean age = 64 ± 1 year) during isometric fatiguing handgrip exercise (Muller 2013). They observed that there was an exaggerated pressor response in older adults, however LV contractility and early diastolic filling was impaired. It was speculated that the reasons for these LV impairments may be due to; (i) cardiac ischemia; (ii) increased afterload and arterial stiffness which are known to affect LV function; (iii) β-adrenergic desensitisation (sympathetic activity increases LV contraction and relaxation via β-receptors). In terms of performance, some extremely sedentary elderly individuals may be at a disadvantage due to baseline impairments in cardiovascular function (Muller 2013) and exercise capacity is attenuated. A greater cardiovascular response to exercise or in fact activities of daily living occurs resulting in higher BP, which could have clinical significance (Muller 2013). The findings of Muller need to be extended, by

consideration of the specific role that the muscle metaboreflex makes in elderly individuals. Elderly individuals who are physically active however may not have cardiovascular impairments to the same extent as sedentary elderly, and may therefore not exert the same magnitude of pressor response. A reduced pressor response to exercise could in part explain higher exercise capacity and improved quality of life observed in active elderly individuals.

6.6.4 Methodological Considerations

There are several limitations to the present work. Handgrip was the only exercise modality used in the present study and although both rhythmic and isometric paradigms were utilized care should be taken in extrapolating our findings to exercise of other muscle groups. Cardiac function was non-invasively investigated using Doppler echocardiography and we recognise that these may be affected by several factors that were not measured, including volume status, central venous pressure, intra-cardiac pressure and arterial stiffness. Furthermore, while TDI measurements are less preload dependent than mitral inflow velocities (George, Naylor et al. 2010), the effect of HR on tissue velocities has been debated (Stugaard, Risoe et al. 1994, Giannaki, Oxborough et al. 2008). Studies in humans examining the influence of muscle metaboreflex activation using more direct methods of assessing cardiac function (Bada, Svendsen et al. 2012) remain to be undertaken. Finally, despite the demonstration in exercising dogs that the cardiovascular response to restricted hindlimb blood flow is virtually abolished with muscle afferent blockade (Pomeroy, Ardell et al. 1986, Kozelka, Christy et al. 1987), we cannot rule out the possibility that ischemic exercise augments central command along with skeletal muscle afferent feedback. Nevertheless, the application of ischemic exercise to augment the activation of the muscle metaboreflex is well

established (Alam 1937) and as it engages metabolically sensitive skeletal muscle afferents while central command and the muscle mechanoreflex are operant, in this respect it is arguably more representative than PEI of normal exercise, particularly in individuals with chronic conditions where skeletal muscle blood flow is compromised (e.g. peripheral vascular disease).

6.6.5 Conclusion

In summary, the results of the present study indicate that the mode of metaboreflex activation determines the mechanism by which BP is elevated, but SV was unaltered by either isolated (PEI) or augmented metaboreflex activation (ischemic handgrip). Furthermore, while LV contractility remained unchanged, LA systolic function was enhanced during IHG and following period of PEI, suggesting that this is a valuable means of preserving SV under these conditions. Additional studies are required in elderly humans examining the influence of muscle metaboreflex activation on cardiac function, and populations where enhanced muscle afferent sensitivity has been identified.

CHAPTER 7:

GENERAL DISCUSSION

As we age we develop dysfunction of multiple systems in the body including the musculoskeletal, immune, and cardiovascular system (Fried, Tangen et al. 2001). Body composition also changes including an increase in visceral adiposity and a decrease in muscle mass and strength (Baumgartner, Heymsfield et al. 1995, Gallagher, Ruts et al. 2000, Harris 2002, Wannamethee, Shaper et al. 2007). Age-related immune dysfunction results in an increase in chronic systemic inflammation and oxidative stress, which has been associated with vascular and cardiac stiffening (Pawelec 2002, Panda, Arjona et al. 2009). These detrimental structural changes result in cardiovascular dysfunction. Attenuated cardiovascular ability coupled with a weakened muscle system limits exercise capacity and even the ability of performing normal daily tasks, e.g. walking, reaching, and carrying. If left un-noticed and untreated, these age-related changes lead to the development of frailty and disease, reducing the quality of life and increasing the risk of mortality. Physical inactivity also increases as we age, and has been associated with the age-related changes discussed above (Mattusch, Dufaux et al. 2000, Taaffe, Harris et al. 2000, Geffken, Cushman et al. 2001). What is unknown however is whether the onset of dysfunction of these multiple systems with age causes reduced physical activity due to increased visceral adiposity, muscle weakness and low exercise capacity, or is the reduced physical activity causing a downward spiral of increased visceral adiposity, muscle weakness and low exercise capacity.

In the first study of this thesis (Chapter 4), a cross-sectional experiment of a cohort of 211 elderly individuals (mean age = 67 ± 5 years) was performed to examine the associations between a number of variables including habitual physical activity, physical functioning, total and visceral adiposity, muscle mass, cardiovascular function and plasma biomarkers regarded to be related to cardiovascular disease development. It was demonstrated that (i) individuals with higher physical activity were associated with faster walking speeds and a faster

completion of the TUG test (Table 4.5); (ii) higher levels of physical activity were associated with a lower total body fat, body fat % and estimated visceral fat mass, however lean body mass was not affected (Table 4.4); (iii) plasma concentrations of the cardiovascular risk factor PAI-1 were lower in higher physically active individuals, however the remaining plasma biomarkers of inflammation (MIF, endothelin-1, C-RP, IL-1β, IL-4, IL-6, IL-10, IL-13, IL-17a) and oxidative stress were not associated with physical activity (Table 4.6 & 4.7); (iv) cardiovascular function represented by resting HR, BP, central pulse pressure and large artery stiffness were not associated with physical activity (Table 4.8). This is the first study to examine the associations between physical functioning, body composition, inflammation, cardiovascular function and habitual physical activity concomitantly in a single cohort of elderly individuals. Although physical activity did have significant beneficial effects on walking speed, whole body and visceral adiposity, and PAI-1 concentration, no identifiable differences were observed for the remaining plasma biomarkers associated with increased cardiovascular risk, cardiovascular function or lean muscle mass, contradicting previous evidence (Paffenbarger, Hyde et al. 1986, Hakim, Curb et al. 1999, Sesso, Paffenbarger et al. 2000, Tanaka, Dinenno et al. 2000, Geffken, Cushman et al. 2001, Myers, Prakash et al. 2002). It is possible to speculate that the physical activity range in this cohort may not be broad enough to expose minor differences of physiological homeostasis in healthy elderly individuals. However, the observation that differences in physical functioning and body composition were present between the lowest and highest physical activity groups, suggests that these age-related changes precede other functional loss such as cardiovascular dysfunction, and that physical activity can attenuate these detrimental changes.

In the second study of this thesis (Chapter 5), a cross-sectional experiment of a cohort of 175 elderly individuals (mean age = 67 ± 5 years) from the original 211 was developed to

examine the associations between LV diastolic function and the following variables: physical activity, inflammation, central and visceral adiposity, and resting arterial stiffness, HR, MAP and TPR. It was demonstrated that (i) LV diastolic function was negatively associated with estimated visceral fat mass, however not associated with waist:hip ratio and BMI; (ii) LV diastolic function was not associated with inflammatory status or oxidative stress, as index using plasma C-RP and lipid peroxide concentration, and plasma leptin:adiponectin ratio; (iii) LV diastolic function was not associated with arterial stiffness, TPR or resting HR, however was negatively associated with MAP; (iv) LV diastolic function was not significantly associated with physical activity. Firstly, this study showsthat MAP was the major negative predictor of LV diastolic function, above all other variables examined. Interestingly, when controlling for age, gender and anti-hypertensive medication, estimated visceral adipose tissue mass also had a significant negative association with LV diastolic function, second only in importance to MAP. This study is the first to measure and demonstrate that visceral adipose tissue mass is an independent predictor of the variance in LV diastolic dysfunction, measured as E/A ratio, in a single elderly cohort, suggesting a detrimental association of visceral adipose tissue mass with LV function in ageing populations. Given that visceral adipose tissue produces inflammatory cytokines, it is somewhat unexpected that in this cohort no association between LV diastolic function and inflammation was observed. Systemic inflammation and oxidative stress have previously been associated with cardiovascular dysfunction (Heitzer, Schlinzig et al. 2001) contradicting the current study findings. MAP was negatively associated with LV diastolic function; therefore it was unanticipated that arterial stiffness and TPR were not related. However, arterial stiffness is known to be associated with an increase in inflammatory cytokines and oxidative stress, tying in with the lack of association of systemic plasma inflammatory and oxidative markers with LV diastolic

function. The results may suggest that an alternative mechanism, possibly renal dysfunction, give rise for the association between MAP and LV diastolic function. Given previous evidence (Arbab-Zadeh, Dijk et al. 2004, Prasad, Popovic et al. 2007), it was expected that physical activity be positively associated with LV diastolic function. The findings of this study reject this hypothesis as physical activity was not associated. A possible explanation for this could be the physical activity range, as in Chapter 4 of this thesis, as none of the participants fell in to extreme activity behaviour such as completely sedentary or highly endurance trained, as was the case in previous research (Arbab-Zadeh, Dijk et al. 2004). The inclusion criteria may be too restricted resulting in exclusion of individuals who live an extremely sedentary lifestyle but have disease diagnosis. However, the approach adopted is that used in many clinical trials where comorbidities represent the main reason to exclude elderly individuals (Ferrucci, Guralnik et al. 2004). Given the findings that MAP and visceral adiposity had significant associations, it is proposed that the underlying mechanism may include the onset of fibrosis potentially resulting from increased inflammation and oxidative stress. However, inflammatory markers and oxidative stress were measured in this cohort and presented in Chapter 4, whereby the only significant association with physical activity was with PAI-1 concentration. It could be possible that a number of predictor associations have been missed in this cohort as the sample size and physical activity range may have been too small, therefore underlying pathologies such as renal dysfunction and variety of inflammatory diseases could have potentially been missed.

The third study of this thesis (Chapter 6) sought to better understand the neural mechanisms underpinning the circulatory responses to exercise in a ‘normal’ young population, as the first step to investigating the effects of ageing. Much scientific interest has been focused on the metaboreflex, due to its role in cardiovascular regulation during exercise

(Crisafulli, Salis et al. 2006, Boushel 2010, Crisafulli 2011), but also its over-activation in heart failure patients, which consequentially limits exercise capacity (O'Leary, Sala-Mercado et al. 2004, Ansorge, Augustyniak et al. 2005, Crisafulli, Salis et al. 2007, Piepoli, Dimopoulos et al. 2008). Previous research has used different approaches to investigate the effects of metaboreflex activation on circulatory responses, providing equivocal results. Our aim therefore was to investigate how the mode of muscle metaboreflex activation (i.e. during [ischemic exercise] vs. following [PEI] handgrip) affects the central hemodynamic response and cardiac systolic and diastolic function in 10 healthy male participants (mean age = 20 ± 1 year). It was demonstrated that (i) during isolated metaboreflex activation (PEI) following handgrip the increase in BP was secondary to an elevation in systemic vasoconstriction (TPR) (Figure 6.1); (ii) the increase in BP during enhanced metaboreflex activation (ischemic handgrip) was secondary to an elevation in CO, driven by increased HR (Figure 6.3); (iii) SV was not altered by either metaboreflex activation mechanism (Figure 6.1 & 6.3); (iv) LV contractility remained unchanged during either isolated or enhanced metaboreflex activation (Figure 6.2 & 6.4); (v) LA systolic function was augmented by the enhanced metaboreflex activation and during PEI following isometric handgrip (Figure 6.2 & 6.4). These findings add to previous research by identifying that the mode of muscle metaboreflex activation (during vs. post handgrip) determines whether the resultant pressor response is flow (CO) or vasoconstriction (TPR) mediated, and that enhanced LA systolic function can serve as a valuable means of preserving SV during enhanced and isolated muscle metaboreflex activation. Activation of the metaboreflex produces a substantial increase in sympathetic nerve activity to the heart and peripheral vasculature. An increase in cardiac sympathetic nerve activity may not only induce coronary vasodilatation via β-adrenergic mediated dilatation and an increase in cardiac work, but has the capacity to induce coronary

vasoconstriction via an effect on α_1-adrenergic receptors (O'Leary, Sala-Mercado et al. 2007, Coutsos, Sala-Mercado et al. 2010). Studies in exercising canines have indicated that muscle metaboreflex mediated α-adrenergic vasoconstriction limits increases in coronary blood flow thus impairing cardiac function (O'Leary, Sala-Mercado et al. 2007, Coutsos, Sala-Mercado et al. 2010). These observations may have particular importance for patient populations in which heightened skeletal muscle afferent sensitivity has been identified, such as heart failure (Piepoli, Clark et al. 1996, Coutsos, Sala-Mercado et al. 2013).

However, it is acknowledged that this study was undertaken in young healthy individuals and additional studies are required examining the links between muscle afferent sensitivity, cardiac function and exercise capacity in patient populations, or populations who are at an increased risk of heart failure, such as elderly individuals. Muller et al. has previously shown that over-activation of the sympathetic nervous system in healthy elderly individuals reduces systolic and diastolic function during exercise (Muller 2013). Exercise capacity in elderly individuals is limited, and has prognostic effects on cardiovascular mortality (Nylen, Kokkinos et al. 2010). Mechanisms by how the metaboreflex increases BP during exercise in ageing may be different than in young due to increased arterial stiffness and reduced LV diastolic function. Inflammatory cytokines such as TNF-α and IL-6, which are elevated during ageing, have been shown to increase activation of the metaboreflex resulting in further vasoconstriction (Vila and Salaices 2005, Granger 2006). Therefore, understanding the mechanisms of metaboreflex over-activity on the BP response to exercise capacity may identify other potential mechanisms of reduced exercise capacity in elderly individuals. Repeating this study in sedentary and highly active elderly individuals would be beneficial to also understand the impact of physical activity on sympathetic control of the pressor response and cardiac mechanics in ageing, due to the inflammatory lowering effect

physical activity has (Mattusch, Dufaux et al. 2000, Taaffe, Harris et al. 2000, Geffken, Cushman et al. 2001).

The work in this thesis has enhanced the knowledge regarding habitual physical activity and multi-system function in a single elderly cohort. For the first time the associations between physical function, systemic inflammation and ROS, body composition including visceral adiposity, cardiovascular function, and physical activity were assessed to better understand the potential complex mechanisms that lead from ageing to cardiovascular dysfunction. First, high habitual physical activity is associated with faster walking speed, lower body fat and visceral adiposity, and lower systemic PAI-1 concentration. Muscle mass, systemic inflammation, ROS and cardiovascular function including arterial stiffness, resting HR, MAP and TPR however were not associated with physical activity. Second, LV diastolic function was associated with higher MAP and visceral adiposity, however physical activity, arterial stiffness, systemic inflammation and ROS, and resting HR and TPR were not associated.

A possible 'tipping point' phenomenon of ageing may be present whereby minor perturbations in individual systems of the body reach a threshold level to cause the onset of physiological function loss, which may not be detected in recreationally active healthy elderly individuals. In order to investigate deeper the multitude of mechanisms that cause cardiovascular dysfunction with ageing, recruiting more directly high physically active individuals and sedentary individuals would be advantageous. Another possibility would be an exercise intervention study on healthy sedentary elderly individuals and investigate cardiovascular function, inflammatory status, visceral adiposity and cardiovascular risk factors pre and post intervention.

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APPENDIX 1

Physical Activity and Ageing Study

Name …….. Hospital Number…….. Date ……./……../……..

INCLUSION CRITERIA

	SIGN
1. Age 60 – 80 years	
2. Able to walk for 2 minutes or 50 metres without stopping (whichever applies)	
3. Patients able and wiling to provide written informed consent for the study	

EXCLUSION CRITERIA

Subjects presenting with any of the following will not be included in the trial

Significant medical history in the last three years as indicated below

	Y	N
1. General history including : Dementia, Parkinson Disease, Stroke, TIAs, Liver Disease, Cancer		
2. Heart disease. Specifically previous myocardial infarct, cardiomyopathy or valvular disease.		
3. Chest pain (angina pectoris). History recurrent (no more than 1 or 2 episodes per month over the last 6 months) or sudden chest pain typically radiating to the left arm or left side of the neck (unless associated with recent cold, cough or episode leading to bruising		
4. Blood Pressure > 190/120 or tendancy to faint		
5. Pulmonary disease including asthma / COPD leading to significant lung function loss and prescription of corticosteroids. Other medication is permitted.		
6. Rheumatoid or osteoarthritis leading to severe stiffness and exercise intolerance (mild stiffness is allowed if exercise criterium above is met)		
7. Current use of corticosteroids.		
8. Type 1 or 2 diabetes (raised blood sugar when measured on capillary sample is allowed)		
9. History of leg pain on exertion, sufficient to limit walking ability to less than 50 metres or < 2 mins		
10. Any cause to consult the G.P or report of feeling unwell in the previous 10 days		
11. Inability to give informed consent		

This patient is able to exercise as part of the Healthy Ageing Study in the Sports and Exercise Science Building (Birmingham University)

Signature of Nurse……Date……

MidREC general practice letter to patient

Patient X
X Street
X Town
X County
XXXX XXX

Dear Mr/Ms. X,

Invitation to take part in Physical Activity and Healthy Ageing study

We are writing on behalf of The University of Birmingham who are running a study to assess whether a high activity level is important to keep healthy. The researchers are currently looking to recruit volunteers between 60 and 80 years old.

Study Summary
The study has been designed to investigate the influence of physical activity levels on health and well-being in individuals between 60 to 80 years of age. We are interested in looking at whether inflammation, which occurs with increasing age, is less pronounced in individuals who incorporate a greater amount of physical activity into their lifestyle. Also we will be looking at whether physically active persons show better functioning in examinations of the heart, blood vessels, muscle, immunity, cognition and psychological status. The research will provide insights into the effects of an active lifestyle on many of the body's systems in individuals in this age range.

If you are interested in this study, please send back the enclosed slip in the freepost envelope with your name and phone number. One of the researchers from the University of Birmingham will then contact you by telephone to ask you some questions related to your health to see if you are eligible for the study. You will also be able to ask any questions you may have about the study. All information will be kept confidential.

Thank you for your time and we hope to hear from you shortly.

Yours sincerely

Please complete the following contact details:
Your initials and surname:
Street address:
City:
Postcode:
Telephone number:

APPENDIX 2

Birmingham
B15 2TH

Research study: Physical Activity and Healthy Ageing

Thank you for taking the time to read this leaflet. We would like to invite you to take part in this study. You Before you decide if you want to participate or not, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends, relatives or your GP, if you wish. Please ask us if there is anything that is not clear or if you would like more information.

1. **What is the purpose of the study?**

To investigate whether physical activity levels with increased age are related to health and well being.

2. **Why have I been chosen?**

You have been chosen because you are:

- Between 60 and 80 years of age
- Able to walk for 2 minutes or more without stopping
- Do not have medical conditions (for instance angina pectoris or high blood pressure) that prevent you from doing exercise and take part in this study
- Able to decide if you want to take part in the study

3. **Do I have to take part?**

No. Taking part in this study is entirely voluntary. If you would like to participate, you will be given this information sheet to keep and be asked to sign a consent form, but you are still free to withdraw at any time and without giving a reason. The care from your GP will not be affected.

4. **What will happen to me if I agree to take part?**

You will be invited to a the Queen Elizabeth Hospital (QE) for a first visit.

During this visit a qualified nurse will first:

- Check your health and ability to do some exercise with a series of questions
- Measure your blood pressure
- Take a drop of blood to measure the sugar (glucose) level in your blood
- Check whether you have understood this leaflet and whether the researchers of the University have answered all your questions
- Check whether you voluntarily wish to take part in this study without pressure from anybody that you spoke before on the telephone
- The nurse will then ask you to sign a form confirming that you take part well informed and voluntarily

If all of the above is fine then your participation in the study starts and a first series of measurements will be made during the remainder of the visit to the hospital (total time of this first visit is about 2 hours). One week later you will be asked to come to a special laboratory in the School of Sport and Exercise Sciences where more measurements will be made (duration of this second visit will be about 2 ½ hours). There also will be 3 questionnaires that you need to complete at home, and you will also be asked to wear a small device at home for a full week that is able to detect movement and can estimate your physical activity (daytime) and sleep pattern (at night).

5. **What do I have to do for the measurements made during the visit to the Hospital (visit -1)?**

Before visit -1

For these measurements you will be asked to not eat anything and only drink water in the previous 8 hours (that is in the overnight period).

During visit-1

Following the health check by the nurse explained above the following will happen:
Blood sample: The nurse will take several tubes of blood from a vein in your arm.
Bone density and body composition: You will have to lay on a bed for approximately 30 minutes and the machine will pass over you to scan the bones, fat and some muscles in your body. The machine uses very low radiation equal to 1/10 of a chest x-ray.
Questionnaire Pack A: At the end of the visit you will be given a questionnaire pack to take home.
Rest: You will be given a small lunch and some time to recover in a comfortable chair before you go home.
Travel home: You either need to arrange that somebody picks you up with a car or we will ring a taxi for you.

After visit-1

In the week after visit 1 you are asked to complete questionnaire pack A at home at your leisure. You are asked to bring the completed questionnaires during the visit to the School of Sport and Exercise Sciences (visit 2).

6. **What do I have to do for the measurements made during the visit to the School of Sport and Exercise Sciences (visit-2)?**

Before visit-2

You will be asked to have your breakfast about 2 hours before you arrive in the School of Sport and Exercise Sciences. For the purpose of this visit, we advise that you wear loose fitting comfortable clothing, shorts and T-shirt would be ideal however loose long legged/armed clothing is acceptable. The reason for

this is explained below.

During visit-2

You will be welcomed by 2 research nurses and a team of 4 University researchers, who are experts in making the special measurements explained below. The reason that we ask you to wear loose fitting clothing on arrival is that it will allow the researchers to conveniently place pads (kind of stickers that pick up signals from your body) on your collar bones and lower ribs, and to assess your heart, and femoral artery (near your hip bone). To ensure you are as comfortable as possible the experimenter will be of the same sex, and the measurements will be taken in privacy. The following measurements will be made:

- **Arterial stiffness measurement:** you will be asked to lie on a bed for approximately 30 minutes while a small light weight plastic pen probe is placed at your wrist, neck and just below your hip bone.
- **Heart scan:** whilst still lying on the bed, a small ultrasound probe will be placed just below the chest area (7^{th}-9^{th} rib space), and then in the high-mid chest area. This procedure will take approximately 20 minutes.
- **Artery wall thickness measurement:** immediately following the heart scan, whilst you are still lying on the bed, a slightly longer ultrasound probe will be placed on your neck, which will take approximately 5 minutes.
- **Brain blood flow measurement:** whilst you are lying down, a small circular probe will be fitted to the side of your head, near the ear. A special holder that fits around your head will be used to keep the probe in place. This procedure will take approximately 30 minutes.
- **Hand-grip and leg muscle strength:** You will be asked to sit in a custom made chair for approximately 10 minutes whilst your strength is measured.
- **4 minute exercise test:** You will be asked to sit on an exercise bike and cycle at a low intensity for 4 minutes. After this, you will remain seated on the bike for approximately 10 minutes. Your heart rate and blood pressure will be measured throughout the test.
- **Mobility and balance measurements:** You will be asked to perform a series of standing balance tests. A measurement of how long it takes for you to stand up, walk a short distance, turn around, walk back and sit down will also be taken. Thes procedures will take approximately 20 minutes.

At the end of the visit you will be given instructions on how to use a device that can measure movement (physical activity monitor) which you will be asked to wear over the following 7 days (explained below) and you also will be given questionnaire pack B with some instructions how to complete it at home in the following week. One of the researchers will also make an appointment with you to come and visit you at home (see home visit below). Finally the nurses will offer you a chance to rest in a comfortable chair while enjoying a cup of tea and a sandwich until you feel ready to go home by car or taxi.

Following visit-2

In the week after visit-2 you will be asked to continuously wear the physical activity monitor (except while showering). The device is very small so nobody will see that you are wearing it and you also will hardly notice it yourself. In daytime the device will be fixed to a belt around your waist to measure daytime physical activity during 7 days covering both week and weekend days. During the night the device will be moved to a strap around your lower leg to measure the sleep pattern. You are also asked to complete the questionnaire pack B at your leisure.

Home visit:

Before the home visit you are asked to collect all the vitamin and nutrition supplements that you are using and keep them at hand for inspection.

One of the members of the research team will visit you at home about 10 days after visit 2. A remainder of this visit will be given via telephone 2 days earlier. During this visit the researcher will collect and check the physical activity monitor and the completed questionnaire pack B. During this visit you will also be asked to go through a questionnaire (pack C), which also includes a series of learning and memory tests, all of which can be done whilst you are seated in the comfort of your own home. At the end the researchers will ask you to show the vitamin and nutrition supplements that you are using and make notes of make and dosage used. This visit should last no longer than 90 minutes.

Optional recall visit and follow up research

The physical activity measurement will tell us how physically active you are. If you are in the group with the lowest or highest physical activity levels (whole group split in 5 activity levels) then we will contact you to ask whether you would like to volunteer for a last and final visit to the School of Sport and Exercise Sciences. Full details of the purpose of this visit will only be given when you are in one of these groups. We also may approach you to enquire whether you would be interested in follow up research that might involve additional specialised measurements or questionnaires in volunteers of the different physical activity levels. For this follow up research we will ask you for your permission to keep your contact details on record for a period of 20 years. An example of a question that we will have is whether the group with the highest physical activity level will develop disease at a higher age, than those with lowest activity level.

7. **What are the possible disadvantages and risks of taking part?**

You may experience some discomfort during the taking of the blood sample. You will feel a sharp pain when the needle is inserted, but this will ease off quite fast. As the blood samples will be taken by an experienced nurse, the risk

that this will be very unpleasant is minimal. We cannot exclude though that a bruise may develop on your arm following the blood taking, which could cause some pain the days after.

The exercise testing may cause you to experience mild fatigue, which will be short lived and you should have fully recovered within 30 minutes. However during any exercise there is a minimal risk of unforeseen heart complications. In order to minimise this risk we will ask you to only perform low-intensity exercise. In addition, you will be carefully monitored throughout by the trained research nurses. During the bone density and body composition measurements there is a minimal exposure to radiation. The risks of this is minimal as the dose received during the measurements is comparable to the dose that you receive during 1 week of the natural background radiation in the UK.

All other procedures and measurements are safe and should not cause pain. However, if at any point during the protocol you feel uncomfortable or unable to continue, testing will be ceased immediately.

8. **What are the possible benefits of taking part?**

There are no direct benefits for research participants.

However, during the testing you will undergo a thorough health check that normally could only be offered by specialised clinics. You also may find it important to know where you are on the physical activity scale and whether you should become more physically active. We will also provide each participant, who expresses this interest, with a brief personalised report, which will be translated into life style advice.

9. **Will my travel expenses be reimbursed?**

For visit-1 and visit-2 we will offer you a contribution of £10 toward transport expenses. The nurses will provide you with a claim form when you ask for it.

10. **What if something goes wrong?**

In the unlikely case that something will go wrong the nurses will be there to provide help is needed. The University of Birmingham has in place a Public Liability Insurance and if you are harmed in any way by taking part in this research project your normal rights apply.

11. **Will my taking part in this study be kept confidential?**

We will only inform your GP of your taking part in this study.

12. **What will happen to the results of the research study?**

The results of this study are expected to be published anonymously in a scientific journal and names of participants will never be published.

13. **Who is organising and funding the research?**

The University of Birmingham is organising this study in collaboration with the University Hospital Birmingham who will provide nurse and clinical support. The study is funded by the Biotechnology and Biological Sciences Research Council (BBSRC) as a doctoral training grant to educate Ageing Researchers.

14. **Who has reviewed the study?**

This study has been reviewed by BBSRC, an internal scientific committee of the University of Birmingham and University Hospital Birmingham and by the Black Country Research Ethics Committee.

15. **Do you have any further questions?**

If you have any further questions about the study please feel free to contact the investigator (s) listed below.

Prof. Anton Wagenmakers
School of Sport and Exercise Sciences
The University of Birmingham
Edgbaston
Birmingham
B15 2TT
T: [redacted]
E: [redacted]

PhD Students:

- David Bartlett: [redacted]
- Clare McNulty: [redacted]
- Rebecca Booth: [redacted]
- Kim Clarke: [redacted]

Independent Contact:
Ros Salter – Primary Care Research Network Manager for Central England
T: [redacted]

Thank you for taking the time to consider taking part in this research

APPENDIX 3

Echocardiography guidelines for valve quantification poster, British Heart Foundation, 2011.