The impact of an 8-week green-exercise programme on systemic health, and on markers associated with cardiovascular disease risk

Author: Jane Elizabeth S. Thompson

Supervisors: Dr Richard Webb, Dr Paul Hewlett, Dr David Llewellyn and Dr Barry McDonnell

A thesis submitted for the degree of Doctor of Philosophy

Cardiff School of Health Sciences,
Cardiff Metropolitan University
Cardiff, Wales, UK.
Declaration

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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STATEMENT 1

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Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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Abstract - The current PhD project aimed to carry out an investigation into the effect of green-exercise programmes on markers of health related to cardiovascular risk. These markers derived from three diverse areas; blood-borne markers of CV risk; vascular haemodynamics and arterial stiffness; and mental health. Exercise is a well-regarded treatment and preventive tool for markers of health related to these three areas and green-exercise has previously been seen to acutely improve markers of mental health and blood pressure, with access to green space being seen to correlate with better health and lower rates of morbidity and mortality. However, the specific impact upon health of regular participation in green-exercise had not before been investigated.

A cross-sectional study was first performed to determine whether there was any effect of regular participation in aerobic exercise upon the measures of health and CV risk that were then to be employed in an intervention study. This study determined that regular participation was associated with reduced expression of the elastin-degrading enzyme, MMP-9, increased levels of HDL, and reduced diastolic blood pressures. With regards to mental health; self-esteem and cognitive function were improved in the regularly active participants compared to those who were sedentary.

For the intervention study, previously sedentary participants in community-based green-exercise walking groups and workplace-based green-exercise walking challenges were recruited. Following the intervention, it was observed that approximately half of participants had not adhered to the green-exercise programme, and the cohort was therefore split in to a non-adherent group and an adherent group. The data suggest that regular attendance (an average of two 45-min sessions per week/8-weeks) of low/moderate-intensity green-exercise programmes is associated with significant reductions in MMP-9, and also increases in CD36 and ABCA1; two genes involved in reverse cholesterol transport. Central and peripheral SBP, DBP and MAP were also reduced, as was AIx (an indirect measure of arterial stiffness). Measures of anxiety, depression, self-esteem, stress and mood were also improved. Correlative associations were identified between AIx and MMP-9 expression, suggesting that exercise-associated MMP-9 down-regulation may be a mechanism contributing towards exercise-associated reductions in arterial stiffness, and hence CV risk.

In conclusion, these findings suggest that CV risk may be combated using free and easily accessible green-exercise programmes.
Acknowledgements

To Gran, because there isn’t a PhD in being nice.

I am very grateful for the advice, patience and guidance that I have received from my supervisors over the last three and a half years, and I thank them for all the time that they have put into this project. Their enthusiasm and energy for the research question has kept me going at times when mine was being sorely tested.

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Thank you to some truly wonderful friends (old and new) who have had my back through this long, arduous journey and have been a massive source of motivation, as well as a much needed distraction. Thanks for putting up with a lot of venting and for feigning interest in, what I deemed to be, really exciting ‘discoveries’.

Maria, we made it!!

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I have (mostly!) enjoyed this PhD journey, and I am now ready for the next big adventure..
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List of Abbreviations

ABCA1 - ATP Binding Cassette, Sub-Family A 1
AIx - Augmentation Index
AIx@HR75 – Alx corrected to a heart rate of 75 bpm
ANOVA - Analysis of Variance
Apo - Apolipoprotein
aPWV - Aortic Pulse Wave Velocity
BMI - Body Mass Index
BP - Blood Pressure
CD36 - Cluster of Differentiation 36
CES-D – Centre of Epidemiologic Studies - Depression
cf. – Compared to
CFPWV - Carotid Femoral Pulse Wave Velocity
CFQ – Cognitive Failures Questionnaire
CHD - Coronary Heart Disease
CRPWV - Carotid Radial Pulse Wave Velocity
CV - Cardiovascular
CVD - Cardiovascular Disease
DBP - Diastolic Blood Pressure
ECG – Electrocardiogram
FMD – Flow Mediated Dilation
GAPDH - Glyceraldehyde 3-Phosphate Dehydrogenase
HDL-C - High Density Lipoprotein-Cholesterol
IL - Interleukin
LDL-C - Low Density Lipoprotein-Cholesterol
LXR - Liver X Receptor
MAP - Mean Arterial Pressure
MI – Myocardial Infarction
MMP-9 – Matrix MetalloProteinase-9
NCD – Non-Communicable Disease
NF-κB - Nuclear Factor-Kappa Beta
NO - Nitric Oxide
oxLDL - Oxidised LDL
PP - Pulse Pressure
PPAR - Peroxisome Proliferator-Activated Receptor
POMS – Profile of Mood States
PPRE - PPAR-Response Element
PSS – Perceived Stress Scale
RCF – Relative Centrifugal Force
RCT - Reverse Cholesterol Transport
RPMI - Roswell Park Memorial Institute
R-SES – Rosenberg Self-Esteem Scale
RSG - Rosiglitazone
RT-PCR - Reverse Transcription-Polymerase Chain Reaction
RXR - Retinoid X Receptor
SBP - Systolic Blood Pressure
SD - Standard Deviation
SES – Socio Economic Status
SNS – Sympathetic Nervous System
SPS – Social Provisions Scale
STAI – State-Trait Anxiety Inventory
T-CHOL – Total-Cholesterol
THP-1 - Human Acute Monocytic Leukaemia Cell Line
TMD – Total Mood Disturbance
TZDs – Thiazolidinediones
Chapter 1 -

Introduction and Literature Review
1.1 **General introduction and rationale**

Lifespans in the United Kingdom are increasing. Between 1990 and 2009, the average life expectancy of males increased from 73 to 78 years, with that of females increasing from 78 to 82 years (Department of Health, 2011a). However, often accompanying longer life-spans are more years of ill-health and disease, and consequently more people are living with *multiple* long-term health conditions. For instance, of those who are over 85 years old, 29% are living with a non-communicable disease (NCD) such as cancer, respiratory disease or circulatory disease, but also have dementia (Warburton et al., 2006). Deterioration and disorder of almost all bodily processes occurs over time and therefore with ageing, thus generating the pathology of many disease states (Harman, 1981), and hence the development of many ‘age-related’ diseases and disorders.

NCDs are a class of chronic diseases that are non-infectious, and the most common forms include cancers, respiratory diseases, cardiovascular diseases and poor mental health, the latter two of which are specific focuses of the current study. Annually, NCDs are responsible for 36 million of 57 million deaths, i.e. 63% of global mortality (World Health Organisation, 2013a). They are largely preventable via tackling modifiable risk factors such as the use of tobacco, unhealthy diet, the harmful use of alcohol, and physical inactivity (World Health Organisation, 2013a). The World Health Organisation states that if these major risk factors were eliminated, then approximately three quarters of heart disease, stroke and type-2 diabetes worldwide would be prevented, as well as the prevention of 40% of cancer (World Health Organisation, 2013a).

A recent systematic analysis demonstrated that a large majority of NCDs are cardiovascular diseases (CVDs), which globally account annually for 17.3 million deaths, whereas 7.6 million, 4.2 million and 1.3 million deaths a year are accounted for by cancers, respiratory diseases and diabetes, respectively (Lim et al., 2013). The term ‘cardiovascular disease’ covers a wide range of disorders that affect the cardiac muscle and the vascular system, and therefore many organs including the brain, kidney, and eyes (O'Rourke and Safar, 2005,
Chapter 1

Literature review

Wong et al., 2001, Wong et al., 2002). CVD manifests most often in the following five main forms: coronary heart disease (CHD), stroke, aortic disease, peripheral artery disease, and cardiovascular events, which include; myocardial infarction (MI), arrhythmias and cardiomyopathy (NHS, 2013a). It should also be noted that CVD often manifests as a complication of type-2 diabetes. This is due to those suffering from type-2 diabetes also suffering from conditions associated with increasing risk of developing CVD, such as hypertension and abnormal lipid profile (dyslipidaemia) (Hopkins et al., 1996, Gray et al., 1998). Hence, CVD and type-2 diabetes are often viewed as linked disorders that are referred to by the collective term ‘metabolic syndrome’ (Mathieu et al., 2010, Mathieu et al., 2008, Després and Lemieux, 2006).

CVD was associated with a cost to the UK economy of £29.1 billion in 2004 with CHD accounting for 29% of this cost and cerebrovascular disease accounting for 27% (Luengo-Fernández et al., 2006). Sixty percent of this cost was spent on health care, whilst 23% was a result of productivity losses (as a result of morbidity and mortality) and 17% was accounted for by informal care-related costs (Luengo-Fernández et al., 2006). More broadly, CVD is the largest cause of mortality worldwide (World Health Organisation, 2009), and it was estimated in 2005 that by 2015, CVD would account for 30% of annual global mortality (World Health Organisation, 2005). However, this milestone was unfortunately reached seven years prematurely; in 2008, 30% of global mortality occurred as a result of CVD (World Health Organisation, 2013b). Risk factors for CVD development include age, gender, diabetes, increased serum cholesterol levels, increased systolic blood pressure (SBP), cigarette smoking and obesity (Stamler et al., 1993, Hubert et al., 1983, Cupples and D’Agostino, 1987). Importantly (with regard to the current study), a sedentary lifestyle is one of the most important risk factors for the development of CVD (Blair et al., 1996), as is ageing (Lakatta and Levy, 2003).

Age-related disease is a critical public health issue because the proportion of the population over the age of 65 is increasing; currently 10 million of the UK population are over 65 years
of age, with this set to increase by 5.5 million within 20 years (Department of Health, 2011b, Parliament.uk, 2010). Age-related diseases and disorders include atherosclerosis, diabetes, vascular diseases, obesity, the metabolic syndrome and dementia and it is well-accepted that age-related diseases such as these are often underpinned by chronic inflammation (Yu and Chung, 2006, Chung et al., 2009). Importantly, many age-related disorders also manifest as physical-inactivity related disorders (Hurley and Reuter, 2011, Ostchega et al., 2000, Terry et al., 2005), as physical activity is able to slow the development of many of the detrimental pathologies associated with ageing, and therefore physical inactivity may contribute to their progression.

Since growing older is typically associated with these disabling age-related disorders, the cost of ageing-associated health care is set to significantly increase in the UK from an annual cost of 6.8% GDP to 9.1% GDP between 2016/17 and 2061/62. This increase is equivalent to an increase of £36 billion in today’s money (International Longevity Centre - UK, 2012). In 2007/08 the average spending of the NHS on retired households was almost double that of non-retired households (Parliament.uk, 2010); thus further highlighting the imperative need to address age-related diseases and their prevention. The biggest killers of the ageing population include CVDs such as heart disease and stroke (World Health Organisation, 2012).

Further to this, poor mental health is associated with CVD (Van der Kooy et al., 2007, Rugulies, 2002, Richardson et al., 2012, Roest et al., 2010, Iso et al., 2002). For instance, in over 3,000 participants, Keyes (2004) demonstrated that the prevalence of CVD was lowest in adults who were classed as mentally healthy, and CVD prevalence was higher in adults with major depression and minor depression.

Therefore, it can be concluded that physical inactivity and sedentary behaviour are two important and modifiable risk factors for NCDs, including global burdens such as CVD (Andersen et al., 2000, Crespo et al., 2002, Warren et al., 2010, Chomistek et al., 2013, Powell and Blair, 1994) and poor mental health (North et al., 1990, Elwood et al., 2013,
Robertson et al., 2012, Heesch et al., 2011, Galper et al., 2006). However, current physical activity levels are low, with only up to 20% of the UK population meeting current recommended guidelines (Farrell et al., 2013). The purpose of this chapter is to review the existing literature and knowledge in the specialist subject areas that are related to the current research project, which is entitled

“The impact of an 8-week green-exercise programme on systemic health, and on markers associated with cardiovascular disease risk”,

and aims to undertake a multi-disciplinary investigation into the effect of an 8-week green-exercise programme on cardiovascular risk and mental health. As health is a multi-factorial phenomenon, with diverse and complex relationships existing between seemingly disparate bodily functions, it is increasingly important to utilise multi-disciplinary approaches in order to investigate the human body as the systemic entity that it is, rather than putting focus on one distinct aspect or mechanism of health. As discussed above, physical inactivity is one of the most important modifiable risk factors for cardiovascular disease; accordingly, the different sections of the literature review will focus on several distinct areas of health that are related to cardiovascular disease. The aspects of cardiovascular health that the current project will focus on are blood-borne biomarkers of cardiovascular risk (i.e. the relative risk of developing CVD or experiencing a CV event based on the presence of risk factors), clinical markers of arterial stiffness, and mental health.
1.2 Biomolecular and biochemical markers of CV risk

Molecular markers of CV risk

There are a number of blood-borne, molecular biomarkers that are useful indicators of CV risk and are routinely used in the clinical setting. Therefore, accounts of markers of this type that are relevant to the current study will be presented below.

1.2.1 Markers related to the pathogenesis of CVD-associated disease

Peroxisome proliferator-activated receptor - gamma

A number of markers of interest in this thesis are directly or indirectly associated with a ligand-activated nuclear transcription factor termed ‘peroxisome proliferator-activated receptor gamma’ (PPARγ). PPARγ is just one of three PPAR isoforms, the other two being PPAR alpha (α) and PPAR delta (δ) (Shi et al., 2002). Despite the three isoforms having similarities structurally, they have diverse biological effects. PPARγ is involved in lipid storage and adipogenesis, whereas PPAR α and δ are involved in the regulation of fatty acid catabolism (Hummasti and Tontonoz, 2006). Importantly, signalling effects triggered by these transcription factors are important in the contexts of suppression of chronic inflammation and of compensating for loss of insulin sensitivity (Lehmann et al., 1995, Gerstein et al., 2006, Saltiel and Kahn, 2001, Dormandy et al., 2005), both of which are relevant to the pathogenesis of CVD; PPARγ is particularly instrumental in this role by regulating genes involved in anti-inflammatory effects, and in glucose and lipid homeostasis (Pineda Torra et al., 1999, Tontonoz and Nagy, 1999). It is most highly expressed in cells directly involved in such homeostatic functions, such as the skeletal muscle and the adipose tissue (Desvergne and Wahli, 1999), but is also expressed in many other cell-types, including in a cell-type of direct interest to the current study; the monocyte (Ricote et al., 1999, Chinetti et al., 1998, Marx et al., 1998).
The importance of this additional cell-type, as well as myocytes and adipocytes, can be explained by the fact that monocytes have the ability to traverse the blood vessel wall and mature into tissue-macrophages that are resident within a wide range of different tissues. Thus, stimuli that affect PPARγ signalling within circulating monocytes have been reported to ‘prime’ these cells for differentiation into tissue-macrophages of differing phenotypes, thus impacting upon systemic characteristics such as chronic inflammation, atherogenesis and insulin sensitivity (Bouhlel et al., 2007, Geissmann et al., 2010, Roca et al., 2009). The importance of the contribution of monocyte-macrophage PPARγ signalling is reflected by the fact that the PPARγ activator Rosiglitazone (see below) is unable to exert its systemic anti-diabetic effects in macrophage-specific PPARγ-knockout mice (Hevener et al., 2007, Odegaard et al., 2007).

PPARγ has been targeted for its therapeutic effect by the development and use of a family of pharmaceutical drugs called Thiazolidinediones (TZDs); which act as potent insulin sensitizers (Rangwala and Lazar, 2004). PPARγ is highly expressed in lipid-accumulating tissue-macrophages and is therefore an important target of TZDs, and one in particular, namely Rosiglitazone (RSG). However, the prescription of RSG has fallen dramatically in recent years due to safety concerns (Leal et al., 2013), most notably a reported increase in the risk of mortality from CV causes (Nissen and Wolski, 2007). The detrimental effects of RSG highlighted the need for greater investigation into, and promotion of, non-pharmaceutical methods of preventing, and alleviating symptoms of, cardiovascular diseases such as type-2 diabetes.

TZDs such as RSG act as ligands for the ligand-binding domain of PPARγ, resulting in a conformational change of the receptor in to an ‘activated state’. Upon activation, PPARγ binds (as a heterodimer with the receptor Retinoid X Receptor (RXR)) to a specific element within the promoter region of its target gene (i.e. the PPAR response element (PPRE)); transcription of the target gene is then initiated (Berger and Moller, 2002).
Amongst the beneficial processes in which PPARγ plays an important signalling role is the anti-atherogenic lipid homeostasis molecular pathway, known as the reverse cholesterol transport (RCT) system. Activation of PPARγ within monocyte-macrophages leads to primary induction of the Liver X Receptor alpha (LXRα), whose promoter contains multiple PPREs (Butcher et al., 2008). The LXRα gene encodes a transcription factor which, when activated, upregulates the expression of the ATP-Binding Cassette transporter A1 (ABCA1) protein, which is key to the export of cholesterol to Apolipoprotein-A1 (Apo-A1) and thus the formation of HDL-cholesterol lipoprotein particles (see below) (Tang et al., 2009, Vaughan et al., 2009). Moreover, PPARγ also plays a role in the uptake of cholesterol by monocyte-macrophages via up-regulation of an additional PPRE-bearing target gene, namely CD36 (Nagy et al., 1998).

Within the context of CVD, this is particularly relevant to the pathogenesis of atherosclerosis, a chronic inflammatory disease of the large arteries which is considered the primary cause of heart disease and stroke (Lusis, 2000). Atherosclerosis occurs as a result of an imbalance in lipid metabolism and the resultant accumulation of lipid-laden foam cells in the arterial wall (Moore et al., 2013, Moore and Tabas, 2011). Cholesterol-rich lipoproteins that are retained in vulnerable areas of the arterial wall are susceptible to modification such as oxidation and aggregation, which causes them to become pro-inflammatory, and to activate the endothelium. The subsequent inflammatory response involves the recruitment of monocyte-derived macrophages, which mature in to mononuclear phagocytes and ingest the normal and modified lipoproteins that have accumulated, while in the process becoming transformed into the lipid-laden foam cells (Moore et al., 2013). The formation of foam cells over time leads to the development of fatty streaks which are precursors for atherosclerotic plaques (although this can take several decades), while foam cells also undergo secondary necrosis which leads to the formation of the lipid core of more advanced atherosclerotic plaques (Tabas, 2005). One of the health concerns associated with atherosclerosis is due to plaque
ruptures and the development of thrombosis (Lusis, 2000). It is still unknown whether stable or unstable plaque build-up is most indicative of risk.

Atherogenesis is the formation of atheromatous deposits during atherosclerosis development and can be interpreted as a ‘response to injury’, with lipoproteins being the injurious agents (Ross, 1993, Libby, 2000). Consequently, the RCT system mentioned above is considered to be anti-atherogenic, as it removes lipoproteins from the potential atherosclerotic site; in contrast, maladaptive lipoprotein-associated inflammatory responses are associated with the pathogenesis and development of atherosclerosis. In atherosclerosis, increased levels of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) and also C-reactive protein (CRP) and matrix metalloproteinase-9 (MMP-9) are associated with the low level grade of inflammation (Ross, 1999, Libby et al., 2009). This is because foam cells secrete pro-inflammatory cytokines that amplify the atherosclerotic response by causing the recruitment of additional monocytes and also the secretion of additional matrix metalloproteinase molecules that further promote lipoprotein retention (Tabas et al., 2007, Lusis, 2000, Glass and Witztum, 2001).

1.2.2 Markers related to symptoms of CV-associated disease

Glucose and insulin

Glucose is a carbohydrate monosaccharide and is a simple sugar in human metabolism (Gropper and Smith, 2012). Diabetes mellitus is diagnosed by elevated glucose levels and it is recommended that plasma levels of glucose be kept between 3.5 and 5.5mmol/L (Diabetes UK, 2012). The regulation of glucose levels occurs as a balance between glucose absorption from the intestine, production of glucose by the liver, and the uptake and metabolism of glucose by the peripheral tissues (Saltiel and Kahn, 2001). Elevated blood glucose levels are indicative of an imbalance in one or more of these mechanisms that regulate glucose levels. Insulin is released postprandially by beta cells of the pancreatic
islets of Langerhans in response to increased levels of circulating glucose and amino acids (Pessin and Saltiel, 2000). In type-I diabetes, it is thought that the beta cells are destroyed as a result of an autoimmune process and are unable to produce sufficient insulin to maintain glucose homeostasis (Tersey et al., 2012). In type-2 diabetes the beta cells are able to release sufficient insulin but the target cells (primarily adipose, muscle and liver cells) are resistant to the normal actions of the hormone and the glucose is therefore not easily absorbed from the blood stream, hence, this condition is termed insulin resistance (U.S. Department of Health and Human Services, 2013).

By stimulating the glucose transporter, GLUT-4, insulin has a major role in glucose regulation as it is instrumental in the uptake of glucose by adipose cells and muscle cells, as well as also inhibiting hepatic glucose production (Saltiel and Kahn, 2001). Insulin facilitates the uptake of glucose from the blood and into the cells for use as an energy source, promotes storage of substrates in fat, liver and muscle; stimulates lipogenesis, glycogen and protein synthesis, and inhibiting lipolysis, glycogenolysis and protein breakdown. Thus, insulin resistance is not only detrimental to glucose levels, but also results in elevated lipid levels (Saltiel and Kahn, 2001) (such as cholesterol and triglycerides; see next section).

With regard to the use of glucose as a biomarker, it should be noted that a meta-regression analysis of 20 studies of 96,000 participants that followed participants over 12 years demonstrated that even below the threshold for diagnosis of diabetes, there is a progressive relationship between glucose levels and CV risk (Coutinho et al., 1999). Some of the mechanisms behind this hyperglycaemia-associated increase in CV risk includes an established association with other CVD risk factors, such as dyslipidaemia and hypertension (Gerstein, 1997, Laws and Reaven, 1993, Isomaa et al., 2001).

Lipoproteins, cholesterol and triglycerides

Many types of lipids, including cholesterol and triglycerides, are transported in the circulatory system, but blood-borne lipids must be transported via lipoproteins, as their organic non-polar nature means that they cannot be transported freely in the aqueous medium of the
blood (Durstine et al., 2002). Lipoproteins are macromolecular complexes that carry lipids and proteins in the plasma. There are a number of different classes of lipoproteins, with the classes based on their physical and chemical characteristics and these include the size and density of the lipoprotein. Lipoproteins mainly carry phospholipids, esterified cholesterol and triglycerides. The core of the lipoprotein is mainly composed of cholesterol esters and triglycerides. Proteins termed apolipoproteins are present on the surface of the lipoprotein, and play important roles in the regulation of plasma lipid levels and the transport of lipoproteins (Miller et al., 2011, Ginsberg, 1998). Lipoproteins are important markers of CV risk, as discussed below.

Cholesterol is critical in ensuring normal growth and survival (Podrez et al., 2000). It is a sterol lipid and a waxy substance found in all parts of the human body. It is an important molecule due to its role in membrane structure and in the synthesis of bile acids and steroid hormones. The body derives cholesterol from the diet and also via biosynthesis in the liver. Cholesterol is transported to the target cells for its role in membrane structure and hormone synthesis via lipoproteins (Ghose et al., 2013, Tabas, 2002). Levels of cholesterol are regulated by a balance between cholesterol synthesis, influx, degradation, by translocation of cholesterol to the plasma compartment for efflux, and via the formation of cholesterol esters (Tang et al., 2004). When in excess, cholesterol is largely excreted from the body in the form of bile salts (Marinetti, 1990).

Thirty percent of cholesterol in the blood is transported by the high density lipoprotein (HDL), and the low density lipoprotein (LDL) transports approximately 60-80%, primarily as cholesterol esters (Ioannou, 2001, Dudek, 2006, American Heart Association, 2009). Due to their role in the development of CVD via pathologies such as atherosclerosis, cholesterol levels are associated with (and hence, often used as markers of) CV risk (Sharrett et al., 2001), but it is important to discern between the LDL-cholesterol and HDL-cholesterol when investigating cholesterol associated CV risk.
As mentioned, LDL is the principal carrier of cholesterol. It is one of the larger, less stable and less dense lipoproteins. Early evidence suggested that LDL-transported cholesterol may contribute to the development of atherosclerosis as the cholesterol is able to permeate the endothelial layer into the intima of the arterial wall (Carew et al., 1976). This is because macrophages are able to take up modified LDL-cholesterol, via scavenger receptors such as CD36 and SR-A1 (Mauldin et al., 2006). This uptake occurs under normal physiological conditions, but is further increased when LDL concentrations are augmented (Schwenke and Carew, 1989). When macrophages are laden with LDL, they are converted to lipid-laden foam cells, a major component of atherosclerotic plaque (Chinetti et al., 2001), and are therefore major contributors to the pathophysiology of atherosclerosis. For this reason, it is recommended that LDL-cholesterol levels are kept below 3mmol/L and total cholesterol levels kept below levels of 5mmol/L (British Heart Foundation, 2007, NHS, 2013b). Reducing LDL-cholesterol by 1mg/dl (or ~0.03mmol/L) has been shown to reduce CV risk by 1% (Brewer, 2004).

In contrast to the above data with regard to LDL, large population studies have demonstrated that high levels of plasma HDL (and its apolipoprotein constituent; Apo-A1) have an inverse association with atherosclerotic mediated CV risk (Castelli et al., 1986, Assmann et al., 1996, Di Angelantonio et al., 2009, Walldius et al., 2001). HDL-cholesterol is smaller and more dense than LDL-cholesterol. It can be synthesised either by the liver or via very low density lipoproteins (VLDL) and chylomicrons in the blood (Brewer, 2004). Therefore, HDL-cholesterol is often referred to as being ‘good’, whilst LDL-C is referred to as ‘bad’. This is due to the apparent beneficial effect of HDL in reducing the susceptibility of an individual to the development of atherosclerosis (Rader, 2006) via hindering the development of macrophage foam cells, and of atherosclerotic fatty streaks and lesions (Lewis and Rader, 2005, Linsel-Nitschke and Tall, 2005, Glomset, 1968), and due to LDLs role in the development of fatty streaks contributing to atherosclerotic lesions, plaque build-up and potential for increased claudication (a symptom of peripheral vascular disorder; a
condition in which a build-up of plaque in the arteries restricts blood flow to the muscles in the leg, causing pain; particular with exercise (NHS choices, 2012a)).

This anti-atherogenic property of HDL is primarily believed to be as a result of its role in the promotion of the efflux of excess cholesterol from the arterial wall. HDL is said to pick up free cholesterol from the arterial wall and other peripheral tissues and then transport it to the liver for safe disposal within bile salts. Further to this, it is also suggested that HDL has antioxidant and anti-inflammatory properties and also promotes the production of a prostaglandin that inhibits the adhesion of platelets to the inner walls of the artery (Paoletti et al., 2004, Mineo et al., 2006). Increasing the plasma concentration of HDL-cholesterol has clinically relevant effects as an increase of 1 mg/dl (or ~0.03mmol/L) reduces the risk of cardiovascular disease by 2 to 4% (Brewer, 2004, Gordon and Rifkind, 1989). Due to the beneficial effect of HDL when associated with atherosclerosis, it is recommended that HDL-cholesterol levels are maintained above 1mmol/L (British Heart Foundation, 2007).

Finally, triglycerides are an additional form of lipid found in the blood stream, they also are either synthesised in the liver, by the body’s fat stores or ingested from the diet. It is well established that an association exists between elevated triglyceride levels and CVD (Sarwar et al., 2007, Austin et al., 1998, Miller et al., 2011) and it is therefore generally recommended that triglyceride levels be kept below a plasma level of 1.7mmol/L (British Heart Foundation, 2007). Hypertriglyceridaemia that occurs as a result of VLDL or triglyceride-rich lipoproteins (TRL), either due to an increased production of TRL or a decrease in the catabolism of TRL, has a direct and negative effect on the composition and metabolism of HDL and LDL. The cholesterol ester transfer protein is activated by higher VLDL triglyceride output, resulting in triglyceride enrichment of HDL and LDL, the triglyceride content of HDL and LDL is then hydrolysed, resulting in small and dense LDL and HDL particles. It has been demonstrated that hypertriglyceridaemic HDL and LDL may be dysfunctional (Greene et al., 2001, Skeggs and Morton, 2002) as smaller, more dense lipoprotein particles may be more prone to
oxidative modification (Chait et al., 1993, Kwiterovich, 2002), adversely influencing CV risk (Ip et al., 2009).

**Markers related to the regulation of cholesterol, and the prevention of atherosclerosis development**

Under normal, non-pathophysiological conditions, cellular cholesterol homeostasis is maintained and cholesterol therefore kept at atherosclerotic-protective levels via cholesterol efflux. Rader et al. (2009) summarised three reverse cholesterol transport (RCT) pathways by which cholesterol could be effluxed and eventually excreted as bile. The first was the efflux of free cholesterol to lipid free lipoproteins such as Apo-A1, mediated by ABCA1 (and controlled by PPARγ signalling; see previous section). The second was efflux to mature HDL molecules via ABCG1. The third mode was efflux to mature HDL particles by other pathways, including Scavenger Receptor class B type I (SR-BI) as well as passive diffusion.

Thus, the RCT system is of major importance in atheroprotection, particularly the role of the ATP-Binding Cassette (ABC) family of proteins (Voloshyna and Reiss, 2011). RCT is a multi-step process of cholesterol metabolism that results in the net movement of excess cholesterol from peripheral cells (including vascular macrophages), for transport to the liver for excretion as bile (following metabolic conversion). The movement of the cholesterol from peripheral tissues includes movement out of arterial wall monocyte-macrophages, and occurs via the plasma compartment (Olchawa et al., 2004, Rader et al., 2009, Lewis and Rader, 2005, Cuchel and Rader, 2006, Wang and Rader, 2007). It is through its involvement in this process that HDL is thought to have anti-atherogenic properties. Lipid poor Apo-A1 mobilises cholesterol and phospholipids from intracellular storage to the plasma membrane (Yamauchi et al., 2004), a transport protein of the ABC family (i.e. ABCA1 and ABCG1) transports the lipid to form nascent HDL (Yokoyama, 2005). Hence, HDL acting as an acceptor of cholesterol in the primary stage of the RCT process is partly the reason for HDL being termed 'good cholesterol' (Glomset, 1968, Fisher et al., 2012).
Thus, ABCA1 may be viewed as a marker of reduced CV risk, as ABCA1 mediates cholesterol efflux to lipid poor Apo-A1 molecules, therefore playing an instrumental role in the formation of HDL. This mediation is promoted by PPARγ (Yokoyama, 2005) and its down-stream target, LXRα (Fu et al., 2001, Venkateswaran et al., 2000, Rigamonti et al., 2005, Butcher et al., 2008), as activation of these transcription factors up-regulates ABCA1 expression and hence macrophage cholesterol efflux (Chinetti et al., 2001). The role of ABCA1 in cholesterol efflux was understood after it was realised that a mutation in the gene of ABCA1 resulted in Tangier disease. This disease is rare and is characterised by factors such as very low HDL plasma levels and Apo-A1 levels, and macrophage cholesterol ester accumulation (Voloshyna and Reiss, 2011).

Also seen to play a role in the RCT system is CD36, an 88kDa glycoprotein that is a member of the scavenger receptor family, which includes other receptors such as SR-B1 (Febbraio et al., 2001). Amongst others cell types, CD36 is expressed in monocyte-macrophages, endothelial cells and adipocytes (Febbraio et al., 2001).

A number of ligands are recognised by CD36, including oxidised LDL (oxLDL) (Nozaki et al., 1995, Endemann et al., 1993). For this reason, CD36 plays an important role in the development of foam cells, as it is able to bind to and internalise oxLDL (Febbraio et al., 2001). Further to this, it has been seen that CD36-deficient macrophages take up significantly less oxLDL (Febbraio et al., 1999). The uptake of native LDL does not stimulate foam cell formation as the unmodified cholesterol is not able to stimulate esterification. In fact, the internalisation of cholesterol via uptake of LDL actually leads to the down-regulation of LDL receptors and therefore of LDL uptake. Hence, a central step in foam cell formation is the oxidative modification of LDL, rendering it immunogenic (Salonen et al., 1992).

Thus, evidence suggests that CD36 plays an important role in atherosclerotic lesion development, via its role in the development of foam cells. However, there is evidence to suggest that an up-regulation of the mRNA expression of macrophage CD36 also has anti-atherosclerotic benefits, as the stimulation of CD36 ligands in macrophages has been
demonstrated to decrease the levels of plasma cholesterol (Marleau et al., 2005). This is likely to be because both CD36 and LXRα/ABCA1 are controlled by the same signalling system in monocyte-macrophages, i.e. the PPARγ signalling system; accordingly, PPARγ provides a mechanism for coupling CD36-mediated uptake of cholesterol within modified LDL to ABCA1-mediated efflux of cholesterol to HDL, and hence to improved anti-atherogenic lipid trafficking by monocyte-macrophages and ultimately reduced CV risk.

Other blood-borne factors that impact upon the arterial wall and affect CV risk include members of the metalloproteinase family.

1.2.3 Markers related to cardiovascular sequelae of CVD-associated pathology

Matrix Metalloproteinase-9

Agents associated with hypertension and arterial stiffness have potential as markers of vascular health and functioning, such an agent is Matrix Metalloproteinase-9 (MMP-9), whose levels are associated with increase isolated systolic hypertension and large artery stiffness (Yasmin et al., 2005).

Interestingly, MMP-9 is indirectly a target gene of PPARγ; in addition to its transactivation role (i.e. initiation of transcription of target genes following binding to PPREs in their promoters), PPARγ also exerts effects via transrepression (in which it denies access to other transcription factors to response elements present in the promoter regions of non-PPARγ target genes (Ricote and Glass, 2007), and PPARγ is known to mediate antagonistic transrepression at nuclear factor-kappa Beta response elements (NFκB-RE) and activator protein-1 (AP-1) sites within the promoter regions of the MMP-9 gene (Novak et al., 1991, Lee et al., 1987).

MMP-9 is a member of the matrix metalloproteinase (MMP) family of enzymes. Members of the MMP family are involved in the breakdown of the extracellular matrix (ECM) (Mott and Werb, 2004, Lau et al., 2008, Nagase and Woessner, 1999). Cells are not the only
component of organ tissue; a large proportion of tissue is composed of extracellular space, which is made up of a variety of components, including polysaccharides and a mixture of both functional and structural proteins, which constitutes the ECM (Alberts et al., 2002).

The MMP family, first discovered in 1962, consists of 66 MMPs (Gross and Lapiere, 1962, Ye, 2000). The family are zinc dependent endopeptidases which are capable of cleaving components of the ECM (Nagase and Woessner, 1999). MMPs are synthesised as zymogens (pro-MMPs); inactive enzyme precursors. They have a common domain structure, consisting of a pro-peptide, the catalytic domain and the heamopexin-like C-terminal domain. The pro-peptide region must be removed prior to activation of the zymogen into an active enzyme (Nagase and Woessner, 1999).

The ECM, amongst other roles, plays an integral role in the strength and structure of the tissue; acting somewhat as a scaffold (Badylak, 2002). Within the vascular ECM, the two proteins, collagen and elastin, are of particular importance and are the main proteinaceous components of the arterial vessels. This is due to the properties they possess and how those properties are essential for the regulation of optimal vascular haemodynamics (Jacob, 2003).

There are 26 different collagen types, and within the arterial vessels type I represents 60% of the collagen found in the vessel wall and type III represents 30% of the collagen found within the vessel wall. The main properties these types exert on the vessel are tensile strength (Jacob, 2003). The elastin protein accounts for 90% of the elastic fibre that exists within the ECM of the arterial walls. The elastic fibres are responsible for the elasticity of the arterial wall (Greenwald, 2007). It is the ratio of collagen to elastin that may largely determine the stiffness of the arterial wall.

It is crucial that MMP activity is regulated due to the role of the enzymatic family in activities such as tissue remodelling and inflammation (Page-McCaw et al., 2007). The MMPs are split into categories, dependent upon their substrate specificity. These categories include the collagenases, the gelatinases, membrane-type MMPs, and the stromelysins. The
gelatinases include the 92-kDa MMP-9 and the 72-kDa MMP-2, and they are known to cleave type IV, V, VII and X collagens, and elastin (Jones et al., 2003). They are regulated at the level of gene transcription, post-translational activation of zymogens and interaction of secreted MMPs with inhibitors such as tissue inhibitor of metallo proteinases (TIMPs) (Brew et al., 2000). Both MMP-9 and MMP-2 have received attention in the context of vascular health and functioning, with elevated levels being associated with increase isolated systolic hypertension (ISH) and large artery stiffness (as measured via aortic pulse wave velocity) (Yasmin et al., 2005). Pro-MMP-9 is secreted by monocytes, macrophages, neutrophils and endothelial cells (R&D Systems, 2012). As discussed above, monocyte-macrophages play a key role in foam cell formation and therefore in the associated inflammatory response and the development of atherosclerosis, and hence CV risk. Further to this, MMP-9 expression is seen to be increased in atherosclerotic lesions and is suggested to play a pathogenic role in acute coronary ischaemia via mediating plaque rupture (Brown et al., 1995). Interestingly, serum levels of MMP-9 have been shown to be a marker of inflammation (Ferroni et al., 2003). In 66 patients following an MI, MMP-9 was significantly elevated compared to control participants and further to this, MMP-9 levels were correlated with IL-6 and CRP. Thus suggesting that there is a link between the inflammatory process and dysfunction in the ECM, and hence a mechanism whereby inflammation may impact upon vascular function.

Therefore, MMP-9 expression appears to have a pathogenic impact on the health and structure of the arterial wall and upon large artery stiffness and CV risk. This increase in CV risk is likely to occur via a stiffening of the arterial wall following an MMP-9 mediated reduction in the elastin component and therefore a reduction in the compliance of the artery, and its ability to buffer flow and arterial pressure (Wagenseil and Mecham, 2012).
1.3 Vascular and haemodynamic markers of cardiovascular disease risk

In addition to the molecular markers discussed in the previous section, CV risk is also investigated via the study of parameters pertaining to the vascular system, hence, these parameters will also be viewed as CV risk markers in the current study. Accordingly, this section will provide a brief account of vascular stiffness and function and its regulation/dysregulation, along with rationales for selecting the specific markers that are to be employed.

1.3.1 The arterial system

It is important to note that approximately 80% of all CVD deaths are associated with arterial disorder and dysfunction (Thom et al., 2006). It can therefore be hypothesised that the ageing process is associated with changes to the arterial system which results in increased CV risk. Although the entirety of the changes that take place are not yet known, it is thought that dysfunction of the vascular endothelium and increases in the stiffness of the large elastic arteries are two of the more important changes that occur to the arterial system (Lakatta and Levy, 2003).

In most cases, the walls of blood vessels are composed of three main components: the endothelial cells, the ECM, and the smooth muscle cells (SMC), with the proportions of each varying within the different layers of the wall (Raines, 2000).

The vascular endothelium is an important regulator in arterial health and functioning as it plays a major role in regulating the agents of vasodilation (Nichols and O’Rourke, 2011). It is a nitric oxide (NO) donor and NO is important in the regulation of smooth muscle tone via inhibition of SMC proliferation (Garg and Hassid, 1989) and therefore of large artery stiffness (Wilkinson et al., 2002a, Schmitt et al., 2005). Vascular NO contributes to arterial function via many roles such as in vasodilation, preventing aggregation and adhesion of platelets, limiting oxidation of LDL, inhibiting proliferation of vascular SMCs and down-regulation of the
expression of genes that are involved in atherogenesis (Avendano et al., 2006, Karp et al., 2004, Mead et al., 2009). Endothelial dysfunction is characterised by a decrease in the bioavailability of NO, and conditions which exhibit endothelial dysfunction (as measured by McEniery et al. (2006)) are associated with increases in aortic pulse wave velocity (aPWV). Conditions associated with endothelial dysfunction include ageing, type-2 diabetes and hypertension (Celermajer et al., 1994, Esper et al., 2006, Brunner et al., 2005, Celermajer, 1997). Further to this, endothelial dysfunction has been proposed as the primary aetiology in atherosclerosis (Verma et al., 2003). Interestingly, it has been demonstrated that therapeutic treatments which improve endothelial function (as measured using flow mediated dilation (FMD), the most common method of measuring endothelial dysfunction (Harris et al., 2010)), are also associated with improvements in arterial stiffness (aPWV) (Mäki-Petäjä et al., 2007).

The arterial wall is composed of three concentric layers; the tunica intima, tunica media and the adventitia (Gasser et al., 2006). The intima consists of the vascular endothelium; in youth this is normally a single lining of endothelial cells and a thin layer of collagen and elastin fibres. These fibres attach to the internal elastic lamina; a dense, elastic membrane. The elastic lamina separates the intima and media. The media is composed of SMC layers and forms the major part of the vessel wall and is a major determinant of the mechanical properties of the vessel. The outer elastic lamina separates the adventitia, which is composed of collagen and some elastin tissue which merge with the surrounding connective tissue, which consists of smaller blood vessels, nerves and fibroblasts (Nichols and O'Rourke, 2011, Raines, 2000).

The ECM is the non-cellular component of all tissues and is fundamentally composed of water, polysaccharides and proteins (most notably collagen and elastin), with the ECM of each tissue being composed uniquely (Frantz et al., 2010), and the precise composition of the ECM contributing to the basis of the mechanical properties of the arterial wall (Wagenseil and Mecham, 2012). Collagen fibrils, elastin fibres and the SMCs bear the majority of the
arterial wall stress, and thus, the proportion of collagen to elastin will determine the stiffness of the vessel. Collagen fibrils bear load in a circumferential direction, whereas elastin fibres bear load in both a circumferential and longitudinal direction (Silver et al., 2001). With each cardiac cycle, there is an increase and decrease in arterial pressure as a bolus of blood is ejected following left ventricular ejection, and a compliant arterial wall that is able to expand and recoil in response to changing pressures is able to buffer this pressure. Both proteins are integral to this buffering function as they are pivotal in supporting the compliant nature of the artery which enables the buffering of pressure (Belz, 1995, McVeigh et al., 1999). Collagen provides tensile strength and is the main structural element of the ECM. Collagen associates with the elastin fibres; which allow tissues that endure repeated stretch and dilation, such as arteries, to recoil. The association between elastin and collagen is important as the stretch innately permitted by the elastin is curtailed and limited by the collagen fibrils (Wise and Weiss, 2009, Frantz et al., 2010). Figure 1.1 displays the stress-strain relationship which describes how under higher pressures, collagen fibres bear more of the stress but are less able to expand in response to this stress and hence, the arteries become stiffer and hence more resistant to extension (McEniery et al., 2007). MMP-9 mediated elastin degradation is one mechanism that is likely to be involved in disrupting the ratio between collagen and elastin.

Hence, these three components of the arterial wall work together to regulate the mechanical properties and functions of the arterial system. Also, the velocity at which blood flows through the arterial tree can impact upon the functioning of these components and hence the physiology of the arteries. The flow of blood is dependent upon its own particulate nature but also upon its interaction with the blood vessels. The ability of the arteries to regulate the calibre of their walls in response to chemical, fluid and nerve signals is vital to the maintenance of an optimal blood flow velocity (Cardiovascular Physiology Concepts, 2007). The conduit arteries form a low-resistance pathway along which blood can travel to reach visceral organs and limbs. The large, elastic arteries such as the carotid and aorta have
compliant arterial walls which buffer flow and pressure when the blood flows under pressure. The walls expand following left ventricular ejection to allow for passage of the bolus of blood that was ejected, and then recoil; propelling the blood forward during diastole. Following left ventricular ejection, a pulse pressure wave is generated. The forward travelling pressure wave travels until it meets a site of impedance mis-match, typically the point at which the low-resistance artery meets a high-resistance, more muscular artery, thus causing the wave to be reflected back to the proximal aorta (Nichols and O’Rourke, 2011, Greenwald, 2007).

Importantly, therefore, the velocity of the pulse pressure wave and the rate of the return of the reflected wave are both key determinants of CV risk (Cruickshank et al., 2002, London et al., 2001) and are often measured as markers of arterial stiffness. This is because as large arteries stiffen, the pulse wave velocity increases as does mean arterial pressure. This stiffening and increase in aortic pulse wave velocity occurs naturally with ageing (McEniery et al., 2005) but is accelerated in disease states such as hypertension (Boutouyrie et al., 2002) and type-2 diabetes (Cruickshank et al., 2002). These increases in large artery stiffness are associated with increases in mean arterial pressure (MAP). These associated increases occur as a result of the increased stiffness increasing the pressure within the arteries. Consequently, the arteries are less able to buffer the pressure that arises from left ventricular ejection, thus, driving up MAP. These interactions will be discussed later in this chapter.

The different components of arterial structure and arterial function as determinants of general arterial stiffness can be seen in Figure 1.2.
Figure 1.1 demonstrates the non-linear stress-strain relationship of the human aorta (taken from (McEniery et al., 2007)). At lower mean arterial pressures, contractile-elastic components are preferentially loaded, whereas as MAP increases the load is transferred progressively to collagen. The shift from elastin to collagen components of the arterial wall leads to an exponential rise in the stress-strain relationship due to collagen having little or no buffering capacity on the pressure.
Figure 1.2 Diagram displaying the major determinants of arterial stiffness, i.e. structure, function and MAP (Adapted from Wilkinson and McEniery (2004))
1.3.2 The arterial system and ageing – arterial stiffness as a marker of CV risk

“Only in the case of young children do we find that the elasticity of the arteries is so perfectly adapted to the requirements of the organism as it is in the case of the lower animals” (Roy, 1880).

Arterial stiffness is a generic term that encompasses a large range of terms that essentially describe how rigid the arterial wall is (Mackenzie et al., 2002). Often the various terms used to describe arterial properties, i.e. distensibility, elasticity and stiffness, are incorrectly used interchangeably. It is important not to refer to these indices interchangeably as interpretation of them is complicated, largely due to many of them being blood pressure dependent. The term ‘arterial stiffness’ will be used throughout this thesis, however where appropriate, the specific indices of stiffness will be highlighted and used for specific interpretation.

Importantly, in a number of populations including hypertensives, patients with end-stage renal disease and patients with type-2 diabetes, increased arterial stiffness as measured using aPWV and augmentation index (Alx) (described in the next section) is associated with increased CV risk (Dabelea et al., 2013, Blacher et al., 1999, Cruickshank et al., 2002, London et al., 2001, Laurent et al., 2001, Boutouyrie et al., 2002, Mattace-Raso et al., 2006). In addition, arterial stiffness has been illustrated to be associated with ageing in healthy individuals (McEniery et al., 2005). aPWV has also been shown to be a good predictor of CV risk in healthy but older adults (Sutton-Tyrrell et al., 2005) and also in the general population (Willum Hansen et al., 2006), suggesting a significant effect of age on the properties of the arterial wall.

Arteriosclerosis and atherosclerosis are examples of age-related cardiovascular conditions that affect the arterial wall. In the literature, the two terms are often incorrectly used interchangeably. Atherosclerosis describes a state in which the arterial wall becomes more stiff due to a build-up of plaque, whereas arteriosclerosis includes vascular remodelling of the arterial wall such as calcification, fibrosis and the degradation of the extracellular matrix.
and therefore the proteinaceous components of the vascular wall, altering the collagen: elastin ratio. This causes the elastic arteries to become more stiff and therefore the compliant nature of the arterial wall is diminished. The arterial wall is therefore less able to expand and recoil in response to changing blood pressures, which has detrimental consequences for blood pressure regulation.

Such vascular remodelling occurs naturally with ageing, but is accelerated in disease states such as type-2 diabetes (Greenwald, 2007) and hypertension (London et al., 1998, London and Safar, 1996). However, it is often difficult to dissociate the normal ageing process from disease-associated changes. In healthy ageing (i.e. in the absence of disease), arteries including the carotid and aortic arteries become increasingly stiff and chronically dilated (Avolio et al., 1985, Nichols and O’Rourke, 2011, Safar and O’Rourke, 2006). These changes are most obvious in the aorta and its proximal arteries, and less obvious in the more muscular, peripheral branches (Avolio et al., 1985, Avolio et al., 1983, Lakatta, 2000, Mitchell and Schwartz, 1965, Wilkinson et al., 2001a, O’Rourke et al., 1968). The elastic arteries are more susceptible to age-related stiffening and undergo vascular remodelling as they are more susceptible to increased fragmentation of the elastic lamina. The chronic state of dilation may be partly explained by dilation of the arteries with each heartbeat. During youth, each heartbeat is associated with a 10% increase in the dilation of the elastic arteries and aorta, however, the more muscular arteries are only associated with a 2-3% increase (Boutouyrie et al., 1992). The differences in the degree of dilation could therefore be accounted for by a difference in the proportions of smooth muscles cells, and a difference in material fatigue between the more elastic arteries and the more muscular arteries (Nichols and O’Rourke, 2011).

As mentioned, elastin is a component of the arterial wall that is involved in maintaining the elasticity of the arterial wall (Wagenseil and Mecham, 2012). Under increased applied forces, vessels become stiffer and therefore become more resistant to extension. The stress–strain relationship, where stress is the force per unit area applied (mean arterial
pressure) and strain is the fractional increase in tissue dimension relative to the unloaded tissue (relative change in arterial diameter), is able to quantify this (see Figure 1.1) (McEniery et al., 2007). During the process of normal ageing, the elastin lamellae become fragmented and consequently the mechanical stress and load is transferred from the elastin and on to the collagen fibres (Robert, 1996). The degradation of elastin fibres together with the fact that collagen fibres are 100-1000 more stiff than the elastin (Greenwald, 2007, Dobrin, 1997) means that the arterial wall becomes more stiff and rigid and therefore the artery presents as chronically dilated. As the large arteries become stiffer, the sites of impedance mis-match become more proximal to the heart causing the forward travelling waves to be more quickly reflected back to the heart, resulting in an increase in the central aortic pressure due to an increase in wave reflection. Thus causing central systolic pressure to rise, diastolic pressure to fall and therefore giving rise to increased pulse pressure (O’Rourke and Hashimoto, 2007). This adaptation has negative implications which include increased pressure and flow pulsations travelling further into the delicate microvasculature of the kidneys, eyes and brain (Mitchell et al., 2011). Other effects of increased arterial stiffening include decreased myocardial perfusion and increased left ventricular load, leading to hypertrophy (O’Rourke and Hashimoto, 2007).

To put this into context, a paper by O’Rourke and Hashimoto (2007) illustrated that when natural elastic rubber is stretched by 10%, fracture is calculated to occur at $10 \times 10^8$ cycles. At an average heartbeat of 70 beats per minute, this would then correspond to 30 years. In the ageing aorta, fracture of elastic lamellae is seen and may account for the increased chronic dilation during ageing and also for the increased age-related stiffening, due to a transfer of the stresses to the more rigid and stiff components of the arterial wall, collagen. However, for the peripheral arteries with approximately 3% dilation and the same number of cycles, fracture would not be expected to occur until at least $3 \times 10^9$ cycles. This then corresponds to over 100 years. This may to some extent explain why the elastin in the media of the proximal aorta has been observed to demonstrate gross damage, whilst there
is little change in the distal, more muscular arteries (Nichols and O'Rourke, 2011, Boutouyrie et al., 1992, Virmani et al., 1991).

In summary, therefore, because vascular remodelling (such as the degradation of the elastin component) causes the artery to become more stiff and thus affects the ability of the arteries in their ability to buffer flow and pressure (Greenwald, 2007), arterial stiffness is often used as an important marker of CV risk. The following section will briefly evaluate the different practical approaches that can be utilised to measure this parameter.

1.3.3 Measurement of arterial stiffness

As can be seen from Table 1.1, there are a number of well-validated, non-invasive measures of arterial stiffness, with some being more applicable to use in clinical and research settings than others. It is important that results obtained from different methods of arterial stiffness assessment are not interchangeably compared as some techniques provide information on systemic arterial stiffness and measures of wave reflection (e.g. AIx), whilst others provide information only on the local stiffness of the vessel under study (e.g. aPWV, brachial PWV, brachial FMD, and femoral FMD).

Table 1.1 describes the various measurements that are widely used in the assessment of arterial stiffness. It should be noted that FMD is a measure of the functioning of the artery rather than arterial stiffness per se, and is assessed using Doppler ultrasound technology. The diameter of the target artery is measured using high-resolution external vascular ultrasound at baseline and then again following an increase in blood flow that occurs as a response to reactive hyperaemia that is induced via cuff inflation (followed by rapid cuff deflation) and therefore occlusion of the artery (Raitakari and Celermajer, 2000). In contrast, applanation tonometry as a method of measuring arterial stiffness, via AIx and aPWV, was employed in this study due to there being more outcome data related to aPWV and AIx compared to that of FMD, and it provided the opportunity to investigate two different areas of
vascular stiffness, i.e., regional and systemic (peripheral) stiffness. Further to this, the
chosen measures of arterial stiffness enabled the measurement of central blood pressure,
as the device used to collect these data (the SphygmoCor) is able to estimate central blood
pressure (as described in detail on pages 34 and 136).
**Table 1.1 Terms and methods used in the measurement of arterial stiffness. Taken from Mackenzie et al. (2002)**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
<th>Methods of Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastic Modulus **</td>
<td>Pressure change required for a theoretical 100% stretch from resting diameter: ((\Delta P \cdot D)/\Delta D) (mm Hg)</td>
<td>MRI Ultrasound</td>
</tr>
<tr>
<td>Young’s Elastic Modulus **</td>
<td>Elastic modulus per unit area: ((\Delta P \cdot D)/\Delta D \cdot h^2) (mm Hg/cm²)</td>
<td>MRI Ultrasound</td>
</tr>
<tr>
<td>Arterial Distensibility **</td>
<td>Relative change in diameter (or area) for a given pressure change; inverse of elastic modulus: (\Delta D/(\Delta P \cdot D)) (mm Hg⁻¹)</td>
<td>MRI Ultrasound</td>
</tr>
<tr>
<td>Arterial Compliance **</td>
<td>Absolute diameter (or area) change for a given pressure step: (\Delta D/\Delta P) (mm Hg) or (cm²/mm Hg)</td>
<td>MRI Ultrasound</td>
</tr>
<tr>
<td>Pulse Wave Velocity</td>
<td>Velocity of travel of a pulse along a length of the artery: Distance/(\Delta t) (m/s)</td>
<td>Pressure waveform Volume waveform MRI, Ultrasound</td>
</tr>
<tr>
<td>Augmentation Index</td>
<td>Difference between second and first systolic peaks as a percentage of the pulse pressure: (((P2-P1)/PP) \times 100)</td>
<td>Pressure Waveform</td>
</tr>
<tr>
<td>Stiffness Index ((\beta))^*</td>
<td>Ratio of logarithm (systolic/diastolic pressures) to (relative changes in diameter): (\beta = \ln(Ps/Pd) / (Ds-Dd)/Dd)</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>Capacitative compliance</td>
<td>Relationship between pressure change and volume change in the arteries during the exponential component of diastolic pressure decay: (\Delta V/\Delta P) (cm³/mm Hg)</td>
<td>Pressure waveform</td>
</tr>
<tr>
<td>Oscillatory Compliance</td>
<td>Relationship between oscillating pressure change and oscillating volume change around the exponential pressure decay during diastole: (\Delta V/\Delta P) (cm³/mm Hg)</td>
<td>Pressure waveform</td>
</tr>
</tbody>
</table>

**P: pressure, D: diameter, V: volume, h: wall thickness, t: time, v: velocity, s: systolic, d: diastolic, **: also requires pressure measurements, \(\Delta\): change
Blood pressure and mean arterial pressure

Peripheral blood pressure is measured easily and non-invasively at the brachial artery using standard, widely available sphygmomanometers. Although peripheral blood pressure has been shown to be associated with CV risk (Franklin et al., 1999, Franklin et al., 2001), the association between central blood pressure and CV risk is far stronger and of greater clinical relevance than when measured peripherally at the brachial artery (Roman et al., 2007, Vlachopoulos et al., 2010a, Vlachopoulos et al., 2010b). Central blood pressure can now also be measured non-invasively using a number of commercially-available devices, including the SphygmoCor device (AtCor Medical, Sydney, Australia).

Central aortic and peripheral brachial pressures may be considerably different as a result of pulse wave velocity, due to its influence on the timing of the return of the reflected wave to the central aorta from the periphery. Despite brachial blood pressure being predictive of cardiovascular risk and outcome, central blood pressure is suggested to better reflect the load placed on the arteries and the pressure placed on the organs and therefore better predict outcome (Vlachopoulos et al., 2010a). More specifically, central pulse pressure has been shown to be more closely correlated with surrogate markers of CV risk including left ventricular mass (Covic et al., 2000).

Mean arterial pressure and pulse pressure

MAP and pulse pressure (PP) are also key determinants of risk (Sesso et al., 2000, Benetos et al., 1997b, Franklin et al., 1999, Haider et al., 2003). MAP is generally regarded as a measure of cardiac output multiplied by total peripheral resistance and is the pressure of the steady flow of blood to peripheral organs and tissues (Schillaci et al., 2009, Safar et al., 2003). MAP is largely determined by large arterial stiffness and is largely consistent throughout the artery tree (Nichols and O'Rourke, 2011, Safar et al., 2003). PP is generally determined by large artery distensibility, the timing and intensity of the reflected waves, the pattern of ventricular ejection and heart rate (Schillaci et al., 2009) and it is the role of the large arteries to minimise this flow and pressure pulsatility from reaching the delicate
peripheral vasculature (Nichols and O'Rourke, 2011). Decreasing diastolic blood pressure (DBP) and increasing systolic blood pressure (SBP) results in an increase in pulse pressure and the main cause of this is increased stiffness of the elastic arteries (Nichols and O'Rourke, 2011) due to PP largely being determined by the summation of the pulse wave velocity of the forward travelling wave and the backward travelling reflected wave (Nichols and O'Rourke, 2011). MAP has been suggested to be predictive of stroke whereas PP might better predict CHD (Verdecchia et al., 2001). In elderly, isolated systolic hypertension patients, Domanski et al. (1999) demonstrated that a 10mm Hg increase in PP was associated with an 11% increase in stroke and a 16% increase in all-cause mortality. Whilst a 10mm Hg increase in MAP was associated with 20% increase in risk of stroke and a 14% increase in the risk of all-cause mortality. Franklin and colleagues suggested from their data that arterial resistance (estimated from MAP) and large artery stiffness (represented by PP) have joint adverse effects on CV risk (Franklin et al., 2009).

**Pulse wave velocity**

The current gold-standard measure of arterial stiffness is aortic pulse wave velocity (aPWV) (Laurent et al., 2006). aPWV measures the time for the forward pressure wave generated from left ventricular ejection to travel between the carotid arterial pulse and the femoral arterial pulse, along the aorta (Laurent et al., 2006). Pulse wave velocity can be measured in any arterial segment between two regions, such as between the carotid arterial pulse and the radial arterial pulse, to measure peripheral PWV.

PWV is measured via applanation tonometry of the arterial sites at their greatest points of pulsatility. Using the Atcor SphygmoCor (Sydney, Australia) equipment and software, the velocity at which the pulse wave travels between the two sites can be measured, in metres per second (m/s). PWV is calculated as the distance travelled by the pulse wave divided by the time taken to travel the distance (Laurent et al., 2006). In order to measure the time taken for the pressure wave to travel between the two sites, either simultaneous
measurement must occur, or by gating the recordings to a fixed point in the cardiac cycle, typically against the R-wave of the ECG.

Rather than systemic stiffness, PWV assesses the regional distensibility of the vessel under investigation. Distensibility of the vessel is the inverse of stiffness and the association between distensibility and PWV can be defined using the Bramwell Hill equation (Mackenzie et al., 2002):

$$PWV = \sqrt{(1/D \rho)}$$

Where $D = \text{distensibility}$ and $\rho = \text{density of blood}$

The prognostic value of aPWV has been demonstrated by Cruickshank et al. (2002), in which 397 patients with diabetes, 55 participants with glucose intolerance, and 119 normal glucose tolerance participants were recruited, and the study employed an ~11 year follow-up. Analysis of the 11 year follow-up data revealed that those who had died had significantly higher aPWV (indicating increased arterial stiffness) than those who had not died. The data also demonstrated that mortality was greater in the diabetic patients than the normoglycaemics, with mortality rates of the impaired glucose tolerance and impaired fasting glucose patients falling between those of the normoglycaemics and the diabetic participants. Thus demonstrating that aPWV was predictive of mortality in populations at increased CV risk.

Increased aPWV and therefore increased stiffening of the aorta is also associated with increased development of left ventricular hypertrophy (LVH); this occurs as a result of the reflected wave reaching the heart during systole and thus augmenting late central systolic pressure and hence cardiac afterload. Diastolic pressure falls, decreasing coronary perfusion pressure and hence increases PP. Aortic and left ventricular end systolic pressure increase, increasing myocardial oxygen demand and the development of LVH (Cheriyan and Wilkinson, 2007). Data from the Framingham investigations demonstrated that LVH is associated with stroke and mortality, with a 35% rate of mortality in men and 20% rate of
mortality in women within five years of LVH diagnosis, this rising to 50% and 35% in the elderly (Levy, 1988). Hence, aPWV appears to be a measureable marker of arterial stiffness and a significantly important risk factor for CV risk, CVD mortality and all-cause mortality.

**Pulse wave analysis**

Pulse wave analysis (PWA), as the name suggests, describes the measurement of the arterial pressure wave form. The aortic pressure wave form is formed as a composite of a forward travelling pressure wave (produced by left ventricular ejection) together with a reflected wave (returned from the periphery): it is the interaction between the forward travelling (incident) and reflected waves which are assessed using PWA. Assessment of the aortic pressure wave allows information on vascular stiffness to be obtained.

The forward travelling wave is reflected back from the periphery upon meeting sites of reflection. The major sites of which are thought to be determined by arterial bifurcations or sites of impedance mis-match (i.e. when proximal and elastic arteries become more distal and muscular). This therefore means that the arterial waveform varies throughout the arterial tree (Nichols and O'Rourke, 2011). Further to this, the timing of the reflected wave will also depend on the speed at which the forward travelling wave has travelled; the greater the stiffness of the artery, the higher the pulse wave velocity and therefore the transmission of the reflected wave back to the heart. Conversely, pulse wave velocity will be significantly slower in a more elastic artery, causing the reflected wave to return to the proximal aorta more slowly. If the reflected wave travels at an optimal velocity, and therefore slowly, it will arrive at the heart during diastole: augmenting diastolic pressure and increasing coronary perfusion pressure (Avolio et al., 2009). With increased stiffness of the arteries, the reflected wave returns to the proximal aorta earlier, during late systole; augmenting central systolic pressure and causing a decrease in diastolic pressure (Avolio et al., 2009). Consequences of the early return of the reflected wave include the development of LVH through the increased pressure exerted on the heart (O'Rourke and Hashimoto, 2007, Epstein and Katz, 1990). Importantly, echocardiographic LVH, after adjustment for CV risk factors, is
associated with a doubling in mortality (Benjamin and Levy, 1999). This is due to LVH leading to cardiac dysfunction as a result of a reduction in coronary flow reserve, tissue ischaemia, the development of arrhythmias and heart failure (Diez et al., 2001).

PWA can be performed using the SphygmoCor system (O'Rourke and Gallagher, 1996) which allows for analysis of the central aortic wave form. This is achieved non-invasively by capturing peripheral pressure waveforms using a high-fidelity applanation tonometer (Kelly et al., 1989b). The tonometer has a Millar micromanometer tip and is used to flatten, (but, importantly, not occlude), a peripheral artery (i.e. the radial or carotid artery); resulting in equalised circumferential pressures, allowing for an accurate recording of the pressure wave form to be obtained. The central arterial waveform is calculated from the peripheral waveform using Fourier analysis and a validated transfer function (Pauca et al., 2001, Segers et al., 2001). Augmentation Index (AIx) a measure of wave reflection (and hence a surrogate marker of systemic arterial stiffness as it reflects the stiffness of the systemic arterial tree), can then be calculated as the difference between the second and first central systolic peaks, expressed as a percentage of the central pulse pressure (SBP - DBP) (see Figure 1.3). AIx therefore measures the enhancement of central aortic pressure caused by the influence of the reflected wave (AtCor Medical, 2011). AIx is affected by reflection timing, ventricular ejection duration, gender, heart rate and the height of participants (Sharman et al., 2009, Wilkinson et al., 2000b). It should be noted that the timing and the return of the reflected wave is driven by heart rate and height and is affected by ventricular ejection duration and gender.

Importantly, increased wave reflection has detrimental effects on health. In end-stage renal failure patients, London et al. (2001) demonstrated that AIx (a measure of wave reflection) is an independent determinant of mortality. 180 patients were categorised in to four quintiles of AIx, with follow-up occurring up to 52 months after the initial observation, and the data demonstrated that those in the quintile with the highest AIx had greater CV mortality and overall mortality, whereas those in the quintile with the lowest AIx had the lowest CV
mortality and overall mortality. Thus, this study demonstrates that increased wave reflection is an important determinant of CV risk and that AIx is a useful method of measuring it.

Figure 1.3 A typical aortic pressure waveform constructed by the SphygmoCor software. Taken from Mackenzie et al. (2002)

Tf: Foot of the forward travelling wave, Tr: time difference between the foot of the wave and the point at which the reflected wave has an effect (the inflection point), P1 & P2: first and second systolic peaks, PP: pulse pressure.
Central blood pressure

Analysis of the derived central wave form can be obtained from the peripheral wave form via use of a generalised transfer function, thus, allowing for determination of central blood pressures. Very recently, a review by McEniery et al. (2014) stated that peripheral SBP can be up to 40mm Hg higher than the SBP in the aorta. This amplification of SBP arises due to an increase in arterial stiffness as the arteries become more distal, causing the upper portion of the pressure wave to become narrower; the systolic portion becomes more prominent, and thus causes a rise in SBP. The kidneys, heart and brain are subjected to aortic pressure rather than peripheral brachial pressure and therefore it is suggested that CV events are more closely related to aortic pressure than peripheral pressure. Laurent et al. (2007) demonstrated, with data collected over 12 years, that surrogate markers of risk and hard CV endpoints supported the notion that central pressure is more strongly associated with CV risk than peripheral pressure. McEniery et al. (2014) also discussed evidence that demonstrated that central pressures (obtained by invasive measures in one study and non-invasive, tonometry based measures in the remaining ten studies) were independently related to future CV events. Four of those studies also demonstrated that only central pressure was predictive of CV risk, and Safar et al. (2002) demonstrated that after correcting for confounding variables, only central pressure remained predictive of CV events in end-stage renal failure patients. Further to this Roman et al. (2007) reported that in healthy individuals, central pressure was a better predictor of future CV events than the predictive value of brachial pressure.

In conclusion, the evidence discussed in this section suggests that with ageing, increases in CV risk occur as a result of increases in large artery stiffness. These increases may occur as a result of reductions in elastin levels in the arterial wall, hence increasing the collagen: elastin ratio, and stiffening the arterial wall. With regard to methodologies, it can be concluded that aPWV is the gold-standard non-invasive method of measuring CV risk, with AIx and central blood pressure also offering important information and prognostic value.
1.4 Mental health and cardiovascular disease risk

1.4.1 The prevalence of poor mental health

“There can be no health without mental health” (Prince et al., 2007)

The above quotation is a World Health Organisation proposition that has been further supported by the EU Council of Ministers, the World Federation of Mental Health, the Pan American Health Organisation and the UK Royal College of Psychiatrists (Prince et al., 2007).

The terms mental well-being, mental health, psychological well-being and psychological health are often used interchangeably. These terms all refer to a

“..state of well-being in which every individual realizes his or her own potential, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to her or his community” (World Health Organisation, 2011).

Mental health describes a continuum, ranging from the worries and concerns people experience from their daily lives, to more chronic and long-term conditions. Mental health problems are common, with approximately a quarter of the British population suffering from some form each year (Office for National Statistics Psychiatric Morbidity report, 2001), and worldwide it is estimated that 450 million people suffer from a mental health problem at any given time (WHO, 2001). Mixed anxiety and depression is common amongst the British population, with almost 9% of the population meeting the diagnostic criteria. In the general population, after 18 months of suffering, these mental health problems will have alleviated in approximately half of those who suffered, however, people with poor physical health, unemployed people and those of low-SES are more likely to still be suffering after this period, compared to the general population (Mental Health Foundation, 2014, The Office for National Statistics, 2001, The Office for National Statistics, 2003).
With regard to the specific focus of the current project, it should be noted that mental health problems such as stress and depression, have strong associations with unhealthy coping resources including lifestyle choices and behaviours like poor diet, alcohol consumption, smoking and physical inactivity (Walsh et al., 2013) which are acknowledged risk factors for CVD development (Mozaffarian et al., 2008). This may be one mode by which there is a positive relationship between poor mental health and CVD (Ford et al., 1998). Poor mental health itself is a less recognised risk factor for CVD (Keyes, 2004), and it is also a consequence of CVD, for instance, in the case of major depressive disorder, depression has been observed to be both a cause and outcome of CVD (Ford et al., 1998, Hemingway and Marmot, 1999, Glassman and Shapiro, 1998, Musselman et al., 1998). In addition, Carney et al. (1997) reported that almost two thirds of MI survivors report depression symptomatology, which has also been observed in patients who have not had an MI or any significant cardiac event, yet exhibit angiographically proven coronary artery disease (Carney et al., 1987). Interestingly, it has also been observed that in patients with no history of CVD, the risk for CVD increases with increasing depression symptomatology (Barefoot et al., 1996, Penninx et al., 2001).

Moreover, atherosclerosis is a leading cause of CVD and presents a possible mechanism for the connection between poor mental health, such as depression, and CVD. In a review by Musselman et al. (1998), it is suggested that the link may be that depression is instrumental in the atherosclerotic plaque development. This link is proposed to be due to enhanced platelet activity and/or alterations in platelet receptors. Platelets have a major role in the development of atherosclerosis due to actions such as stimulating macrophages to uptake lipoproteins and hence contributing to foam cell formation (Siegel-Axel et al., 2008, Gawaz et al., 2005, Weyrich et al., 2007) (as discussed previously). Interestingly, Musselman et al. (1996) demonstrated that, compared to healthy control participants, participants with depression exhibit enhanced baseline platelet activation and responsiveness. Laghrissi-Thode et al. (1997) demonstrated that patients with comorbid CVD and depression
demonstrated increased platelet activation, as determined by measurement of products secreted by platelets. This was compared to healthy control participants and also compared to participants with CVD but without depression.

Another suggested mechanistic link between poor mental health and CV risk is via pro-inflammatory cytokines such as IL-6. As discussed briefly earlier in this chapter, IL-6 is also present in atherosclerosis and levels of this cytokine are generally increased in CVD development (Jenny et al., 2002). Interestingly, levels of IL-6 are also increased in depressed populations and those suffering from chronic stress and distress, in both men and women (Kiecolt-Glaser et al., 2003, Lutgendorf et al., 1999).

The sympathetic nervous system (SNS) poses a third mechanism by which the association between mental health and CVD exists. The SNS is a sub-division of the autonomic nervous system and is involved in the regulation of blood pressure, with over-activity of the SNS playing a role in the development of hypertension (Joyner et al., 2008, Charkoudian and Rabbitts, 2009). Mental stress has been shown to increase the activation of the SNS (Noll et al., 1996). This is thought to occur via the hypothalamic-pituitary axis (HPA) (Black, 2006).

Finally, stress has been shown to be a risk factor for cardiovascular disease (Greenwood et al., 1996). Ghiadoni et al. (2000) demonstrated in a healthy, middle-aged male cohort that transient mental stress was associated with endothelial dysfunction. Participants carried out mental stress inducing speech tasks and had endothelium-dependent and -independent dilation assessed non-invasively before and 30, 90 and 240 minutes after the stress task, via high resolution ultrasound. The results demonstrated that the very brief stress-inducing tasks resulted in endothelial dysfunction that lasted up to four hours. Endothelium dysfunction is associated with a decrease in the bioavailability of anti-atherogenic NO and it may be that a mechanism underpinning the acceleration and initiation of atherogenesis in pre-clinical participants is via recurrent bouts of mental stress. Therefore, it appears there are a number of mechanisms which contribute to the association between mental health and CV risk.
1.4.2 Constructs of mental health

Similarly to the previous sections, the approach taken in the current study is to focus on measurable parameters that act as markers for the aspect of CV risk under consideration. Therefore, in this section, accounts will be presented of experimental tools which may be used as markers quantifying different aspects of mental health that are relevant to CV risk.

Mental health is a central component of health related quality of life (HRQoL), which can be measured using scales such as the Short-Form 36 (SF-36). Such scales relate a person’s happiness with their life to perceptions of their health, and can be defined in terms of an individual’s own perceived function (Rejeski et al., 1996). Perceived health is a measure of one’s perceptions of their own health and can be influenced by the various constructs of mental health. The perceptions of health can vary, depending on the level of importance a construct of health is perceived to be by an individual. Perceptions of health have been shown to be associated with mortality (Kaplan and Camacho, 1983). Using a 9-year follow-up study, Kaplan and Camacho demonstrated that the association between increased risk of death and poor perceived health is strong and consistent.

HRQoL consists of the following constructs; anxiety, depression, self-esteem, mood, stress, social support, cognitive functioning, and perceived health (Biddle and Mutrie, 2008). These terms will be defined below.

Anxiety

Anxiety is defined as an emotional state of nervousness, apprehension, tension, and worry, accompanying a state of physiological arousal. Importantly, anxiety is separated into state anxiety and trait anxiety, with state anxiety being a transient emotional state and trait anxiety being a fixed trait of personality (Spielberger, 2010, Tilton, 2008). It is one of the most prevalent psychiatric disorders, with simple phobia (an unreasonably strong fear) being the most common, followed by social anxiety (Rowney et al., 2010). The pathophysiological mechanism behind the disorder is, as yet, unknown but it is proposed to be as a result of
disrupted modulation of the central nervous system (CNS). Anxiety disorder is a clinical condition that is diagnosed in patients that meet the Diagnostic and Statistical Manual of Mental Disorders (DSM IV-TR) criteria (Sadock et al., 2007, Kessler et al., 2005). A review conducted by Player and Peterson (2011) stated that psychosocial stressors that are associated with anxiety disorders increase autonomic arousal, via the HPA and thus increase levels of circulating catecholamines. The heightened arousal is associated with a pro-inflammatory state, hypertension and therefore risk of CHD. The review discussed observations that have correlated anxiety with hypertension; with increased prevalence of anxiety being more common in those with hypertension and vice versa, with longitudinal studies further demonstrating greater risk of hypertension development in those with anxiety disorders. These associations remain after controlling for depression.

**Depression**

Depression, in its mild form, is characterised by frequent periods of unhappiness. It is quite common and often undiagnosed, however, clinical depression is determined and diagnosed against a specialist diagnostic questionnaire; the Becks Depression Inventory (Beck et al., 1961) or via the use of questionnaires such as the Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 1994) (Fox, 1999). The DSM is currently in its fifth version (the fifth edition was released in 2013).

Depression is often secondary to other medical conditions such as alcohol addiction and is often associated with chronic diseases including type-2 diabetes and cardiac disease (Biddle and Mutrie, 2008). Further to this, as discussed at the beginning of this section, an interesting observation regarding depression and CV risk was that in patients with no history of CVD, the risk for CVD increases with increasing symptoms of depression (Barefoot et al., 1996, Penninx et al., 2001). Depression and anxiety commonly occur together (Kessler et al., 1996, Hirschfeld, 2001) and both feature heavily in individuals suffering from low mental well-being, which is characterised by emotional distress, poor body image, low self-esteem, a sense of hopelessness and chronic stress and anxiety (Fox, 1999).
Self-esteem

Self-esteem is an internal resource and an important construct of mental health. It refers to the value placed on aspects of the self, and can be restricted to individual domains of one’s life, such as social acceptance. Self-esteem can also be a global construct made up by a multi-dimensional structure, i.e. domains such as academia, sport ability, and physical appearance contribute to global self-esteem (Biddle and Mutrie, 2008). Global self-esteem has been suggested to be a relatively stable construct, with the specific domains being more susceptible to change and influence (McAuley et al., 1997). Self-esteem and mood are important determinants of both short-term and long-term mental health, as discussed briefly by Barton and Pretty (2010). From two large, longitudinal studies, Orth et al. (2009) demonstrated that low self-esteem is a risk factor for depression, rather than a consequence of depression. This was seen across all age ranges, and in men and women alike. Carver et al. (1989) demonstrated a significant relationship between active coping and self-esteem. Self-esteem may act as a buffer between stress and depression development, in that in cases of high self-esteem and high stress, depression is less likely to develop than in cases of high stress and low self-esteem (Brissette et al., 2000, Carver et al., 1989). Carver et al. (1989) suggested that those with low self-esteem may be distracted from active coping by the negative emotions experienced as a result of stressful situations.

Mood - Mood is a state that is composed of a number of constructs, such as tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia, and confusion-bewilderment. Mood describes the set of states we experience on a day-to-day basis, with the various states lasting hours, days, weeks or months (Oatley and Jenkins, 1996). The mood states do not result from a specific event, but are more likely to have stemmed from a generic feeling (Biddle and Mutrie, 2008). Self-esteem and mood are both state measures that, in the short term, can be easily manipulated. Barton and Pretty (2010) described self-esteem and mood as early indicators of long-term morbidity and having important implications for health behaviours, motivations, and lifestyle choices, and therefore CV risk.
Perceived stress

Stress can cause individuals to perceive their lives as being uncontrollable, unpredictable and overloaded (Cohen, 1983). Stress can be acute and influenced by the daily hassles that everyone experiences, such as missing the bus, but it can also be chronic, which has negative implications on health, as chronic stress has large associations with poor general health (Rainford, 2000). Stress is generally a response to the perceived inability to cope successfully with the environmental demands (Herbert and Cohen, 1996). Bovier et al. (2004) demonstrated that stress was a strong correlate of mental health in a large sample with a mean age of 26 years. Care giving for family members is associated with the experience of long-term and chronic stress, which has been associated with increased risk of mortality (Schulz and Beach, 1999). Chronic stress has also been reported to be a cause of essential hypertension (Esler et al., 2008). Therefore, it may be important to determine if reductions in perceived stress are associated with a reduction in the development of hypertension as improved stress levels could have important consequences for CV risk.

Perceived social support

Social support is another construct of well-being that has important implications for health and is an external resource of coping. It has been reported that women with fewer social relationships experience more strokes than women with more social relationships (Rutledge et al., 2008). Another study demonstrated that larger social circles and therefore less social isolation is associated with lower mortality rates (Rutledge et al., 2004). Social support also appears to have a buffering effect in that it has protective effects on mental health which are particularly pronounced in those who suffer from high amounts of stress or under stressful situations (Cohen and Wills, 1985). There are two hypotheses behind the beneficial effect of social support on health, the stress-buffering model being one and the main effects model being the second (Kawachi and Berkman, 2001). The stress-buffering model postulates that social support can only have a beneficial effect under stressful circumstances and for those under stress, whereas the main effects model suggests that social support and relationships
can have a beneficial impact regardless of whether the individual is under stress. However, the two models are not mutually exclusive and they might together explain how certain characteristics of social support influence mental health. For instance, it is suggested that the more structural components of social support, such as social integration and networks, may operate via the main effects model whereas the functional components of social support, such as perceived support, operate via the stress-buffering model (Kawachi and Berkman, 2001). In this way, it is thought that the degree to which an individual perceives there to be social support increases the individual’s coping resources and abilities, and hence other constructs such as perceived stress.

**Cognitive functioning**

Cognitive functioning refers to an individual’s ability to process thoughts, memories, ideas and involved perception. It embraces a spectrum of tasks from the more simple such as reaction time, through to complex information processing (Biddle and Mutrie, 2008). With ageing, cognitive functioning declines and cognitive impairment becomes more prevalent (Barnes et al., 2003). Cognitive impairment can be classified, with a slight impairment in a cognitive process (such as memory), but otherwise normal performance, being termed mild cognitive impairment (Petersen et al., 2001). Cognitive impairment is associated with co-morbid disease (Ferrucci et al., 1996), increased risk of dementia (Petersen et al., 1999) and mortality (Bassuk et al., 2000).

From this section, it appears that mental health status has strong implications for one’s health and well-being, and even CV risk. Importantly, lifestyle choices are strongly advocated as a means of improving these factors; in research literature, exercise in particular is highlighted as being a lifestyle improvement with the potential for large benefits on mental health, and more broadly on CV risk as a whole. Accordingly, the next section will review this aspect of the literature in detail.
1.5 **Exercise and its impact on cardiovascular related health**

As briefly discussed in the introductory section (section 1.1), physical inactivity is one of the most important modifiable risk factors for CVD (Safar and London, 2000), as regular aerobic exercise is associated with reduced risk of CVD (Blair et al., 1989, Manson et al., 2002, Mora et al., 2007). It has recently been demonstrated that over a 15-year period, recreational physical activity independently predicted reduced CV mortality (Dhaliwal et al., 2013). Therefore, after focusing sections 1.2-1.4 of this literature review on several distinct areas of health related to cardiovascular disease (i.e. blood-borne biomarkers of CV risk, clinical markers of arterial stiffness, and mental health, respectively), the next sections of the literature review will discuss how physical activity impacts upon these three CV-risk associated parameters. It should be stressed that the mechanisms by which exercise lowers CV risk have not been fully elucidated; for example, although it has been known for some time that moderate-intensity, regular exercise has beneficial impacts upon blood pressure (Nelson et al., 1986), it is unlikely that this accounts for all of the risk reduction that is observed. Indeed, it is likely the exercise exerts its effects via a multi-factorial repertoire of distinct mechanisms including, but not limited to, reduction in traditional CV risk factors such as blood pressure, hyperglycaemia and hypercholesterolaemia (Huonker et al., 2002). Traditional risk factors such as these account for approximately 59% of the decrease in CV risk observed during increased physical activity, thus leaving 41% of the exercise-associated reduction in CV risk unaccounted for (Mora et al., 2007). The unaccounted-for reduction in risk could be as a result of improvements in factors such as novel bio-markers of risk, arterial stiffness, and mental health.

1.5.1 **Physical activity and exercise programmes**

Physical activity refers to bodily movement that is produced by skeletal muscle contraction and requires energy expenditure greater than that expended at rest, whilst exercise is a subset of physical activity, and comprises structured, planned and repetitive bodily movements,
that is carried out in order to improve or maintain aspects of physical fitness (Sigal et al., 2004, Howley, 2001). Exercise programmes are typically split into aerobic exercise and resistance exercise programmes. Aerobic exercise involves large muscle groups in dynamic activities such as walking, cycling, swimming and running. Resistance exercise is designed to increase muscular strength, power and endurance and generally includes weight bearing exercise; either using one’s own body weight or using external resistance, such as purpose-built machines and dumbbells. Aerobic exercise mainly stresses the cardiorespiratory system whilst resistance exercise primarily works the musculoskeletal system, i.e. the bones, joints and muscles (Howley, 2001). Both aspects of fitness are important for health and this was reflected in the most recent set of UK physical activity guidelines published by the Department of Health (Department of Health, 2011a). The guidelines set standards for various age groups, as opposed to one guideline for all. The new guidelines also put emphasis on taking part in physical activity every day. It is recommended that adults (19-64 years old) should be carrying out moderate intensity activities for at least 150 minutes over a week (in bouts of 10 minutes or 5 sessions of 30 minutes) (Department of Health, 2011a). Alternatively, adults can partake in 75 minutes worth of vigorous intensity exercise over a week (or combinations of moderate and vigorous intensities) (Department of Health, 2011a). New to the guidelines was the recommendation that adults partake in physical resistance training activities that aim to increase muscle strength on at least two days of the week (Department of Health, 2011a).

Previously it was thought that the major factor of importance with regard to reducing CV risk was the frequency and duration at which exercise took place, however, large emphasis has recently been placed upon the importance of reducing sitting and sedentary time (non-exercising waking hours) as a means of reducing chronic disease risk (Owen et al., 2009). This is due to evidence highlighting the association between sedentary time and obesity, abnormal glucose metabolism and the metabolic syndrome (Dunstan et al., 2004, Dunstan et al., 2007, Dunstan et al., 2005, Foster et al., 2006, Hu et al., 2003, Jakes et al., 2003).
is suggested that the most efficient manner of doing this is by breaking up sedentary time (Healy et al., 2008). Healy demonstrated that independent of total sedentary time, time spent participating in moderate/vigorous intensity activities and the mean intensity of breaks, frequent interruptions in sedentary time were beneficially associated with CV- and metabolic risk factors, such as waist circumference, BMI, triglycerides levels and glucose levels, but not associated with improved SBP or DBP.
1.5.2 The impact of exercise on blood-borne markers of CV risk

As described in section 1.2, molecular markers of CV risk include inflammatory signalling agents (such as IL-6 and PPARγ), and also blood-borne factors relevant to pathologies associated with increased CV risk (such as glucose, insulin, blood lipid profiles and expression levels of MMP-9). The evidence for exercise’s impact on each of these will be presented in turn in this section.

PPARγ and anti-inflammatory signalling agents

There is limited in vivo data stemming from investigations into the effect of exercise on PPARγ. The effect of exercise in rats was investigated using an 8-week voluntary wheel running study by Petridou et al. (2007). The data demonstrated that the DNA-binding activity of PPARγ was significantly higher in the adipose tissue of the physically active rats, suggesting greater activation of PPARγ following regular exercise. PPARγ was first identified as an exercise-activated signalling agent by Mahoney et al. (2005), however, this was in skeletal muscle cells. Butcher et al. (2008) then demonstrated that PPARγ is an exercise-activated signalling agent in vascular cells also.

As discussed further below, following an 8-week low-intensity, treadmill-based walking study in healthy humans, there was an increase in the leukocyte mRNA expression of PPARγ and its target genes, and also a significant increase in the DNA binding activity of PPARγ following four weeks of exercise (Butcher et al., 2008). As discussed in section 1.2 of the current chapter, activation of PPARγ is associated with many diverse beneficial effects, including suppression of chronic inflammation, and also increased transcription of genes involved in forming HDL and exporting cholesterol from the arterial wall.

Increased resting levels of IL-6 are associated with increased risk of type-2 diabetes and of insulin resistance (Bastard et al., 2000, Kern et al., 2001, Fernandez-Real et al., 2001) and resting levels of IL-6 correlate with obesity measures such as BMI (Kern et al., 2001). The majority of data investigating exercise and IL-6 has used acute bouts of exercise as a
stimulus, however Oberbach et al. (2008) conducted a 12-month aerobic exercise intervention or RSG-treatment intervention in 60 impaired glucose tolerance (IGT) participants. The exercise intervention resulted in significantly decreased resting serum IL-6 levels. Interestingly, there was no change in IL-6 levels of the RSG treatment group, which suggests that exercise may be more beneficial at reducing CV risk than pharmaceutical drugs designed for that specific purpose. Other studies have also demonstrated the effect of chronic exercise on reducing resting IL-6 levels (Jankord and Jemiolo, 2004, Hamer et al., 2012).

**Glucose and insulin**

Age and physical inactivity have detrimental effects on glucose tolerance (Shimokata et al., 1991). A number of studies have investigated the effect of exercise programmes on glucose and on insulin sensitivity. Knowler et al. (2002) demonstrated in a cohort of 3,024 participants at high risk of type-2 diabetes development, that the progression to diagnosis of type-2 diabetes could be better prevented with lifestyle improvements that addressed diet and exercise, than with anti-diabetic metformin treatment. 2.8 years from baseline and the incidence of type-2 diabetes diagnosis was 11.0, 7.8, and 4.8 cases per 100 person-years for the placebo, metformin, and lifestyle-intervention groups, respectively. Thus, the study demonstrates that, in the present case, lifestyle interventions are more beneficial than the pharmaceutical treatment at delaying or preventing the progression of type-2 diabetes. Furthermore, the exercise treatment has the added benefit that the impact of the exercise programme would aid systemic health and not solely the ailment being targeted by the drug.

In addition, Oberbach et al. (2008) conducted a 12-month aerobic exercise intervention in 60 IGT participants. Following the 12-month intervention, plasma levels of glucose and insulin were significantly decreased. Another 12-month exercise study by Seals et al. (1984) demonstrated a beneficial effect of a high-intensity endurance exercise programme on insulin sensitivity in an older population. There was also a beneficial effect for those participants who were in a low-intensity training programme group; however, the effect was
not as great as that which was attained in the group undertaking the high-intensity training. A 16-week cycle ergometer aerobic exercise programme in 65 healthy, sedentary men and women was conducted by Short et al. (2003). In contrast to Seals et al, Short and colleagues reported an improvement in insulin sensitivity in a younger population (20-39 years) but not for the older population (60 years and older). This suggests that with ageing, cells become increasingly resistant to insulin and less able to improve insulin sensitivity with therapeutic agents such as exercise.

Interestingly, in a cohort of 154 overweight/obese and dyslipidemic participants, Houmard et al. (2004) indicated that if training sessions per week accumulated 170 minutes, then the intensity and weekly training volume improved insulin sensitivity to similar degrees. The literature regarding age and insulin sensitivity is somewhat contrasting, however, Seals and colleagues used a cohort size of just $n=11$, and there was also a considerable difference in the length of the programme and therefore results are not directly comparable. Broadly, however, these data discussed above suggest that improvements in glycaemia and insulin sensitivity can be accrued by various intensities and modes of exercise and that the effects can occur in older and younger populations.

Lipid profile and RCT

LDL-cholesterol, total cholesterol and triglycerides are increased in healthy people who consume too much saturated fat. Upon diagnosis of hyperlipidaemia, patients are advised to increase their levels of exercise and reduce their levels of sedentary behaviour (NHS, 2013b). However, the evidence regarding exercise and its impact upon total-cholesterol and LDL-cholesterol levels is conflicting (Durstine et al., 2001) and studies demonstrating the impact of exercise on markers related to RCT are limited, although studies such as Olchawa et al. (2004) (as mentioned above) and Leaf (2003) have used measurement of HDL to determine RCT efficiency (Haskell, 1986).

Durstine et al. (2001) conducted a large quantitative meta-analysis in which they stated that cross-sectional studies typically demonstrate a 9% to 59% greater concentration of HDL in
active populations as compared to their sedentary counterparts. Similarly they showed that triglyceride levels in over half of the studies reviewed demonstrated that triglyceride levels were between 19% and 50% lower in the physically active populations when compared to sedentary participants. The review also demonstrated a dose response effect of exercise on HDL-C and triglycerides, with greater energy expenditure having greater effects. However, there was little evidence from cross-sectional studies to demonstrate any effect (independent of differences in body weight and body fat) of regular exercise compared to sedentary behaviour on LDL-cholesterol or total-cholesterol levels. When reviewing 100 longitudinal studies, the paper claimed that only in 25% of studies did an exercise intervention have a beneficial effect on LDL-cholesterol and total-cholesterol levels. They suggested that the effects on males and females were similar and that in the 25% of studies that reported beneficial effects on LDL-cholesterol levels, the decreases ranged from 5 to 19%, and total cholesterol decreases ranged from 4 to 20%. Further to this, the most effective training programmes for the lowering of LDL-cholesterol and total-cholesterol levels are suggested to be endurance aerobic programmes undertaken by previously sedentary participants.

Results of a review by Leon and Sanchez (2001) supported what Durstine et al reported regarding exercise and HDL-cholesterol, LDL-cholesterol and total-cholesterol levels, but reported little evidence to support exercise and its beneficial impact on triglyceride levels. Specifically, studies that have shown exercise-associated improved lipid profile include that by Seals et al. (1984). They demonstrated a beneficial impact of a high intensity endurance exercise programme on HDL-cholesterol and triglyceride levels in an older, healthy cohort. This data was supported with data from a younger cohort, the study by Olchawa et al. (2004) carried out a cross-sectional investigation into the effect of fitness on lipid profile. The study recruited 25 endurance-trained males (from triathlon, biathlon, swimming and running teams) and 33 normally-active men, as a reference group. The mean age was 34 years and 31 years, respectively. The study found that physical fitness was related to HDL-cholesterol levels in the plasma. Interestingly, the results also demonstrated that the ability of plasma to
promote cholesterol efflux from the macrophages was improved in the physically fit men. Therefore, it would appear that chronic exercise via endurance training has a beneficial impact upon blood lipids and cholesterol efflux above that of normally-active individuals.

Intensity effects were investigated by Kraus et al. (2002) who recruited 159 participants aged between 40 and 65 years, whom were sedentary and either overweight or mildly obese, with dyslipidaemia. Participants were either placed into a control group, a low amount of moderate-intensity exercise group (approximately walking 19.2km per week), a low amount of high-intensity exercise group (approximately jogging 19.2km per week) or a high amount of high-intensity exercise (approximately jogging 32 km per week). The intervention had a two to three month period of gradually increasing participants to their exercise intensity, followed by a 6-month period of them exercising at their prescribed intensity. Lipoproteins were profiled into 11 classifications based on their density. The results demonstrated that benefits of exercise were largely upon the lipoprotein particle size rather than concentration of the lipoproteins, with greater beneficial changes occurring in the high amount of high-intensity exercise group than the other two exercise groups (in 10 of the 11 lipoprotein variables assessed). Further to this, the lower intensity exercise groups consistently demonstrated beneficial impacts upon the 11 lipoprotein variables that were assessed.

In fact, previously published data from our own group, Butcher et al. (2008), reported the effects of an 8-week long treadmill-based study on lipid profile, in which 34 healthy, sedentary adults (mean age, 46 years) were recruited. The cohort was split into a no exercise control group and an intervention group with equal numbers of males and females. The treadmill programme had participants walk 10,000 steps three times a week at their chosen speed. At eight weeks, no significant changes from baseline in the anthropometric measures were observed. However, in the serum there was a significant increase in HDL-cholesterol levels and a significant decrease in total-cholesterol levels, and non-significant decreases in LDL-cholesterol and triglyceride levels. In contrast, data from a meta-analysis by Kelley et al. (2012) reported beneficial effects on total-cholesterol, LDL-cholesterol, total-
cholesterol: HDL-cholesterol and triglyceride levels, but not on HDL-cholesterol levels, in overweight and obese adults following a combined aerobic exercise and diet intervention. The inconsistency in the literature is likely due to the different ages and obesity status of the cohorts, the variety of training undertaken, the baseline lipid and lipoprotein characteristics and levels, and diet.

Nevertheless, following the findings of Butcher et al., it is suggested that beneficial effects of exercise training on lipid profiles occur as a result of an improvement in the ability to synthesise, transport and catabolise the various lipoproteins (Haskell, 1986). Importantly, another aim of the Butcher et al. (2008) study was to demonstrate the ability of exercise to activate the ligand-activated transcription factor PPARγ within leukocytes and to also assess the PPARγ-regulated genes; LXRα, CD36 and ABCA1, all of which are involved in the RCT system. Following the 8-week intervention, exercising participants demonstrated up-regulated monocytic CD36 and PPARγ expression, with ABCA1 and LXR alpha being significantly up-regulated at week eight. These findings were supported by the same group’s subsequent observations that exercise appeared to be associated with generation of PPARγ ligands in the plasma (Thomas et al., 2012), and with up-regulation of anti-inflammatory leukocyte genes/down-regulation of pro-inflammatory leukocyte genes (Yakeu et al., 2010).

As described in section 1.2, because monocytes have the ability to traverse the blood vessel wall and mature into tissue-macrophages, stimuli that affect PPARγ signalling within circulating monocytes have been reported to ‘prime’ these cells for differentiation into tissue-macrophages of differing phenotypes, thus impacting upon systemic characteristics such as inflammation and insulin sensitivity (Bouhlel et al., 2007, Geissmann et al., 2010, Roca et al., 2009). Accordingly, recent unpublished data from the same research group has indicated that exercise-associated increases in monocyte PPARγ activity correlate inversely with systemic fasting glucose levels (Ruffino et al., in prep).

In a broader sense, alongside these emerging developments in the field of exercise-associated PPARγ signalling, these studies provide evidence that a structured, lab-based,
low-intensity, 8-week walking programme carried out by previously sedentary individuals can bring about improvements in serum levels of total-cholesterol and HDL-cholesterol, and can also up-regulate the expression of transcription factors and target genes involved in peripheral cell RCT (Butcher et al., 2008, Thomas et al., 2012, Yakeu et al., 2010).

In support of this suggestion, a wrestling based study over eight weeks had participants conduct two relatively short training sessions per day. Compared to the sedentary group, the exercise group demonstrated significantly up-regulated ABCA1 gene expression levels following the intervention, and a decrease in LDL-cholesterol levels was also observed (Rashidlamir et al., 2011). Similarly, Hoang et al. (2008) conducted a cross-sectional study that split participants into three training status categories; inactive, minimally active and ‘health enhancing physical activity’ active. These data demonstrated that higher levels of physical activity were related to greater expression of ABCA1 and of plasma Apo-A1, further suggesting the link between regular exercise and removal of excess cholesterol from the arterial wall via the transport protein ABCA1, and Apo-A1.

In conclusion, despite some limitations and variability in the findings reported in the literature, there is evidence to suggest that regular aerobic exercise has a beneficial impact on RCT (and hence on risk of atherosclerosis development) via a reduction in inflammation, activation of up-regulation of PPARγ signalling and up-regulation of ABCA1 and CD36 within leukocytes (most likely specifically within the monocyte subset of the general leukocyte population). Thus, the data discussed above suggests that exercise is associated with an activation of PPARγ which results in improvements in plasma and serum markers of CV risk via the increase in leukocyte mRNA expression of two genes involved in the RCT system.

**MMP-9**

Interestingly, as described in section 1.2, another gene relevant to CV risk and vascular health is MMP-9. The metalloproteinase is also regulated by PPARγ, although the nature of the regulation is PPARγ-dependent down-regulation of MMP-9 (Luo et al, 2009; Hetzel et al, 2003). Little research has been conducted on the effect of exercise training on levels and
expression of MMP-9. Previous reports have linked exercise to reductions in circulating levels of MMP-9, but these reports have been limited to resistance training programmes in men only (Cook et al., 2013), or exercise interventions in men with metabolic syndrome (Roberts et al., 2006) and children with atherosclerotic risk factors (Roberts et al., 2007). Importantly, these interventions were diet based as well as exercise (Roberts et al., 2007, Roberts et al., 2006) which makes it difficult to interpret whether exercise or diet had the greatest effect on MMP-9 levels. However, Kadoglou et al. (2010) demonstrated that in a diabetic population, a 16-week exercise-only programme resulted in decreased plasma levels of MMP-9.

To conclude, data accruing from previous investigations of the molecular markers of CV risk discussed in this section appear to demonstrate that exercise has beneficial effects on the development of CV risk at a molecular level, that such beneficial effects may stem from deterrence of foam cell formation, inflammation and elastin breakdown, that various intensities and doses appear to be beneficial, and that improvements in markers related to CV risk can be accrued in both healthy and clinical populations.
1.5.3 The impact of exercise on vascular/haemodynamic markers of CV risk

As described in section 1.3, the arterial system is comprised of three main components; the endothelial cells, the ECM and the SMCs (Raines, 2000). In the current section, evidence will be presented suggesting that all three of the components are influenced by the extent to which the individual participates in physical activity.

As recently described in the previous section, through its impact on MMP-9 expression/activity, exercise is suggested to influence the characteristics of the ECM and hence the physiology of the arterial system. Also, regular physical activity has been shown to be beneficial in the context of the vascular endothelium. A study by Pierce et al. (2011) recruited 28 healthy, sedentary middle aged/older men, 13 middle aged/older, healthy, habitually active men and 20 young healthy, sedentary control participants. Brachial artery FMD was carried out as a measure of endothelial function. Pierce et al. demonstrated that the habitually active older group had a significantly greater brachial FMD than their sedentary, older counterparts, but was not different from the young, sedentary participants. The greater brachial FMD suggests an improved vascular endothelium associated with regular physical activity, comparable to that of a young individual. To ensure this was an effect of the endothelium, FMD was carried out following an endothelium-independent dilation of the brachial artery using sublingual nitroglycerin (GTN), for which all groups had similar results suggesting that the age- and exercise- associated effects observed were attributable to the vascular endothelium. The same study also investigated differences in the expression of NADPH oxidase and NFκB, as markers of oxidative stress, an important risk factor for vascular pathophysiology and disease (Cai and Harrison, 2000), which is associated with reduced FMD and therefore endothelial dysfunction (Loffredo et al., 2007). The endothelial cells from the older exercising men did not display age-related increases in these markers compared to their sedentary counterparts, therefore suggesting a molecular mechanism (i.e. prevention/minimisation of oxidative stress) by which physical activity exerts its beneficial effect upon the preservation of the vascular endothelium and therefore upon
age-related CV risk. Endothelium-dependent posterior tibial FMD has been seen to improve in 40 participants following a 10-week exercise programme, compared to 18 matched controls (Gokce et al., 2002), and therefore suggesting an improvement in endothelial function.

Finally, Kearney et al. (2010) reported that participants of an exercising programme underwent significant decreases in peripheral PWV (compared to baseline, and compared to control group participants); as peripheral PWV is a measure of the more muscular arteries, it might suggest that exercise is exerting some effects on SMC function.

With regard to investigation of these three components on arterial health and function, it should be noted that FMD provides information only on one particular point in the arterial tree, however, FMD and aPWV (as a marker of large artery stiffness) have been observed to have a significant inverse association in patients with ISH (versus healthy participants) and in older (versus younger) participants. Soltesz et al. (2009) also demonstrated an inverse relationship between brachial FMD and Alx (as a marker of wave reflection). Regular exercise participation is also associated with improved arterial stiffness (aPWV and Alx) (Tanaka et al., 1998, McDonnell et al., 2013) and therefore FMD as a marker of endothelial function appears to be associated with other markers of vascular health, and hence endothelial function is suggested as one mechanism by which exercise reduces CV risk.

The impact of exercise on blood pressure

A large scale meta-analysis reviewed 54 randomised, controlled trials, which involved 2,419 participants, in order to investigate the effect of aerobic exercise on blood pressure (Whelton et al., 2002). The meta-analysis determined that aerobic exercise resulted in a mean SBP reduction of 3.84mm Hg and a mean DBP reduction of 2.58mm Hg. BP dropped in both normotensive and hypertensive populations; however, the review was unable to determine the ideal intensity or frequency at which people should carry out aerobic exercise for maximal BP lowering effects (Whelton et al., 2002).
A study that attempted to investigate intensity and duration of aerobic exercise programmes on blood pressure was carried out by Braith et al. (1994). They investigated the impact of a 6-month walking programme in an older cohort (60 – 79 years old) of sedentary normotensives. Participants were grouped into exercising at 70%, and 80-85% of maximal heart rate reserve. Neither of the groups showed a reduction in body weight, and both training groups demonstrated a significant decrease in SBP and DBP by the end of the 6-month period. The study demonstrated that exercise in the form of walking at 70% and 85% of HR reserve is capable of moderately reducing blood pressure in elderly, normotensive participants. However, the mechanism as to how blood pressure was reduced was unknown.

Further to this, an investigation into the impact of walking on borderline hypertensive women also attempted to determine how long it took for a walking programme to positively impact upon blood pressure by including data collection mid-way through the intervention. The 24-week walking programme recruited post-menopausal women with systolic BP of 130–159mm Hg and/or diastolic BP of 85–99mm Hg (Moreau et al., 2001). There was an exercise group in which women walked an extra 3km per day in addition to their daily lifestyle physical activity and a control group. At baseline, the exercise group walked an average of 5,400 steps per day and the control group walked 7,200 steps per day. This was equivalent to walking 3.4 and 4.7km per day, respectively (significantly different between the two groups). Women in the exercise group increased their daily walking by 4,300 steps (2.9 km per day; significantly different from baseline and from the control group) and were walking an average of 9,700 steps per day, with no change in walking per day for the control group. By week 12, SBP and MAP were reduced in the exercise group (SBP reduced from 142 to 136mm Hg, MAP reduced from 103 to 98mm Hg). Between week 12 and week 24, SBP fell a further 5mm Hg, with no further change in MAP. The intervention meant that participants were meeting the American College of Sports Medicine and the Centers for Disease Control and Prevention minimum physical activity recommendations and that this
was effective in significantly lowering blood pressure. Interestingly, the changes in blood pressure were independent of changes in diet, body composition, or insulin levels (Moreau et al., 2001).

In order to understand these parameters further, Cornelissen and Fagard (2005) carried out a meta-analysis of aerobic endurance training on resting BP and ambulatory BP (AMBP). Seventy-three randomised controlled studies were included in the review, which concluded that chronic dynamic aerobic exercise lowers BP, and that the effects are more pronounced in hypertensive participants than normotensives. It also concluded that the change in BP is based on the observed reduction in systemic vascular resistance. The authors suggest that the sympathetic nervous system is involved, as is the renin-angiotensin system. This was suggested due to an average reduction of 29% in plasma noradrenaline and an average 20% reduction in plasma renin activity following the training periods. Interestingly, the reduction in plasma renin activity suggests that the reduction in SNS activity also affects the kidney. The meta-analysis also observed a reduction in insulin resistance, weight, body fat and waist circumference and an increase in HDL-cholesterol levels. The average training programmes consisted of three bouts of forty minute training sessions a week, for 16 weeks, at 65% HR reserve. The authors observed that the magnitude of the reduction in blood pressure was related to the increase in VO$_2$ max, and therefore related to an increase in fitness. However, investigations into the impact of exercise on markers of vascular health were not included in this study. Further, due to the observed suggested reduction in SNS activity, it would have been interesting to measure change in perceived mental stress and its association with SNS and BP, as discussed in section 1.4.

Thus, published research consistently supports the view that exercise can lower blood pressure. As exercise has been suggested to also affect vascular health, it is important that these factors discussed above are considered when interpreting how blood pressure is lowered.
The impact of exercise on arterial stiffness

As described earlier, central arterial stiffness and wave reflection increases with normal ageing, as demonstrated by an increase in aPWV and Alx, respectively, and this stiffness of the arteries is suggested to contribute to age-related increases in CV risk (Vaitkevicius et al., 1993, Avolio et al., 1985, Kelly et al., 1989a). McEniery et al. (2005) have demonstrated that in a younger population (i.e. younger than 50 years) Alx is a more sensitive marker of arterial stiffness and risk and aPWV is more sensitive a marker in an older population of 50 years and older, when Alx as a marker of stiffness is less pronounced. This is most likely because Alx is a marker of arterial function, whereas aPWV is a marker that is influenced by structural change; importantly, such structural change is unlikely to have generally occurred sufficiently before the 5th decade in order to significantly affect aPWV.

With regard to the effects of exercise on arterial stiffness, Tanaka et al. (1998) conducted a cross-sectional analysis comparing effects of being physically active with being sedentary, in a cohort of healthy women (n= 53). The active women had been active for at least the previous two years (mean, 13 years) and on average they trained for six hours a week. The study recruited pre- (younger) and post-menopausal (older) women of sedentary and highly physically active status. They demonstrated that aPWV and Alx was ~30 to 50% lower in the physically active older women compared to the sedentary older women. There was no significant difference in aPWV or Alx between the physically active younger and the physically active older participants. This was despite the older active women having higher SBP, MAP and PP. As the sedentary older women had higher aPWV and higher Alx than the sedentary younger women, these data representing the active, older women suggests exercise has a protective effect against age-related structural and functional changes of the arterial wall, in women.

Vaitkevicius et al. (1993) demonstrated similar findings in a healthy, older male cohort (54-75 years); endurance trained men (n= 14) had a 26% lower aPWV and an average 36% lower Alx than their age-matched, sedentary counterparts. However, these two studies were both
relatively small. McDonnell et al. (2013) conducted a large, cross-sectional study that recruited 1,036 healthy male and female participants. They demonstrated that aPWV did not differ between young highly active individuals and age-matched controls, as expected, based on the McEniery et al. (2005) paper. McDonnell et al. (2013) also demonstrated that older participants (> 50 years old) who regularly participated in physical activity had lower aPWV (and therefore lower CV risk) compared to age-matched sedentary counterparts. These data therefore are suggestive of an exercise-associated delay in the age-associated stiffening of the large arteries in older participants who are regularly physically active. Further to this, AIx (as a measure of peripheral arterial function and wave reflection) was lower in the young physically active group compared to their sedentary, age-matched counterparts which suggests an exercise-associated improvement in arterial function in young, physically active participants.

In summary, these three cross-sectional studies suggest that regular exercise has a beneficial protective effect against the structural and functional changes on the arterial wall that would normally occur with ageing. These data also suggest that age differentially impacts how exercise affects the vascular system. However, the Northern Ireland Young Hearts Project (NIYHP) is a large, cross-sectional study and from which Boreham et al. (2004) investigated the relationship between fitness and arterial stiffness (measured via aorto-iliac and aorto-dorsalis pedis PWV) and demonstrated an inverse relationship between cardiorespiratory fitness and both the elastic aortoiliac segment and the muscular aorto-dorsalis pedis indices of arterial stiffness, and an inverse relationship between sports-related physical activity levels and peripheral arterial stiffness (aorto-dorsalis pedis PWV). This is contrary to the cross-sectional studies discussed, which demonstrated exercise-associated reductions in aPWV, as Boreham observed the inverse relationship in a peripheral, muscular segment rather than the elastic. However, the cohort was young (n= 405; equal mix of males and females, with a mean age of 23 years) and may therefore fit with the data from McDonnell et al. (2013) that demonstrated that wave reflection (to some extent a marker of
peripheral function) was significantly lower in the physically active young individuals when compared to their sedentary counterparts.

Therefore, exercise interventions (both resistance exercise training programmes and aerobic exercise training programmes) have largely shown similar effects; Havlik et al. (2005) conducted a study as part of the Activity Counselling Trial (ACT) as part of which 464 baseline measures of aPWV were taken. Following an increase in self-reported walking over a 24-month period, there was a significant reduction in large artery stiffness (aPWV). However, SBP was observed to be a significant contributor to the change in aPWV, further highlighting the need to adjust for changes in blood pressure when aiming to determine the impact of regular exercise on large artery stiffness. Furthermore, Yoshizawa et al. (2009) ran an investigation into the impact of a 12-week moderate-intensity resistance exercise programme and a 12-week moderate-intensity aerobic exercise programme on aPWV. Participants recruited were 35 healthy females with a mean age of 32.9 years, and were randomly selected to join the resistance training programme, the aerobic training programme or the control group. There was no change in blood pressure for either of the intervention groups. The aerobic exercise group demonstrated a significant decrease in aPWV following the intervention, with no change observed in the resistance training programme. It is interesting that a significant, blood pressure independent reduction in aPWV was observed in a relatively young population considering the findings of McEniery et al. (2005) and McDonnell et al. (2013). However, the size of the cohort was relatively small and the effect seen therefore could be due to the time of assessment in the menstrual cycle affecting NO bioavailability and therefore endothelial function (Giusti et al., 2002, Hashimoto et al., 1995).

Since different types of training seem to induce differential effects, it is important to note that Collier et al. (2008) conducted a study to determine the impact of resistance training on aPWV compared to an aerobic exercise programme. The interventions were four weeks long and cohorts were a mix of pre- or stage 1- hypertensives, not on any medication and a mix of men and women with a mean age 48 years. Following the programmes, both groups
demonstrated a significant reduction in SBP; however, the resistance group demonstrated a significant increase in aPWV whereas the aerobic group demonstrated a significant reduction. These two studies suggest that resistance training does not have the same beneficial impact on arterial stiffness that regular aerobic exercise has been seen to have. However, four weeks is a short period of time for a structural change to occur and the study does not mention whether or not the analysis adjusted for the change in blood pressure. If not, it is likely that the observed reduction in aPWV following aerobic exercise is mediated by the blood pressure improvement and therefore the functioning of the artery, rather than in improvements in structure, i.e. in elastin: collagen ratio. Further to this, the disease state of the groups is likely to impact the ability of the exercise to have an effect.

Further to the suggestion that a 4-week exercise intervention was possibly too short a period of time for structural changes to have occurred in the Collier et al. study, in a small cohort of 10 healthy males with a mean age of 21 years, the effect of an 8-week endurance training programme on aPWV was measured. Prior to the study, participants were largely sedentary as they only undertook exercise once a week during gym class. The participants trained at a moderate intensity on a cycle ergometer three to four times a week, for 60 minutes each time. Following the 8-week training programme, SBP and DBP remained the same as at baseline but aPWV was significantly reduced. Following the training period, participants undertook a detraining period which consisted of being sedentary for 8-weeks. Four weeks into the detraining period, aPWV had returned to baseline. This suggests that the improvement in aPWV following the relatively short-term exercise programme had been a result of functional changes to the vasculature and that the exercise had not resulted in structural changes (Kakiyama et al., 2005).

Hayashi et al. (2005) measured central and peripheral PWV in 17 sedentary and middle-aged men pre- and post- a 16-week, moderate-intensity exercise training programme. This involved walking and light jogging. Following the training programme, aPWV had significantly decreased, but peripheral PWV had not. Kearney et al. (2010) measured
peripheral (carotid-radial) PWV in 70 sedentary and overweight participants who were randomly assigned to either a control group or walking groups. Those who were placed in a walking group were consequently assigned to carrying out 3 bouts of 10 minute walking each day on 5 days of the week. PWV was measured at baseline and following the 6-month intervention. The study found that those in the walking groups had a significant decrease in their PWV compared to baseline and compared to those in the control group. This effect was found independent of any changes in weight or blood pressure. This study was similar to many studies which have found long-term exercise programmes to be effective in reducing aPWV but not in the more muscular vascular bed, as measured via peripheral PWV. These data suggest that exercise affects different areas of the vascular system and that this may differ further depending on the characteristics of the cohort such as age and factors such as the duration, modality and intensity of the exercise.

Finally, Tabara et al. (2007) carried out a study investigating the effect of acute and long-term aerobic exercise on AIx as a marker of arterial stiffness, and more specifically; wave reflection. Ninety-nine healthy participants took part in an acute study, and a sub-set of 40 participants also participated in a long term study; 30 minutes of aerobic exercise twice a week for six months. The mean age of the participants was 67 years. The study showed that following the acute bout of exercise, SBP and DBP were significantly reduced (measured once heart rate had returned to baseline heart rate). There was no change in AIx. Following six months of regular aerobic exercise, SBP and DBP had significantly reduced. AIx was also significantly reduced (significance remained when heart rate was adjusted for) and there was no change in heart rate. The authors suggest that heart rate not reducing may be as a compensatory response to the reduced blood pressure. The authors highlighted for particular interest the finding that the acute effect of aerobic exercise was significantly and independently associated with the outcome of long term exercise on AIx, suggesting that the baseline values determined the extent of the change that could be achieved. Those with highest baseline AIx scores showed greater reductions in AIx.
following the acute bout of exercise. Similarly, those with highest BP’s had the largest reductions in both the long-term and acute studies, with this change being correlated with the change in Alx. However, the reduction in Alx is likely to be as a result of the reduction in BP. Unfortunately, aPWV was not measured in this study and therefore there was no data on large artery stiffness.

A dose-response effect of aerobic exercise was suggested by Edwards and Lang (2005), who recruited healthy male and female participants (age range 18-45 years) and compared Alx in 16 recreationally active participants with 16 competitive endurance athletes. In the endurance trained athletes (who had higher fitness and trained more regularly, for longer duration and at a higher intensities), Alx was lower than in the recreationally active participants. Thus, suggesting a dose response effect on Alx and therefore upon the functioning of the artery, likely as an improvement in endothelial function.

Thus, in summary, the literature demonstrates that exercise is beneficial in improving markers of vascular health, but that there is some confliction with regard to the exact impact exercise training has on markers of arterial stiffness, such as FMD, aPWV, peripheral PWV and Alx. Nevertheless, it appears that the majority of studies have demonstrated that exercise training is associated with a beneficial effect upon markers of arterial stiffness and therefore upon CV risk. Therefore, it is suggested that a longitudinal study is needed to determine the mechanisms that occur in order for these exercise-associated benefits on markers of arterial stiffness to be achieved. Such a study should be conducted on a multi-disciplinary basis, so that it could also investigate the impact of exercise on other confounding factors associated with CV risk such as the biomolecular markers discussed previously, and also mental health (see next section).
1.5.4 Exercise and markers of mental health associated with CV risk

“I found that I worked better and thought more clearly when I was in good physical condition” (Mandela, 1994)

Exercise is known to improve quality of life and subjective well-being, indirectly, by preventing diseases and morbidity, and therefore it is now considered imperative to determine its direct effect on mental health (Fox, 1999).

The case for increasing exercise levels for health has largely been built based on the evidence demonstrating the benefits that can be accrued upon one’s physical health and function (Fox et al., 2007). However, large-scale prospective studies have demonstrated the beneficial impact of regular exercise on the prevention of depression (Strawbridge et al., 2002), cognitive impairment, development of dementia and Alzheimer’s disease in older adults (Abbott et al., 2004, Colcombe and Kramer, 2003, Laurin et al., 2001, Rovio et al., 2005, Yaffe et al., 2001). Moreover, a meta-analysis reported that the current literature suggests that regular exercise is associated with better health, quality of life outcomes and better mood states (Penedo and Dahn, 2005). Hence, as will be discussed in detail further below, there is a growing body of evidence demonstrating the beneficial effect of exercise on the following range of mental health makers.

Anxiety

Literature regarding exercise and mental health suggests that there is an anxiolytic (anxiety relieving) effect of exercise. A review of the effect of exercise on various markers of mental health discussed how regular exercise reduces and improves symptoms of anxiety, with acute anxiety responding better to the exercise than chronic anxiety (Paluska and Schwenk, 2000).

An aerobic exercise programme was seen to improve state anxiety (as measured using the State Trait Anxiety Inventory (STAI) questionnaire) in a study conducted by Blumenthal et al. (1999). There were 156 participants aged between 50 and 77, who all met the criteria for
major depressive disorder (MDD). They were randomly assigned to a 4-month course of aerobic exercise, a 4-month course of sertraline therapy (medication used for the treatment of depression) or a 4-month course of aerobic exercise plus sertraline therapy. Following the intervention, all three treatment groups demonstrated significant improvements in anxiety; however the change was not significantly different across groups. These data suggest that aerobic exercise is as beneficial at reducing anxiety as the pharmaceutical treatment.

Also investigating the effect of aerobic exercise on general well-being and anxiety was a study undertaken by Cramer et al. (1991). The study included a 15-week, moderate-intensity exercise training programme that recruited 35 sedentary and mildly obese women and grouped them into an exercise group or sedentary group. The programme consisted of five, 45-minute brisk walking sessions per week. The exercise group had improved general well-being, and improved ‘energy levels’ were significantly correlated with improvements in sub-maximal cardio-respiratory fitness. State anxiety was improved at the mid-point of the programme but had returned to baseline by the 15th week. This could be a result of tedium borne from increasing fitness levels but no increase in the intensity of the exercise.

Based on both clinical and non-clinical populations, it has been suggested that the largest improvements in anxiety occur when an exercise programme has run for at least ten weeks. Despite this, it is also surmised from the literature that even short bursts of exercise are efficient in reducing anxiety (Scully et al., 1998). Another review concluded that regardless of the measure of anxiety (i.e. state or trait, self-report, physiological or behavioural), or the mode and method of exercise (i.e. chronic or acute), the results are consistent in suggesting that there is a beneficial relationship between exercise and anxiety reduction (Landers and Petruzzello, 1994).

**Depression**

Exercise and its effect on depression has also been investigated. It has been observed that people with depression tend to be less physically active than those who do not suffer with
depression (Paluska and Schwenk, 2000). Cardiorespiratory fitness was observed to have an inverse graded dose-response relationship with the Center for Epidemiologic Studies Depression scale (CES-D) scale for depression measurement. Thus, suggesting improved depression symptomatology with increased fitness (Galper et al., 2006). Further to this, those with depression who increase either aerobic exercise training or strength training have been shown to have significantly reduced depressive symptoms. A review by Martinsen (1990) demonstrated a similar relationship between levels of clinical depression and the number of patients who reported being physically sedentary. The review also found non-aerobic exercise to be as effective as aerobic exercise in the treatment of clinical depression. These data demonstrate that the two modes of exercise are beneficial in terms of reducing depressive symptomatology; however, the data doesn’t describe the effect on healthy populations.

Using 80 studies, North et al. (1990) conducted a meta-analysis investigating the effects of exercise on depression and found a significant effect of \( r = 0.53 \), suggesting a moderate association between depression and exercise. They found that those suffering from clinical depression had significant reductions in levels of depression as a consequence of the increased physical activity, whereas those from the general population illustrated more moderate improvements. Further to this these data showed that both acute and chronic exercise were effective at reducing levels of clinical depression. Another aspect of physical activity that was investigated in the meta-analysis was the impact of the duration of exercise on depression. Although a 4-week programme was effective in reducing levels of depression, it was found that the greatest improvements were after 17 weeks of exercise. This suggests that long-term adherence to aerobic exercise programme is most effective in reducing clinical levels of depression, though the study was not able to determine whether the effects on depression levels were comparable to that of the usual therapeutic or pharmaceutical treatment.
Blumenthal et al. (1999) investigated the effects of a 16-week aerobic exercise training programme on depression (measured using BDI). As described in the section on exercise and anxiety, the exercise intervention included exercising via either brisk walking/jogging or on a cycle ergometer. Following the 4-month intervention, depression levels in the exercise group improved to the same degree as a pharmaceutical treatment group and an exercise-plus-pharmaceutical group. In order to determine the long-term effects of the significant reductions in depression levels observed, a follow-up investigation was conducted. Blumenthal and colleagues carried out an investigation into the effects of the treatment 6-months after completion of the programme. They found that amongst those who were in full remission from MDD (i.e. no longer met criteria for MDD) those in the exercise-only group were less likely to relapse than those in the other two groups. Combining exercise with medication had no additive effect to that of medication alone (Babyak et al., 2000). These results suggest that regular exercise is as beneficial as pharmaceutical interventions for depression during the treatment phase, but more beneficial in the long-term.

Another study that recruited MDD patients involved a relatively short exercise programme demonstrating the beneficial impact of exercise on depression, as measured using the CES-D (Knubben et al., 2007). Participants were recruited following a major depressive episode and were randomly assigned to either an endurance training programme (daily treadmill-based walking according to an interval-training pattern) or a placebo activity group (10 minutes stretching per day). Following the 10 days, the endurance training group demonstrated significantly greater reductions in the CES-D measured depression than the placebo group, demonstrating that a walking-based exercise programme is beneficial in relieving depression, even after a relatively short period of time.

The effect of different types of walking programme interventions on participants with depression has also been investigated by a recent meta-analysis that was conducted by Robertson et al. (2012). The meta-analysis included 341 participants from eight studies. All studies included participants who had a form of depression, with only studies with cohorts of
participants who had been recruited because they suffered from bi-polar disorder or a chronic medical condition being excluded. The trials also varied in the walking intervention itself, for instance; some walks were supervised, walks were either indoor (i.e. on a treadmill) or outdoors, and the duration and frequency of the walks varied. The walks ranged from 20 to 50 minutes and some programmes lasted up to six months. The control groups included groups that were assigned therapies that included usual care, relaxation and stretching therapies, or being involved in a support group. When the data were pooled, the results demonstrated that the walking programmes significantly reduced depressive symptoms. The data was also split to include only those studies that had recruited people with high BMI’s; only indoor walking; only outdoor walking; and only group walking. All four methods of splitting the data resulted in the walking programmes alleviating the symptoms of depression.

Thus in conclusion, these data together suggest that exercise is capable of reducing levels of depression in healthy and clinical populations, and that anaerobic and aerobic exercise programmes of both long and short intervals are beneficial.

Self-esteem

Exercise and its impact on self-esteem has also been investigated. An aerobic exercise programme was seen to improve self-esteem (as measured using the Rosenberg Self-Esteem Scale) in a study conducted by Blumenthal et al. (1999). As described in the exercise and anxiety section, a 4-month long programme of treadmill-based brisk walking, jogging or cycle on a cycle ergometer was employed, and participants with MDD demonstrated improved levels of self-esteem following the intervention. Self-esteem was improved to the same degree as a pharmaceutical treatment group and an exercise-plus-pharmaceutical. These data demonstrate that exercise is as beneficial as pharmaceutical treatment in the improvement of self-esteem, with the added benefit of likely improvements to systemic health, unlike the pharmaceutical treatment.
Using a relatively healthy cohort of participants, a study by McAuley et al. (2000a) recruited 174 older, sedentary participants (mean age 66.7 years) on to a walking group in order to investigate how exercise affects levels of self-esteem. Data illustrated that participant’s self-esteem increased over the six month intervention, and then decreased during the non-intervention follow-up period of six months. The frequency of the activity and changes in physical fitness, body fat, strength and physical condition were associated with increases in measures of self-esteem that were related to perceptions of physical self-worth. A follow-up to this study was carried out over the four year period following the intervention. The study investigated self-efficacy (as defined in this study by the ability to take part in moderate intensity exercise three times a week, for 40 minutes). Over the period of the study, participants who reported greater reductions in their self-efficacy and physical activity levels, demonstrated significant deteriorations in their levels of self-esteem (McAuley et al., 2005). Therefore, not being able to take part in physical activity as frequently as they had previously, had a negative impact on their self-esteem. Whether this is as a result of not taking regular physical activity or the perception of not being able to is unknown.

Thus, the literature supports the view that physical activity has a beneficial impact upon self-esteem, while also highlighting the need for participants to adhere to exercise in the long term in order to gain and maintain the improvements associated with regular exercise.

**Mood**

Mood has been studied extensively in the context of exercise. Using the Profile of Mood States (POMS) questionnaire, Starkweather (2007) demonstrated that a 10-week treadmill-based walking programme was associated with significant improvements in mood in an exercise group (n= 10), with no improvements observed in the non-exercising control group (n= 10). Acute bouts of treadmill-based walking were also seen to improve mood (using the POMS scale) in Pretty et al. (2005b).

Another 10-week exercise programme was undertaken by Moses et al. (1989) and recruited 96 healthy participants to investigate the effect of moderate-intensity aerobic exercise and
high-intensity aerobic exercise on mental well-being and mood markers and compared the results to a non-exercising control group. Following the 10-week intervention, VO$_2$ max (a measure of cardio-respiratory fitness) improvements were observed in both of the exercising groups. However, general mental well-being improvements were observed only in the moderate-intensity group.

Thus, these data suggest that mood has been observed to improve following both acute bouts of aerobic exercise and long-term aerobic exercise programmes, also that that is the form of exercise rather than the improved cardiorespiratory exercise that impacts upon the construct of mental health and well-being. Further to this, different intensities of exercise may affect mental health constructs differentially.

**Perceived stress**

Similar effects of different intensities of exercise have been observed in investigations of perceived stress. Norris and colleagues demonstrated that aerobic exercise is more beneficial than resistance training for improving levels of perceived stress in an adult cohort (Norris et al., 1990). Participants in the aerobic training group evinced larger positive changes in self-reported mental health and stress measures than those in the resistance training group. However, both exercise groups improved compared to the control group. In contrast to what Moses and colleagues reported on mood, Norris et al. (1992) demonstrated that a 10-week high-intensity training programme resulted in greater reduction in stress than a moderate-intensity exercise programme. However, the cohort of the study were adolescents, which may suggest that high-intensity exercise may be more beneficial to mental health in that age group, than moderate-intensity exercise. This could be due to what a group perceives to be the outcome of an exercise programme, for instance, in a young group, high-intensity training with the potential to build more muscle might be more important than the benefits gained from a moderate-intensity exercise programme.

Importantly, regular aerobic exercise has also been compared to ‘usual care’ stress management training in ischaemic heart disease patients, in order to determine the
difference in effectiveness. In 134 ischaemic heart disease patients, Blumenthal et al. (2005) investigated how an aerobic exercise intervention (plus usual care) affected stress, compared to the effect of usual care or usual care plus stress management training. Participants undertook three bouts of 35 minutes training per week over 16 consecutive weeks. The participants completed depression, trait anxiety, hostility and general distress questionnaires. Participants in the exercise intervention and in the stress management training showed greater reductions in the overall distress measure and in the depression measure than the usual care group did.

A 10-week intervention recruited 10 healthy participants to walk for 30 minutes, five times a week and 10 sedentary control participants. Participants were aged between 60 and 90 years. Stress, assessed using the Perceived Stress Scale (PSS) and perceived health, measured using the SF-36, were measured via questionnaires at baseline and after the intervention. The exercise group had significant reductions in perceived stress scores and demonstrated significant improvements in the quality of life measure via improvements in perceptions of their own health (Starkweather, 2007). These data demonstrate that adhering to national government guidelines regarding target exercise levels is associated with improvements in perceptions of one’s health and in perceived stress levels. It is suggested that improvements in perceived stress levels lead to improvements in levels of perceived health.

Thus, the data regarding exercise and perceived stress is encouraging and suggestive of a beneficial effect of aerobic training and anaerobic training, and beneficial effects in younger and older populations.

**Social support**

Taking part in an exercise programme is often associated with interaction with other individuals. A combination of aerobic exercise training and resistance training has been seen to improve levels of perceived social support, as measured using the Social Provisions Scale (SPS) in a study conducted by McGale et al. (2011). 104 sedentary males (aged 18-
40 years) were recruited on to a 10-week study investigating the effect of participation in a team based sport plus cognitive behavioural techniques, a combination of aerobic and resistance training, or a no exercise control group on mental health. The exercise training programme demonstrated greater increases than the team based sport plus cognitive behavioural techniques at week 5 of the intervention, and compared to the control group at the 8-week follow-up. The study would have benefited from employing a cohort of participants who exercised but not as part of a team. This would have allowed the authors to discern whether the effect of the exercise improves perceived social support or whether the interaction with other people as a result of the exercise groups resulted in the observed improvements.

Cognitive functioning

Finally, as exercise has been shown to improve and impact upon mental health, it is suggested that increased physical activity levels also aids cerebral function (as measured using cognitive functioning questionnaires). A prospective study undertaken by Lytle et al. (2004) examined how self-reported exercise habits in a cohort of 1, 146 participants affected cognitive function. The participants were all aged 65 years or older. Global cognitive function was assessed using the Mini Mental State Examination (MMSE). Three groups were composed based on the self-reported physical activity levels; high exercise group (aerobic exercise of ≥ 30 minute duration, ≥ 5 times a week), low exercise group (all other exercise groups) and a sedentary control group. Results showed that both low exercise and high exercise participation was inversely associated with cognitive decline, i.e. both exercise frequencies were protective against cognitive decline. Weuve et al. (2004) also investigated the relationship between exercise and cognition and recruited 16, 466 women, aged 70 years and older. A cognitive assessment, based on the MMSE, was adopted, as were memory and attention tests. As well as completing the cognition tests, participants reported their physical activity levels over the last year. The results showed that the women who undertook regular physical activity had less cognitive decline than less physically active
women. Similarly, using the Cognitive Failures Questionnaire (CFQ), regular exercise has been associated with significantly fewer cognitive failures by Froeliger et al. (2012), while Donta et al. (2003) demonstrated significant improvements in the score of CFQ following a 12-week aerobic exercise programme. Potential mechanisms for these inverse associations may include increased cerebral blood flow associated with improved vascular haemodynamics and vascular health (i.e. increased cardiac output, increased blood volume and improved vascular function).

Thus, based on the evidence discussed above, it may be concluded that exercise programmes appear to be associated with improvements in many of the common constructs and easily measured constructs of mental health. Importantly, exercise appears to be beneficial at improving the measured levels of poor mental health in clinical populations (i.e. depression in clinical depressives) and also at improving mental health in the general population; and therefore can be seen as both a treatment and preventative tool. However, it should be acknowledged that there does not appear to be one optimal form, intensity, mode, duration, or frequency that is successful in improving all mental health constructs in all populations, and therefore, it is perhaps the case that the effect of exercise on mental health is rather subjective as its effectiveness will be largely determined by the goals an individual aims to achieve from the exercise. Further to this, it is likely that the exercise programme needs to be tailored to each individual in order to provide a challenging but achievable stimulus that increases self-efficacy and hence does not negatively impact self-esteem.

Some evidence has suggested that as little as five minutes exercising in an outdoor, natural environment is sufficient to induce anxiolytic effects, demonstrating a dose-response relationship (Barton and Pretty, 2010). Exercise in an outdoor setting is an area of research that has recently gained a lot of interest and research input, and is termed green-exercise.
1.6 Exercise and green space

With regard to the ‘tailoring’ of exercise to the needs of the individual, an important aspect of physical activity and exercise which was not considered in the studies reviewed in section 1.5 should be considered, i.e. the potential importance of a natural, outdoor environment in triggering (and possibly adding to) the benefits associated with participation in exercise. This will be reviewed in this penultimate section of the current literature review.

Physical inactivity and sedentary behaviour have slightly different definitions, and the distinction between the two is considered to be important in light of recent research which distinguishes between the two behaviours (Sedentary Behaviour Research Network, 2012, Tremblay et al., 2010). Physical inactivity is a state of behaviour in which people are not sufficiently active to maintain good health (World Health Organisation, 2008). Sedentary behaviour is defined as a class of behaviours that involve sitting and activities that require very low levels of energy expenditure, i.e. less than 1.5 metabolic equivalents (METs) (Marshall and Ramirez, 2011, Pate et al., 2008, Marshall and Welk, 2008, Tremblay et al., 2010). Both behaviours are recognised as being important, but distinct, modifiable CV risk factors (U.S. Department of Health and Human Services, 1996, World Health Organisation, 2013a), i.e. a risk factor that can be changed by increasing levels of physical activity. It was estimated that if everyone in England met the recommended physical activity levels of 150 minutes of moderate intensity exercise per week (Department of Health, 2011a), then 37,000 deaths per year would be prevented (Network of Public Health Observatories, 2013). Further to this, the effect of meeting the physical activity guidelines yet remaining sedentary and not sufficiently active for the other waking hours is now an area that is receiving interest (Ellingson et al., 2014, Peddie et al., 2013).

Current UK guidelines state that adults (aged between 19 and 64 years) should aim to be active daily; accumulating at least 150 minutes worth of moderate intensity exercise, or 75 minutes worth of vigorous intensity exercise, in one week (Department of Health, 2011a). However, the proportion of the global and local population undertaking enough exercise to
gain the associated benefits is low and therefore the incidence of physical inactivity related to CVD remains high (Barengo et al., 2004). Data collected from the population in Wales reported that at least 36-38% of women and 30-32% of men undertake no exercise, (NatCen Social Research, 2012) and data on 1 million adults living in England concluded that 80% do not exercise enough (Farrell et al., 2013). However, the paper did not advise as to which guidelines they determined ‘enough exercise’ to be. The paper by Farrell also observed that the less well educated and those with lower socio-economic status (SES) were more likely to be physically inactive, while, in contrast, only 12% of those with a university degree were likely to be physically inactive. The paper reported that in the previous weeks, 46% of people had not walked continuously for 30 minutes for leisure purposes and that 10% of people had not walked continuously for 5 minutes over the previous 4-week period.

In Wales, the counties with the largest rates of physical inactivity are all in the region of the south Wales valleys, where, additionally, rates of physical inactivity were higher than rates of physically activity. The highest rates of physical activity were in the more affluent counties in mid and west Wales, where rates of physical inactivity were lower than rates of physical activity. Interestingly, all but one of the counties (Monmouthshire) fit this mid Wales: south Wales trend (NatCen Social Research, 2012). Of the 22 unitary authorities in Wales, those five authorities with the lowest percentage of people reporting ‘good health’ are all the same areas as those reporting the highest rates of physical inactivity, namely Blaenau Gwent, Merthyr Tydfil, Neath Port Talbot, Rhondda Cynon Taff and Caerphilly (The Office for National Statistics, 2013a). These five counties are the five counties of Wales with the lowest SES as determined by the Welsh Index of Multiple Deprivation (WIND) (The Office for National Statistics, 2011). Hence, local data published on CVD-associated mortality by local authority is in agreement with Mackenbach et al (2000)’s demonstration in general terms that incidence of morbidity and mortality from CVD is higher in areas of low SES (Scarborough et al., 2010).
As demonstrated above, there are five counties within Wales that all exist within the south Wales valleys region; they all suffer from the lowest rates of physical activity in Wales, low SES and high CVD-associated mortality. Based on data demonstrating the beneficial impact of exercise on CVD (Kohl, 2001), it is plausible that the disease burden in these five counties could be alleviated via increasing physical activity levels. However, as these areas are of low SES, levels of unemployment are high and therefore disposable income to spend on the costs associated with exercising, i.e. gym membership, travel to gym/exercise classes, clothing associated with exercising, does not exist. This information fits with previous work that has demonstrated that those from a low SES background are less likely to be physically active (Popham and Mitchell, 2007), which would be true of the south Wales valleys region.

Saelens, Sallis and Frank (2003) suggested that this may occur particularly in areas with poor connectivity and single land use. Importantly, green space is abundant in the five areas mentioned above; for example 80% of the county borough land of Caerphilly is classified as countryside (Caerphilly County Borough Council, 2011). As a method of addressing the low SES associated CV risk in these areas, initiatives that encourage habitants of these areas to take part in physical activity in the green spaces that surround their homes have been set up. In particular, these initiatives have been implemented by Groundwork Wales (GWW, a national charitable organisation with many aims regarding greener living and the environment) and their partners, collectively the Valleys Regional Park (VRP). The VRP is a collaborative initiative whose partners include organisations such as British Trust of Conservation Volunteers (BTCV, recently TCV [The Conservation Volunteers]), Ramblers Cymru and Sport Wales. GWW and VRP’s aims include changing behaviour, limiting the impact of people and businesses on the environment and encouraging walking and cycling in one’s community (Groundwork Wales, 2013). The programmes developed by GWW within the VRP remit are themed around ‘green-exercise’, and making use of the natural environment.
1.6.1 Green-exercise

"Nature and green space can be seen as a great outpatient department whose therapeutic value is yet to be realised" (Bird, 2007).

Over the last few years, an increasing amount of research has investigated the synergistic effect of exercising whilst in a natural environment, on markers of health. This has been termed green-exercise. Green-exercise is defined as any form of exercise or physical activity that takes place within a relatively green setting (Pretty, 2003, Pretty et al., 2005b). The study of green space, green-exercise and its effect on health, in particular mental health, has to some extent stemmed from the Biophilia hypothesis (Wilson, 1984). The hypothesis suggests that between human beings and nature there exists an innate and instinctive bond which causes humans to feel the urge to affiliate with other life forms. It is suggested that the affiliation with nature and green space has a rejuvenating effect on mental health (Hartig et al., 1991).

Nature and green space is thought to have this effect partly via sensory stimulation (Clayton, 2007) and partly as a result of evolution; humans were hunter gatherers and farmers for a far longer period of time than being an industrialised population, and humans therefore connect with nature which causes relaxation. Further to this, the natural environment provides an opportunity from which to recover from mental fatigue (Herzog et al., 2003) and for attention restoration (Kaplan and Kaplan, 1989, Kaplan, 1995) as it is an environment which does not require effort and direct attention. Time away from activities and thoughts associated with daily routine is suggested to allow for attention restoration (Kaplan, 1995). These theories have spawned much research, such as that conducted by Vries et al. (2003) whom showed a positive relationship between people’s health and green space; in a greener environment, inhabitants reported fewer symptoms of ill health, better perceived general health and better mental health.
Across the south Wales valleys (and more broadly elsewhere in the UK, and internationally), this evidence has led to the development of ‘blue gyms’; which is exercise in the presence of water environments (Depledge and Bird, 2009), ‘green gyms’; which involves tackling physical jobs in the outdoors as a means of improving one’s own health as well as the health of the environment (Yerrel, 2007), and green-exercise walking programmes, which take advantage of the footpaths that travel through rural and picturesque landscapes. Green-exercise programmes such as these are proposed to overcome many of the barriers associated with not exercising. For instance, in the rural areas of the south Wales valleys there is no need to have a car or the money to take the bus to get to the exercise venue, as it is on the doorstep of the people who live in these areas. This also overcomes the ‘not having enough time’ barrier that is often cited as a reason for not exercising (Salmon et al., 2003). Green-exercise can be carried out at any age and level of fitness as it is easily tailored to fit all abilities and fitness levels. Finally, it does not require specific equipment or clothing and therefore incurs no additional cost.

Thus, when the health benefits associated with time spent outdoors and living in and around green space (Ryan et al., 2010, van Dillen et al., 2011, De Vries, 2001) are put together with the fact that adults only spend 20% of their time outdoors and children only spend 9% of their time outdoors (HPA, 2008), green-exercise seems to be a logical step towards increasing physical activity levels, particularly when considering evidence bases such as that which suggest that the nearer to the coast people live, the more active they are (Bauman et al., 1999); which appears to be mirrored by green space accessibility (Lachowycz and Jones, 2011). Interestingly it has recently been demonstrated that the least physically active areas of large English cities had twice the housing density and 20% less green space, with the more healthy authorities having half the housing density and a fifth more green space (Royal Institute of British Architects, 2014).

Furthermore, as will be discussed in the current section, observational data demonstrating a positive effect of being amongst nature and being able to view nature on health supports the
Biophilia hypothesis. Importantly, however, there is currently a lack of evidence demonstrating that green-exercise programmes are associated with similar health benefits to those seen on laboratory/gym-based exercise programmes. To date, the impact of green-exercise on health is limited mostly to cross-sectional observation investigations and to interventions using acute bouts of green-exercise as the stimulus, and with the main focus of the research being on mental health (Barton and Pretty, 2010, Pretty et al., 2005b).

A study conducted in the Netherlands investigated the relationship between the amount of green space in people’s living environments with their perceived health (n= 250,782). Perceived health was better for those living in greener environments; 10.2% of residents felt unhealthy in areas where 90% of the space around them is green, whereas in areas where only 10% of the surrounding environment is green, 15.5% of residents felt unhealthy. Data from urban households demonstrated that the degree of urbanity is less strongly related to perceived health than the amount of green space. These data demonstrated that people of all education levels and all ages had better perceived health when there was access to more green space. Green space and its effect on health was also associated with the proximity to their home and the data demonstrated that the effects of the green space being within a 1km radius or a 3km radius were considered equal, however, the proximity became more important in highly urban areas (Maas et al., 2006). In addition, the same group (Maas et al., 2009b) carried out an important study that compared the percentage of green space within 1km and 3km for each household against GP-assessed rates of morbidity (n= 345,143). Their results found that having 10% more green space than the average (of the whole cohort) within a 1km radius of one’s home was associated with lower rates of 15 of the most prevalent 24 disease clusters. This relationship was strongest for those who typically spend more time in the area local to their home, i.e. children less than 12 years old and people from low SES backgrounds, and also for people aged between 46 and 65 years. The disease clusters for which these relationships existed included CHD, depression, anxiety disorder and diabetes. Interestingly, there were also strong relationships between more
green space within 1km of people’s homes and morbidity rates in urban areas, with the strongest correlations occurring in slightly urban areas compared to strongly urban areas. Interestingly, a study conducted in California investigated the effect of stress on psychological and physiological recovery in natural and urban settings. Sitting in a room with a view of the natural environment resulted in a more rapid decline in DBP following a ‘mental-stress-inducing task’ condition and a ‘no-task’ condition, compared to sitting in a room with no view. Further to this, walking in a natural environment following a ‘mental-stress-inducing task’ condition and a ‘no-task’ condition resulted in rapid decline in SBP and DBP in the first 10 minutes, which was not evident in the urban environment groups (Hartig et al., 2003). These data suggest that there is a buffering effect of viewing nature on blood pressure in response to stress tasks, perhaps mediated by the view of green space dampening the stress experienced by the stress task, via attention restoration. Further to this, walking in a green space after completion of the stress task resulting in a more rapid decrease in BP may be due to a restorative effect of the green environment on stress and hence dampening of SNS activity and therefore a more rapid reduction in BP. The effect of access to green space has also been investigated in terms of its buffering effect on low-SES associated mortality, as discussed below.

In a UK context, Mitchell and Popham (2008) investigated whether income-related health inequality in England is affected by exposure to green space, as the literature discussed might suggest. Populations of low-income families were classified into four groups, ranging between ‘least deprived’ and ‘most deprived’. The areas in which they lived were classified into five groups, ranging between ‘least exposed to green space’ and ‘most exposed to green space’. The most deprived groups suffered the greatest rates of all-cause mortality and circulatory disease mortality across all five levels of green space exposure, with the opposite true for the most deprived groups (i.e. the least deprived groups suffering the lowest rates of mortality). Interestingly, the steepness of the income-related mortality gradient between the four deprivation groups was lower for the populations with the greatest
exposure to green space. The four different populations were privy to the same welfare state, national income distribution and health service but the natural environments in which they were resident differed. Therefore, access to green space appeared to reduce SES-associated mortality compared to areas with less green space access.

Further to these observations, it must also be noted that recent research demonstrates that women are more likely than men to be affected by stress if living in an area with the lowest levels of green space (Roe et al., 2013). The study was conducted in 106 men and women (equal gender split), aged between 35 and 55 years, from a socio-economically deprived region of Scotland (Carstairs Index). Green space was determined using the Census Area Statistic Ward data. Participants completed the Perceived Stress Scale questionnaire, and more green space was associated with lower perceived stress. For both men and women, perceived stress was higher in low green space regions, but perceived stress was significantly higher in low green space areas in women than in men. In areas with high green space, perceived stress levels between men and women were more similar and it was only in low green space areas that stress levels between men and women were significantly different. However, these studies are cross-sectional in nature and therefore it is difficult to conclude cause and effect.

Hence, given that exercise can positively impact upon both physical and mental well-being (Scully et al., 1998) and the premise that the natural and built features of the environment affect mental state and behaviour as well as interpersonal relationships (Frumkin, 2001, Kellert, 1993, Tuan, 1977), it would appear that the environment can have either a positive or pathogenic effect on our well-being (Lewis and Booth, 1994, Gesler, 1992, Burgess et al., 1988).

Thus, scrutiny of the literature broadly supports the view that access to – and exercise in – green space is beneficial to health (albeit with the proviso that the evidence base is not yet fully comprehensive in many respects). There are three mechanisms proposed by which green space is suggested to be associated with better health. The first is through improving
social contact and creating a sense of belonging within a community (Maas et al., 2009a), and the second mechanism is thought to occur as a result of the psychological restoration, one theory of which was mentioned previously (Kaplan’s attention restoration theory). The second theory (of the second mechanism) is Ulrich’s psychoevolutionary model which suggests that green space aids stress reduction via the array of visual stimuli in the natural environment causing an immediate, affective response which impacts upon the neuroendocrine system and the brain (Ulrich et al., 1991). A third mechanism by which green spaces are correlated with improved perceived health and lower rates of morbidity is by providing salutogenic environments, i.e. by providing environments which are conducive to exercise, as green spaces independently promote physical activity (Humpel et al., 2002, Kaczynski and Henderson, 2007, Lachowycz and Jones, 2011).

Pretty (2004) defined three levels of engagement with nature: viewing nature (as if looking at nature through a window, on the television or in a painting), being in the presence of nature (may be incidental to another activity such as walking through fields or sat reading in a garden), and active participation and involvement with nature (such as gardening, farming or horse-riding). The first of the three levels (viewing) has been studied in an observational and an experimental manner. Ulrich (1984) carried out an observational study that demonstrated that patients who had a view of trees from their hospital window spent fewer post-operative days in hospital than those who had a view of a wall out of their hospital window. Furthermore, the patients with a wall-view required stronger doses or narcotic pain drugs compared to patients with a view of nature. Similar work has been undertaken in prison settings (Moore, 1982). Prisoners who had a view of nature had fewer symptoms of stress such as headaches, digestive illness and made fewer ‘sick calls’ compared to prisoners who had views that included buildings and walls. Therefore, views of nature were associated with higher levels of prisoner health and well-being. An example of an experimental study investigating how ‘viewing nature’ and its effect on physiological and psychological responses was conducted by Ulrich et al. (1991). The study instructed
participants to watch a stressful film for 120 minutes. Participants then watched six videos (with colour and sound) depicting natural and urban settings. Physiological measures including heart period, muscle tension, skin conductance and pulse transit time, and measures of psychological state, including fear, positive affect, anger/aggression, attentiveness/interest and sadness were employed. Findings indicated that participants recovered faster and recovery was more complete when participants had been engaged with the natural-setting videotapes as opposed to the urban-setting videotapes. The findings were consistent with the psycho-evolutionary theory that nature has a restorative influence that involved an improvement towards a more positive emotional state (Ulrich et al., 1991).

The influence of being in the presence of nature is of considerable focus in the current research project. Pretty et al (2005b) tested the green-exercise hypothesis by testing the effects of viewing nature whilst exercising, on mental and physical health. Pretty and colleagues were the first group to assess the synergistic effect of physical activity and viewing nature on health and well-being. Participants completed self-esteem (Rosenberg Scale), mood (POMS scale) and general health questionnaires and had resting heart rate and BP assessed. They then exercised on a treadmill at an intensity that they reported as being ‘fairly light’, for most participants this intensity was achieved at a light jogging, for others it was fast walking. The exercise session lasted 20 minutes. During this time, participants were shown a series of randomly allocated pictures on a projector screen (the experimental group), whilst the control group were shown a blank white screen. The pictures were made up of four categories of photographs. The categories were ‘rural-unpleasant’, ‘rural-pleasant’, ‘urban-unpleasant’ and ‘urban-pleasant’. Participants were split so that they viewed only one category of picture. Immediately following the exercise, participants completed the mood and self-esteem questionnaires and five minutes after exercise they had their peripheral BP monitored. Post-exercise, only those who viewed rural-pleasant scenes had significant decreases in SBP, DBP and MAP. Interestingly, urban unpleasant scenes demonstrated slightly increased MAP. The results suggested that for
BP, exercise in a pleasant setting may have a greater positive effect than exercise alone. Self-esteem increased in those viewing rural- and urban-pleasant scenes to a greater extent than exercise alone. When viewing the urban- and rural-unpleasant scenes, self-esteem increased to a lesser extent than exercise-alone, suggesting a depressive effect on self-esteem of exercising whilst viewing unpleasant scenes.

An additional study by Pretty et al. (2005a) examined the effect of carrying out ten different types of physical activity in the countryside. The activities adopted by the study caused volunteers to participate in the last two types of engagement with nature, i.e. being in the presence of nature, and active participation and involvement with nature. Examples of the activities include horse riding, walking groups and mountain biking. Significant improvements in self-esteem were seen in 9 out of the 10 activities (the exception being conservation work, in which participants had encountered a long and hard day). Mood was also investigated. The measurements of mood; anger-hostility, confusion-bewilderment, depression-dejection and tension-anxiety, all improved post-activity; leading to an improvement in total mood disturbance. As results were similar for all ten activities, it suggests that self-esteem and mood measures were not affected by the type or intensity of the green-exercise activities, only that greater improvements were made with longer visits. This was an encouraging result as it suggests that mental health improvements can be gained regardless of the duration, type and intensity of the activity being undertaken. Further to this, the effects appear to be beneficial in populations other than those considered healthy, as described next.

A collaborative study between the green-exercise research department in Essex University and Mind (leading mental health charity) investigated the effect of an indoor walk against a green walk (Mind, 2007). The study recruited a cohort of 20 Mind members (suffering from low mental well-being), whom took part in two organised walks. The study investigated the effect of the walks on self-esteem, mood and enjoyment. The first walk undertaken was in a country park which had surroundings consisting of grasslands, woodlands and lakes.
Exactly a week later the same group took a walk through a large indoor shopping centre. Both walks were 30 minutes long. Participants were encouraged to walk continuously and a certain level of social interaction was also encouraged. Participant’s ages ranged between 31 and 70 years. Results showed that following the green walk, there was an 11% improvement in self-esteem, whereas there was a 4% decrease in self-esteem following the indoor walk. In terms of overall mood, following the walks there were significant differences between walks for the constructs of anger, confusion, depression and tension. All of these differences were in a beneficial direction with regard to the green walk, i.e. the green walk was associated with significantly better improvements in mood post exercise, compared to the indoor walk. Following the green walk, tension fell on average 13%, whilst tension increased an average 2% following the indoor walk. Both walks were followed by a fall in depression, but the green walk had an average decrease in depression of 6%, whereas the indoor walk only had an average drop of 1%. Anger-confusion dropped 8% after the green walk, with only a 1% drop following the indoor walk. However, the two walks were not directly comparable if wanting to compare the direct effects of indoor and outdoor exercise. This is because a certain level of stress would have been induced in the shopping centre that would be above that of other forms of exercising indoors, on a treadmill for instance. Therefore, the difference observed between the two environments may also be a reflection of the indoor environment that the participants were subjected to, rather than independently the restorative effect of exercising in a natural setting.

More recently, Barton and Pretty (2010) carried out a multi-study analysis to investigate what the most beneficial dose of green-exercise is for improving mood and self-esteem. An analysis was performed on 10 studies undertaken by their green-exercise research department over the previous 6 years. The 10 studies were selected as they performed the same method of measuring self-esteem and mood following an acute bout of green-exercise. All 10 studies had adopted the Rosenberg Self-Esteem Scale and the POMS mood questionnaire. The analysis illustrated that both self-esteem and mood were
significantly improved following the green-exercise bout, and that mood had a slightly greater effect size than self-esteem. For both the mood and self-esteem measures, the analysis demonstrated that undertaking the acute bouts of exercise in natural, waterside habitats evinced the largest changes. The results also show that for both self-esteem and mood, the effects are immediate, i.e. occur after only 5 minutes of the activity, and the greatest effects occur with light intensity work. Men and women appear to incur the same degree of improvement in the markers assessed. Healthy populations and those with mental health problems incurred the same improvements for the measure of mood; however the improvements for self-esteem were greater in those who self-diagnosed themselves as having a mental health problem than for the healthy population.

Overall, the above results demonstrate that improvements in self-esteem and mood occur irrespective of the duration, intensity and location (i.e. woodland or park) of the green-exercise and irrespective of age, gender and health status. Importantly, however, despite the interesting and compelling data on the impact of green-exercise on these markers of health, the data on green-exercise, to date, lacks clinical measures of vascular health, and biomolecular markers of health. Such investigations are required in order to determine whether there is a measureable systemic effect of green-exercise or if it is limited to improvements in mental health.

In conclusion, the literature discussed in this section implies that there is an additive effect on mental health and blood pressure when exercise is carried out in a natural setting (whether it’s a natural, green setting or simulated). However, the impact of green-exercise has not as yet been investigated on a biomolecular and/or vascular haemodynamic basis. Hence, given the potential impact of green-exercise programmes upon health, particularly with regard to prevention of global burdens such as CVD, there is a need to undertake multi-disciplinary investigations (ideally including biomolecular and vascular/haemodynamic elements) in order to evaluate green-exercise’s possible potential as a useful tool to use in
the prevention and alleviation of CV disease burden and therefore needs further investigation.
1.7 Aims of the PhD research project

The literature review has identified two major areas relevant to the impact of exercise on CV risk which have previously been relatively neglected. These are:

a) Multi-disciplinary investigations. Exercise is known to be a systemic multi-factorial phenomenon that impacts on many aspects of human health, and as such, different forms of exercise are likely to exert their effects by triggering a wide variety of distinct mechanisms. Therefore, the current author would contend that a multi-disciplinary approach (i.e. measurement of blood-borne markers of CV risk, vascular markers of CV risk and markers of mental health), such as that which is to be employed in the current study, is appropriate for the investigation of this area of interest.

b) Green-exercise. Exercise in natural, green settings has been reported to be associated with additional benefits above and beyond those seen in laboratory-based studies. Moreover, the beneficial impact of green-exercise upon markers associated with CV risk is important from a public health/policy context, as green-exercise provides a route to exercise that overcomes most of the reported barriers to exercising in that it is free, requires no special equipment or clothing, can be carried out at any age or fitness, and is easily accessible. However, few studies have investigated the impact specifically of green-exercise on markers of cardiovascular health (such as blood-borne biomolecular and biochemical markers, vascular stiffness and mental health).

Therefore, the overall aim of the current project was to employ a community-based strategy to conduct a multi-disciplinary investigation into the impact of low/moderate-intensity, aerobic, community-based green-exercise programmes on important health parameters that are related to cardiovascular disease risk.
Chapter 1

Aims

The specific aims of the project were:

- To investigate the impact of the green-exercise programmes on the expression of PPARγ-regulated genes involved in RCT (i.e. CD36 and ABCA1).

- To investigate the impact of the green-exercise programmes on markers of arterial stiffness, and on the expression of a PPARγ-regulated gene involved in degrading the elasticity of the arterial wall (i.e. MMP-9).

- To investigate the impact of the green-exercise programmes on markers of mental health.

- To determine whether any changes in the parameters investigated are inter-related (i.e. whether changes in one parameter have any significant associations with changes in any other parameter(s))

- To identify and understand any potential mechanistic links between these three diverse exercise-associated benefits.

- More broadly, to use results regarding the impact of green-exercise programmes on parameters of health associated with CV risk as an evidence base with which policy makers can address worrying physical activity levels and rates of cardiovascular related diseases, particularly in areas of low socio-economic status.
Chapter 2 - Methodology
2.1 Introduction

The current chapter will discuss the methodologies that were employed for the recruitment and selection of participants onto the studies performed within the current PhD project. It will also describe the structure of the data collection consultations that were carried out with the recruited participants. As will be described in detail below, the project consisted of one cross-sectional study and one longitudinal, intervention study. All data collected in the cross-sectional study was also collected in the intervention study, however, the intervention study had additional types of data collected.

Further to this, the selection and optimisation of the various techniques used for the collection of data from participants will be described. First, the biomolecular methodologies will be discussed, followed by the description of the anthropometric and vascular measures of health, which will be followed by a description of the measures used in the mental health assessment of participants. As the current project consists of just one cross-sectional study and one longitudinal, intervention study, the current methodology chapter will describe in depth all of the methods used and then each specific method will be briefly referred to as appropriate elsewhere in this thesis.

2.1.1 Ethics

The current research project was approved under the Cardiff School of Health Sciences ethics committee. All participants completed informed consent forms, and ethical approval was granted by Research Ethics Committee; thus the study conforms to the principles outlined in the Declaration of Helsinki. Participants consented to taking part in up to two consultations of up to 1 hour each, before and after an eight week exercise programme. They consented to the taking of a venous blood sample, to being subject to non-invasive measures of health including blood pressure, weight, height, waist circumference and vascular stiffness, and to completing mental health questionnaires. They also consented to
the samples of blood being subjected to analytical experiments via which the benefits of exercise within blood cells could be characterised.

2.1.2 Inclusion criteria for the cross-sectional study

In order to be eligible for the cross-sectional ‘arm’ of the current research project, all participants were required to be 18 years of age or older, capable of providing verbal and written informed consent, and not pregnant. Participants also needed to be healthy in the sense that they were not currently receiving any primary care for chronic ill health or disability. \( n = 55 \) of the participants were staff of Cardiff Metropolitan University who signed up to undergo a free health ‘MOT’ check on the University premises, set up as part of the University’s Health and Well-Being sub-committee initiative. \( n = 65 \) of the participants were the baseline readings of those participants that signed up to the longitudinal, intervention study (see section 2.1.5).

2.1.3 Recruitment process of the cross-sectional study

Those participants who were recruited via the staff health ‘MOT’ on to the study had responded to an email that was sent University wide, advertising the health ‘MOT’. Those who expressed an interest in having a health ‘MOT’ were sent a participant information sheet detailing the health ‘MOT’ and were asked if they were willing for the data accrued to also be used for research purposes. If they were willing, they signed a consent form on the day of the health ‘MOT’ consultation.
2.1.4 Overview of consultations – cross-sectional study

Consultations were each allotted a period of one hour. Participants were asked to fast for four hours on the day of the consultation, and to abstain from caffeine and tobacco products on the day of their consultation, to not have any alcohol for 12 hours, and to not have carried out any strenuous exercise for 24 hours prior to their consultation. Were participants not able to provide a blood sample, they were asked to refrain from having a large meal in the four hours preceding the health consultation, rather than being asked to fast for four hours.

Participants had height, weight and waist circumference measured (see section 2.3 on anthropometric measurements). Blood pressure measurements were then carried out. Following blood pressure measurements, participants underwent two measurements of arterial stiffness; namely pulse wave analysis (to derive central blood pressure and augmentation index) and pulse wave velocity (see section 2.4 on vascular/haemodynamic measurements). \( n=65 \) of the participants were provided with questionnaire booklets that contained quantitative measures of mental health (see section 2.5).

Finally, a 16ml sample of venous blood was obtained from each participant. All samples were obtained via a trained phlebotomist, using venepuncture at the ante-cubital vein with minimal tourniquet. Three Vacuette® vacutainers (Greiner Bio-one, Gloucestershire, UK) of blood were taken from participants; 1 x serum separator tube (SST) (6ml), 1 x heparin (6ml), and 1 x potassium oxalate (4ml). Blood samples were centrifuged for fractionation immediately upon collection, except the SST tube, which was fractionated after 30 minutes rest (see section 2.2.4 on blood fractionation and subsequent biomolecular analyses).

2.1.5 Inclusion criteria and recruitment process for the intervention study

Sedentary participants were recruited on to the intervention ‘arm’ of the research project via a collaborative partnership between Cardiff Metropolitan University and Groundwork Wales
LTD, and their affiliates (many of which are encompassed within the Valleys Regional Park initiative which aims to promote activities related to the environment across the south Wales valleys (Valleys Regional Park, 2013)). These partners were host institution and external partner, respectively, for the ESF KESS grant which funded the current PhD project. This arrangement facilitated collaboration with Communities 1st, the British Trust for Conservation Volunteers (BTCV), Cardiff Council’s Health and Well-Being sub-committee, Let’s Walk Cymru, Ramblers, Coed Lleol/Small Woods Association, Interlink RCT, and others that are involved in the promotion of sustainable living and physical activity in south Wales.

The aims and objectives of the current research project were disseminated to the above organisations, and to Cardiff Metropolitan University’s own Health and Well-Being sub-committee. The organisations mentioned were all involved in running either green-exercise walking programmes or pedometer challenges (whereby staff were encouraged to increase their number of steps per day in order to meet the British Heart Foundations recommended 10,000 steps per day, primarily by active commute and lunchtime walks in natural, green settings). Each time a new walker joined an established walking group under the auspices of the above organisations, the individuals concerned were evaluated to determine whether they fit the project’s inclusion criteria. If they were suitable, the respective walk leader or health and well-being coordinator would describe and explain the research project and ask the individual if they would like to take part. The contact details of those individuals who did wish to participate in the current study were then passed on to the current research project. Following this, individuals were provided with information on the project and their potential involvement. Finally, informed consent (see above; section 2.1.1) was obtained and the consultations sessions were organised.

In order to be eligible for the intervention ‘arm’ of the current research project, all participants were required to be 18 years of age or older, capable of providing verbal and written informed consent, and sedentary but about to embark on either a green-exercise walking programme, or a pedometer challenge. Participants needed to be literate in English in order
for them to be able to complete the mental health questionnaire. Participants also needed to be healthy in the sense that they were not currently receiving any primary care for chronic ill health or disability.

In order to ensure that participants didn’t introduce a large amount of heterogeneity into the sample population and to ensure that they were suitable to answer the research question, all participants included were:

- Between the age of 18 and 85
- Literate
- Not pregnant
- Not physically active at the beginning of the project
- Not immobile/physically disabled, or suffering from any health problems that would affect their walking ability/mobility
- Not a current hospital inpatient
- Not receiving any psychiatric health care
- Not mentally incapacitated

2.1.6 Overview of consultations - intervention study

Two data collection consultations took place, each approximately eight weeks apart. The first consultation took place when the participant was sedentary; the second took place eight weeks into the green-exercise programme (see Figure 2.1 for an overview). The study was conducted between the months of April and December over three consecutive years.

Consultations were each allotted a time of 45 minutes. Both consultations took the same format (the first consultation differing slightly in that it also included a verbal description of the project and the signing of consent forms and blood donation forms). Participants had been instructed to abstain from caffeine and tobacco products on the day of their consultation, to not have any alcohol for 12 hours, and to not have carried out any strenuous
exercise for 24 hours prior to their consultation. Finally, participants had been instructed to be at least 4 hours fasted on the day of their consultation. Were participants not able to provide a blood sample, they were asked to refrain from having a large meal in the four hours preceding the health consultation, rather than being asked to fast for four hours. All consultations took place in the morning.

Participants were first provided with a personal medical history questionnaire, a mental health questionnaire (see section 2.5 on mental health measurements) and a diet questionnaire; together these were estimated to take 30 minutes to complete. Participants were instructed to fill the forms out in their own time but it was stipulated that they needed to complete them within 48 hours of the time and date of the consultation in which they were given them.

Following this, participants' height was measured, they were then weighed and had their waist circumference measured (see section 2.3 on anthropometric measurements). Blood pressure measurements were then carried out. Following the blood pressure measurements, participants underwent two measurements of arterial stiffness; namely pulse wave analysis (to derive central blood pressure and AIx readings) and pulse wave velocity (see section 2.4 on vascular/haemodynamic measurements).

Finally, a 16 ml sample of venous blood was obtained from each participant. All samples were obtained via a trained phlebotomist, using venepuncture at the ante-cubital vein with minimal tourniquet. Three Vacuette® vacutainers (Greiner Bio-one, Gloucestershire, UK) of blood were taken from participants; 1 x serum separator tube (SST) (6 ml), 1 x heparin (6 ml), and 1 x potassium oxalate (4 ml). Following collection, blood was kept on ice until it could be taken to the lab to be fractionated. The time that samples were kept on ice varied depending on where the consultations took place. In all cases, samples were taken to the blood for fractionation from between 30 minutes and 5 hours of collection (see section 2.2.4 on blood fractionation and subsequent biomolecular analyses).
Recruitment: Healthy but sedentary

Regular participation in green-exercise

Baseline consultation

Mental health questionnaire
Anthropometric measures of health
Vascular haemodynamic measures
Blood sample

8 weeks consultation

Figure 2.1 Diagram displaying the structure of the consultations in the intervention study over the 8-week period.
2.2 Biomolecular and biochemical methods

2.2.1 Preliminary in vitro investigations – THP-1 monocytic cells as a ‘model system’ for primary leukocytes

Prior to the recruitment of participants on to the studies of the current PhD research project, a series of in vitro experiments was first run in order to determine which laboratory methodologies and techniques would be most suitable for addressing the biomolecular arm of the research project. These experiments also allowed for the validation and optimisation of reagents prior to using human-derived tissue samples.

For these investigations, cultured cells from the THP-1 cell line (obtained from the Health Protection Agency Culture Collections (Salisbury, UK)) were used; THP-1 cells are generally considered to be a model system for investigating monocytes and macrophages in the cardiovascular system (Qin, 2012). THP-1 cells are human monocyte cells which were derived from the peripheral blood of one year old acute monocytic leukaemia patient (Tsuchiya et al., 1980). The passage number refers to the number of times the cell sample has been sub-cultured, and all experiments were conducted using THP-1 cells that had not exceeded a passage number of 25. Cells were passaged when growth was approximately 80% confluence which was typically after a 48 hour growth period.

THP-1 cells were maintained in RPMI-1640 media (Sigma Aldrich, Dorset, UK) following its supplementation with 10% foetal calf serum, 1% non-essential amino acids, 1% sodium pyruvate and 1% penicillin streptomycin mix. The cells were cultured at a temperature of 37°C in a humidified atmosphere with 5% CO₂ to encourage an optimal rate of proliferation. Trypan Blue solution (Sigma Aldrich, Dorset, UK) was used to determine the viability of the cells. The method works on the principle that live cells possess an intact cell membrane and are therefore unable to absorb the blue dye solution. However, apoptopic cells have damaged membranes and therefore the cytoplasm is able to absorb the dye and will hence appear blue under light microscopy. This allows for the estimation of the number of
dead cells present in a sample of viable cells (Sigma Aldrich, 2014). Cells were incubated with 0.4% w/v Trypan Blue for 10 minutes. Following incubation, the sample was pipetted on to a haemocytometer slide and observed using a microscope. The viable cells and non-viable cells were counted and the percentage of stained (or non-viable) cells against the number of non-stained (or viable) cells was determined. The cells were only sub-cultured prior to an experiment if cell viability was above 95%.

2.2.2 Treatment of cells with Rosiglitazone as an ‘exercise mimetic’

Following sub-culturing of cell samples for each investigation, equal volumes of cells were either left untreated or were treated with the agent Dimethyl Sulfoxide (DMSO) to act as a negative control. The experimental samples were treated with the RSG. RSG (discussed in Chapter 1) is a TZD class drug that was previously frequently prescribed as an antidiabetic drug (Lebovitz et al., 2001, Raskin et al., 2001). RSG works by binding to ligand-activated PPARγ in a variety of cell-types including leukocytes (specifically monocytes), where it exerts its effects via regulation of PPARγ target genes. RSG in the present investigations was used as an ‘exercise mimetic’ - a method of activating similar cell-signalling effects in the cells as a low-intensity exercise programme has been observed to do, i.e. activating PPARγ and regulating the mRNA gene expression of proteins involved in beneficial processes such as the reverse cholesterol transport system and paracrine control of chronic inflammation (Butcher et al., 2008, Thomas et al., 2012, Yakeu et al., 2010).

In order to confirm that PPARγ-mediated effects on leukocyte mRNA expression of the genes of interest (see section 2.2.6 below) were also seen at the protein (and therefore the functional) level, cell surface expression of CD36 in RSG-treated and control samples was assessed. This was achieved using Fluorescence Activated Cell Sorting (FACS) flow cytometry; which uses light scatter technology to carry out simultaneous quantitative measurement of multiple physical characteristics (such as the size and granularity) of
individual cells, or of the expression of proteins recognised by fluorescently-labelled antibodies. FACS is a laser-based technology which is used in biomarker detection, cell counting and cell sorting. The flow cytometer device is composed of three systems. These are the fluidics, the optics and the electronics. The fluidics component transports the molecules in a fluid stream to the laser beam. The optics system is composed of lasers which illuminate the molecules in the fluid stream as they pass the lasers. Optical filters direct the resulting light signals to the appropriate detectors. The electronics system then converts the detected light signals into computer-literate electronic signals. Molecules moving past the laser beam cause laser light to be scattered and the detectors then produce electronic signals proportionate to the optical signals that are striking them. Parameters are set up in order to collate data on events that fall within specific characteristics (abcam, 2010).

RSG treatments were conducted at RSG concentrations of 1µM (i.e. addition of 2µl of stock 1mM RSG solutions to 2ml THP-1 samples). DMSO treatments were conducted at 0.1% v:v concentration of DMSO, therefore, the volume of DMSO being used for each sample was matched to the volume of RSG used in the sample for which the DMSO was a control.

For this investigation, THP-1 cells were treated with DMSO or RSG, and left to incubate for 24 hours. Following the incubation, cells were seeded at various cell counts in order to provide samples with varying cell concentrations. These cell counts were 0.8 x 10⁶, 1.0 x 10⁶, 1.5 x 10⁶, and 10 x 10⁶ /ml. Each cell concentration was prepared in triplicate.

RNA was extracted from samples (as described in section 2.2.5.2); samples were analysed for quantity of RNA (as described in section 2.2.5.3) and then converted to cDNA (as described in section 2.2.6.1). RT-PCR analysis was run on these samples using CD36 as the gene-of-interest, and GAPDH as the endogenous reference gene (as in the section 2.2.6.4; Semi-quantitative RT-PCR analysis). In parallel samples, CD36 surface protein levels were determined using FACS; cells were treated with RSG at a concentration of 1µM.
and left to incubate for 24 hours. Following the 24 hours, cells were split in order to obtain samples at cell concentrations of $0.1 \times 10^6$, $0.8 \times 10^6$, $3 \times 10^6$ and $10 \times 10^6$ /1ml.

Following the splitting of cells into experimental sample size, the samples were spun in PBS at 300xg for 10 minutes. The supernatant was removed and each pellet was resuspended in 100µl of FACS buffer (containing phosphate buffered saline (PBS), 0.5% bovine serum albumin and 2mM EDTA). Each sample then had 10µl of CD36-FITC antibody mixed into it. Samples were covered in foil and incubated at 4°C for 10 minutes. Following this, the samples were spun at 3000xg for 10 minutes. The supernatant was removed and the pellets were resuspended in 250µl of FACS buffer and transferred to glass FACS tubes and FACS analysis run (Beckman Coulter FC500, High Wycombe, UK). The baseline settings were established using negative control samples (i.e. un-labelled cells) to adjust the PMT voltages associated with the fluorescent parameter under investigation. Fluorescent intensity readings were acquired (~5000 events), and analysed using FlowCytomix pro 1.0 Software (eBiosciences, Hatfield, UK) for quantification of the cell surface protein expression of CD36.

Cell-populations of interest (i.e. intact, healthy monocytic cells) were identified based on combination of forward scatter (which reflects cell size) and side scatter (which reflects cell granularity) characteristics; meanwhile, fluorescent intensity via the CD36-FITC antibody combination reflects the number of CD36 protein molecules expressed on the surface of the cells.

**Results**

RSG treatment resulted in an up-regulation of CD36 mRNA in THP-1 monocytes suggesting a significant increase in the activation of PPARγ. As presented in Figure 2.2, the RSG-treated THP-1 cell sample sizes of 0.8 and 1.5 million cells per sample resulted in significant increases in the expression of CD36 ($P<0.05$) when compared to untreated control samples of similar cell volumes. As presented in Figure 2.3, consistent with mRNA expression detected by RT-PCR, the greatest increase in CD36 protein expression was detected in cell samples of 0.8 million cells and above.
Figure 2.2 RT-PCR analysis of changes in CD36 mRNA expression in RSG-treated THP-1 cells using different cell number samples. Varied cell number samples of THP-1 cells were incubated for 24 hours after RSG (1μM) treatment. CD36 mRNA expression was analysed via RT-PCR using GAPDH as an endogenous reference gene and DMSO-treated cells used as control. Control THP-1 cells expressed little detectable CD36 mRNA, however, cells treated with RSG (1μM) showed a marked increase in CD36 mRNA expression. Compared to control samples, the cell volumes of 0.8 and 1.5 million cells per sample produced a significant up-regulation of detectable CD36 (P < 0.05, Student's t-test) while the other two cell volume samples did not (demonstrated wide standard deviation). For subsequent analyses and RNA isolation, a target cell sample volume of 0.8-1.5 million cells/sample would be suggested based on these data. Results are expressed as the mean± SD of three replica experiments. * indicates statistically significant results (P < 0.05).
Figure 2.3 Flow cytometry analysis of changes in CD36 surface protein expression on RSG-treated THP-1 cells, in various cell number samples. Changes in CD36 surface protein expression on THP-1 cells with different cell number samples were analysed using FACS flow cytometry following treatment with RSG (1μM) and conjugating with CD36-FITC antibody. THP-1 cell numbers of 0.8, 3.0 and 10 million/sample rendered dramatically increased up-regulation of CD36 surface protein after RSG treatment. (A) Line graph shows changes in percent CD36 fluorescent intensity depending on number of cells present in samples after treatment with RSG. (B) Representative logarithmical histographical fluorescence expression of baseline control (unstained cells), negative control (untreated cells), vehicle control (DMSO-treated cells) and RSG-treated cell samples.
Discussion

Three of the four cell volumes used to assess the effect of RSG on CD36 expression demonstrated agreement when assessed via RT-PCR and FACS methodologies. Both methodologies demonstrated an up-regulation of CD36 expression following treatment with the exercise mimetic, RSG (see Figures 2.2 and 2.3). A Pearson’s bivariate correlation analysis of the RT-PCR and FACS data demonstrated a significant and positive relationship; \( r = 0.886, \ P < 0.05 \). These data suggest that the RSG-induced increase in the transcriptional up-regulation of CD36 correlated with cell surface protein expression of CD36. Thus, in the current in vitro study, a detectable increase in the transcription of a gene was correlated with a functional change in that gene at the protein level.

It is important to note that FACS methodology requires that samples are not frozen prior to use in the analysis, whereas for RT-PCR samples can be frozen following the many steps involved in preparing the samples for analysis. In the current in vivo study, data collection was required to fit in around participant’s everyday lives and at the time of determining laboratory techniques and methodologies, it was not certain what time of day the participant consultations would take place. Since there appears to be agreement between the mRNA expression of at least one of the genes of interest with its expression at a cell surface (and therefore functional) level, it is concluded that RT-PCR would be most appropriate in the current study due to the inflexibility afforded by employment of the FACS technique.

2.2.3 Blood collection

As an objective of the study was to investigate the impact of exercise on gene expression patterns in circulating leukocytes (which has been shown to have consequences with regard to several relevant clinical outcomes (Yasmin et al., 2005, Chawla et al., 2001)), fasting venous blood was collected from participants, via venepuncture from the antecubital vein, using minimal tourniquet. Blood was collected into Vacuette (Gloucestershire, UK) heparin
tubes (6ml capacity), serum separator tubes (SST) (6ml capacity) and oxalate tubes (4ml capacity), to full capacity in order to ensure the blood was at the optimal ratio of blood: clot-activator (SST) or blood: anti-coagulant (heparin tubes and oxalate tubes). Immediately following collection, all tubes were gently inverted manually eight times in order to ensure thorough mixing of blood with the clot-activator or anti-coagulant present in the tube. The tubes were then rested upright, either on ice or in a refrigerator at 4°C, except for the SST tubes which were kept upright and at room temperature. When participant data collection was off-site, the length of time between blood collection and transporting it to the laboratory to begin the fractionation process varied, but was always between 1 and 5 hours. During this time, all samples were kept at optimal conditions, as described above. When data collection occurred on-site, samples were invariably in the laboratory and undergoing the initial stages of the fractionation process within 3.5 hours of blood collection. All blood collected in to the SST tubes were incubated for 30 minutes at room temperature in order to clot before any agitation, as per the manufacturers’ guidelines.

2.2.4 Blood fractionation – serum, plasma and mixed leukocyte samples

To obtain serum and plasma from the blood samples, the oxalate and SST vacutainers were spun at a speed of 400 relative centrifugal force (RCF) for 10 minutes at 4°C in a Hettich Rotina 380R (Buckinghamshire, UK) centrifuge. Serum samples were aliquoted into polypropylene microcentrifuge tubes (Invitrogen, Paisley, UK) from the SST tube, and plasma samples were aliquoted into polypropylene microcentrifuge tubes from the oxalate tube. Both the serum and plasma samples were stored in a -80°C freezer. Each sample type was stored as two aliquots, as a method of limiting repeated freeze-thaw cycles as much as possible.

In order to obtain a sample of mixed leukocytes, Histopaque-1077 (Sigma Aldrich, Poole, UK) was used as a separation medium. It was added to a 15ml centrifuge tube, following
which, blood from the heparin tube was added to it via gently pipetting the blood down the inside of the centrifuge tube so that it formed a layer on top of the Histopaque-1077 solution. The Histopaque-1077 to blood ratio was 1:1 v:v. The centrifuge tube was then spun at 400 RCF for 30 minutes, at 4°C.

Following centrifugation, samples were observed to have partitioned into four layers (see Figure 2.4). A plasma layer had formed on the top which was aliquoted into two polypropylene microcentrifuge tubes, and stored in the -80°C HTA freezer, as per the university’s HTA guidelines. The ‘buffy layer’ interface (containing mixed leukocytes) was transferred to a centrifuge tube, using a Pasteur pipette. To this centrifuge tube, 10ml of isotonic PBS was added and mixed, via gentle aspiration. The centrifuge tube containing the buffy layer and PBS was then centrifuged at 250 RCF for 10 minutes, at 4°C. Following the spin, the supernatant was discarded and the cell pellet was re-suspended in 5ml of isotonic PBS and mixed via gentle aspiration. This was then centrifuged at 250 RCF for 10 minutes, at 4°C. These two wash steps were then repeated. The cell pellet was finally re-suspended in 1ml of Trizol® Reagent (Invitrogen, Paisley, UK) and stored at -80°C.
Figure 2.4 displaying partitioning of heparin Vacuette blood sample into four layers following addition of Histopaque-1077 to whole blood and subsequent centrifugation, Cardiff Metropolitan University Research Laboratories.
2.2.5 Isolation of RNA

2.2.5.1 Selection of optimal RNA/cDNA isolation methodology

An investigation was run in order to determine whether a technique that requires less manual input during the RNA extraction process (Miltenyi Biotec [Surrey, UK] “µMACS” mRNA-to-cDNA isolation kits) yields enough mRNA and cDNA to run efficient RT-PCR experiments.

µMACS Column technology allows for the direct isolation of mRNA, without the need for prior total RNA preparation. Cell separation is achieved by retaining the magnetically labelled mRNA within a µMACS column situated within the magnetic field of a MACS Separator unit.

Venous blood samples were collected as described previously (section 2.2.3), buffy coat samples were fractionated from the whole blood (section 2.2.4), and also from cultured THP-1 cell samples. These mixed leukocyte samples then underwent mRNA isolation using µMACS mRNA isolation kit, according to the manufacturer’s instructions. Briefly, cells were lysed with 1ml of Lysis/Binding Buffer and vigorously vortexed for 5 minutes. The lysate was cleared by applying it to a LysateClear Column, which was placed in centrifuge tube and spun at 13,000xg for 3 minutes. 50μl µMACS Oligo (dT) Microbeads was added to the cleared cell lysate (which causes binding to the target poly-A tail of mRNA) and the solution was then applied to µMACS column which was placed in the magnetic field of the thermoMACS separator (a permanent magnet housed on a stand). Subsequently, magnetically labelled mRNA was isolated within a ‘µ Column’ placed in the MACS separator. It was then washed twice with 200μl of Lysis/Binding Buffer and then washed four times with 100μl of Wash Buffer, in order to remove proteins, rRNA and DNA. Purified mRNA was then eluted from the column with 50μl of pre-heated (70°C) Elution Buffer, in order to undergo further applications.
In order to then convert mRNA into cDNA, 20μl cDNA synthesis Enzyme Mix resuspended in buffer was applied to the column for reverse transcription. Following thorough washing of the enzyme-mix components with 100μl Wash Buffer, and an addition of 20μl cDNA Release Solution, newly-synthesized cDNA was eluted with 50μl cDNA Elution Buffer for downstream analysis.

At the mRNA and cDNA stages, samples were analysed spectrophotometrically using Nanodrop analysis (as described in section 2.2.5.3) in order to determine quantity of RNA and cDNA, respectively. Following this, the cDNA samples derived from the Miltenyi Biotec μMACS mRNA-to-cDNA isolation kits and cDNA samples derived using traditional methods which include the Trizol Reagent/chloroform and Applied Biosystems® High-Capacity cDNA Archive Kit techniques (described in sections 2.2.5.2 and 2.2.6.1.) were used to run RT-PCR experiments (see ‘Semi-quantitative RT-PCR analysis’ section; 2.2.6.4). This allowed for comparison of RNA yield between methodologies and comparison of the efficiency of RT-PCR assay between methodologies. The CD36 gene was used as a model.

**Results**

Since a low value of $C_T$ correlates with a higher cDNA yield, Figure 2.5 demonstrates that μMACS One-step cDNA kit produced higher yields of cDNA than the Trizol reagent method and μMACS mRNA isolation column method. There was no significant difference between μMACS One-step cDNA kit and the Trizol reagent method for CD36 and GAPDH mRNA yields ($P > 0.05$, Man-Whitney Test), whereas significant differences were observed using μMACS mRNA isolation column compared to the former two ($P < 0.05$, Two-Sample T-Test in both cases).
Figure 2.5 displaying data obtained from comparing RNA isolation and cDNA synthesis protocols for THP-1 cells. The graph illustrates mean C_T values of CD36 quantified in triplicate RT-PCR assays. Among three different methods, CD36 cDNA yields appear to be significantly higher levels in both µMACS One-step cDNA kit and Trizol reagent method than that produced from µMACS mRNA isolation column, as indicated by lower C_T values ($P<0.05$, Two-Sample T-Test). * indicates statistically significant results. Error bars indicates SD.
Discussion

As can be seen in Figure 2.5, the traditional Trizol reagent/chloroform method and the µMACS One-step cDNA kit produced the lowest $C_T$ values and therefore the highest RNA yields. These two methods produced significantly greater RNA yields than the µMACS mRNA isolation column, however, the µMACS One-step cDNA kit produced the greatest RNA yield of all three methods. This procedure also minimises the risk of losing any sample, and once the skill is mastered it would also be more time effective. However, it is relatively expensive when compared to the Trizol reagent/chloroform method, and the Trizol reagent method does produce comparable results. As the Tri reagent method is cost effective and an efficient method of yielding RNA, and subsequently cDNA, for RT-PCR assays, it was deemed appropriate to use this methodology in the current in vivo study.

2.2.5.2 Phenol/chloroform extraction of RNA

Within a maximum period of three months of storage in the freezer, the leukocyte samples stored in Trizol Reagent were removed from the -80°C freezer and left to thaw at room temperature. Once thawed, they were vortexed for approximately 5 seconds and then incubated for a further 5 minutes, at room temperature. 200 µL of chloroform was added to the sample and the tube was then shaken vigorously by hand, for 15 seconds. Samples were then incubated for a further 3 minutes before being spun at 12,000 RCF for 15 minutes, at 4°C. This resulted in the separation of the sample into three phases: the phenol/chloroform phase containing proteins and lipids (lower phase), the interphase containing DNA and the upper aqueous phase containing RNA (see Figure 2.6).

The aqueous phase was transferred into a new polypropylene microcentrifuge tube, to which 500 µL of isopropanol was added, and mixed via gentle aspiration. The solution was incubated for 10 minutes at room temperature and then spun (12,000 RCF; 15 minutes; 4°C) to obtain an RNA pellet. Following this, the supernatant was removed, and the RNA pellet
was washed in 1ml of 75% ethanol (ice cold), mixed using the vortex for approximately 5 seconds, and then spun at 7,500 RCF for 5 minutes, at 4°C. Immediately following this, a second spin was conducted at 12,000 RCF for 5 minutes, at 4°C. The ethanol was removed via pipette and the remaining RNA pellet left to air dry for ~30 minutes, with the polypropylene tube lid off. Once dry, the RNA pellet was re-suspended in 50µl of ddH$_2$O and stored on ice until quantification of RNA, which was carried out on the same day in all cases.

Figure 2.6 displaying samples separated into three layers following mixing of the leukocyte sample suspended in Trizol Reagent, with chloroform (Suckale, 2008).

2.2.5.3 RNA quantification

The RNA concentration of each sample was quantified, and its RNA purity was assessed by determining the ratio of its absorbance at 260:280nm and 260:230nm using a Thermo Scientific Nanodrop spectrophotometer (Delaware, USA). To ensure there was a high enough concentration of RNA for samples to be suitable for use in semi-quantitative PCR experiments, only samples with a concentration of ≥ 5ng/µL were used for further analysis. With regard to absorbance ratios at 260nm:280nm, only samples of ratio > 1.8 were deemed suitable for use, while with regard to absorbance ratios at 260nm:230nm, only samples with
a ratio > 2.0 were deemed suitable for use (any ratios lower than 1.8 and 2.0, respectively, indicate the presence of contaminants which absorb at either 280nm (e.g. un-removed sample proteins) or 230nm (e.g. un-removed phenol from the Trizol reagent), respectively).

### 2.2.6 RT-PCR-based gene expression assays

#### 2.2.6.1 Conversion of RNA to cDNA

In preparation for analysis of the samples using semi-quantitative PCR, RNA samples were reverse-transcribed to cDNA samples using an Applied Biosystems® High-Capacity cDNA Archive Kit (Warrington, UK). Before reverse transcription, samples were first diluted from their starting concentration (as determined during the RNA quantification stage), as displayed in Table 2.1. Samples were diluted with ddH₂O to workable and common RNA concentrations (as displayed in Table 2.1), all samples were diluted to final volumes of 20µL.

**Table 2.1 displays the RNA and cDNA concentrations that samples were diluted to depending on their initial starting concentration**

<table>
<thead>
<tr>
<th>Range of starting concentrations from Nanodrop (ng/ µL)</th>
<th>RNA concentration after dilution with ddH₂O (ng/ µL)</th>
<th>cDNA concentration following cDNA conversion process (ng/ µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;5, &lt;10</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>&gt;10, &lt;15</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>&gt;15, &lt;25</td>
<td>15</td>
<td>7.5</td>
</tr>
<tr>
<td>&gt;25, &lt;30</td>
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<tr>
<td>&gt;200</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

In order to prepare the samples for cDNA conversion, the manufacturer’s guidelines were adhered to, briefly; 20µL of RNA was added to 20µL of 2X Reverse Transcriptase (RT)
Master Mix (which was prepared as per the manufacturer's guidelines). Samples were mixed via pipette aspiration and then using a vortex machine, for approximately 5 seconds. Polypropylene PCR tubes containing the mixed solution were then transferred to an Applied Biosystems 96-Well GeneAmp® PCR System 9700 machine (Warrington, UK) and the following thermal cycling was applied: Step 1: 10 minutes at 25°C; step 2: 120 minutes at 37°C; step 3: 5 seconds at 84°C and step 4: 4°C for ∞. Once the thermal cycling steps were complete, the samples were retrieved from the machine and stored in a -20°C freezer until later analysis. Following this step, it was assumed that the cDNA concentration would be approximately half the RNA concentration that was achieved in the dilution step that followed the Nanodrop analysis, due to the dilution with the 2X RT Master Mix (see Table 2.1).

2.2.6.2 Design of primer pairs for use in semi-quantitative PCR

Semi-quantitative real-time PCR (RT-PCR) was performed against the following genes: CD36, ABCA1, MMP9, and GAPDH. CD36 and ABCA1 have previously been investigated at an mRNA expression level in the context of an exercise programme and CV risk (Butcher et al., 2008). Circulating levels of MMP-9 have been investigated following a treadmill-based exercise programme, however, the mRNA expression of MMP-9 does not appear to have been investigated before in a healthy population, in the context of exercise (Roberts et al., 2007, Roberts et al., 2006). GAPDH served as the endogenous reference/housekeeper gene as its expression remains relatively stable and consistent under normal and pathophysiological conditions (Dheda et al., 2004).

GAPDH, ABCA1 and CD36 primer pair sequences that had previously been used within the research group to which the current project is affiliated were used (Butcher et al., 2008, Thomas et al., 2012). When designing primer pair sequences for MMP-9, sequencing of primer oligonucleotides (Sigma Aldrich, Poole, UK) was carried out using OligoPerfect Designer (Invitrogen, Paisley, UK); the respective sequences are displayed in Table 2.2.
In the designing of the primer pairs, the following guidelines were adhered to: primer sequences should have comparable melting temperatures within a range of 55-60°C, and an identical (or as close as possible) % GC content. Also, the last 5 nucleotides at the 3’ end of the primer should not contain more than two GC bases. Further to this, it should be ensured that sequences of identical nucleotides (especially G’s) were avoided; this is in order to minimise the risk of primer dimer formation.

Finally, all primer sequences were screened against the National Center for Biotechnology Information (NCBI) (Nagase et al., 2006) database of human genomic sequences using the Basic Local Alignment Search Tool (BLAST) search tool, in order to ensure that they were specific only to the desired amplicon within the gene in question in each case. Once the sequences were finalised, primers were obtained as desalted, lyophilised oligonucleotide samples (Sigma-Aldrich Ltd, Poole, UK), and were reconstituted in sterile, RNase-free water (ddH₂O) in order to provide a stock solution of 100 µM. Primers were then stored at -20°C.

2.2.6.3 Primer validation and optimisation analyses

Working concentrations, for both the forward and reverse primers of each of the genes being quantified, were optimised as follows. RT-PCR experiments were run with varying forward and reverse primer concentrations, and optimal primer concentration combinations were selected based on the concentrations which resulted in the highest ΔRn values and the lowest threshold cycle (Cₗ) values (see Figure 2.7). 

ΔRn is calculated as: \( (R_{n+}) - (R_{n-}) \), and is the magnitude of the reference dye signal generated by each pre-determined set of PCR conditions.
Also, for each PCR reaction, a dissociation step was included to confirm that the reaction involved the amplification of one single PCR product (see Figure 2.8).

*Figure 2.7 displaying optimisation of MMP-9 primer pair concentration.* Sample with highest $\Delta R_n$ and lowest $C_T$ is highlighted and was used in all future analyses. Optimisation carried out using cDNA derived from THP-1 cells.
Figure 2.8 displaying dissociation curve obtained from primer optimisation of MMP-9. Sharp peak at ~83°C, suggesting a lack of primer dimer products and therefore suggesting specificity of primers. Optimisation carried out using cDNA derived from THP-1 cells.

Figure 2.9 displaying RT-PCR relative fluorescence vs. cycle number. Amplification plots are created when the fluorescent signal from each sample is plotted against cycle number; therefore, amplification plots represent the accumulation of product over the duration of the real-time PCR experiment. The samples used to create the plots are a dilution series of the target DNA sequence. [Taken from Life Technologies (2012)].
2.2.6.4 Semi-quantitative real-time-PCR analyses

Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) is currently the most sensitive technique available for the detection and quantitative analysis of mRNA gene expression. The current project employed SYBR-Green chemistry for PCR analysis. It works due to the action of the SYBR-Green dye in binding double-stranded DNA and emitting fluorescent light upon excitation. As PCR product accumulates with amplification of cDNA template, fluorescence increases (see Figure 2.9) (Life Technologies, 2012).

During each PCR reaction, SYBR-Green fluorescence, and hence amplification of the selected amplicon, was monitored on an Applied Biosystems 7500 Real-time PCR system (Warrington, UK). Each reaction was carried out in triplicate. Each reaction consisted of 5µL of Fast SYBR® Green Master Mix (Applied Biosystems) $n_A$ µL of reverse primer, $n_B$ µL of forward primer, $n$ µL of ddH2O and 1µL of cDNA sample (diluted with ddH2O to a concentration of approximately 5ng/µL) and 10-(5+$n_A+n_B$). $n_A+n_B$ were dependent upon the results from the optimisations that were carried out on each individual primer for each gene that was quantified (as discussed in section 2.2.6.3). Therefore, individual PCR reactions were designed such that each well on a MicroAmp® Fast Optical 96-Well Reaction Plate (Life Technologies) contained 10µL of reaction mixture. Reactions were carried out in triplicate.

Upon complete loading of all wells with the required components for their respective PCR reactions (as described above), each plate was then centrifuged for 2minutes at 2000RCF, in order to mix the solution and to eliminate any air bubbles. The plate was then transferred to an Applied Biosystems 75000 Real-time PCR system for amplification. For each PCR reaction, a dissociation step was included to confirm that the reaction involved the amplification of one single PCR product (see Figure 2.10).
Also, prior to running individual participants' samples, validation experiments were performed in which cDNA abundance was varied (by serial dilutions of a single reference sample), and plotted (as a log input amount) versus the C<sub>T</sub> differences between gene of interest and endogenous reference gene in the resulting PCR reactions. If this resulted in a slope of approximately 0, this was taken to indicate that the amplification efficiencies were approximately equal for the 2 genes, and hence that semi-quantitative comparative analysis of the expression levels of the 2 genes was valid (see Figure 2.11). As can be seen in Figure 2.11, in the case of MMP-9 cf. GAPDH, a gradient of 0.0188 was obtained; this falls below the recommended threshold of ±0.1, and so it was concluded that this method of analysis WAS appropriate in this case. Similar results were obtained for the other two genes-of-interest (i.e. CD36 and ABCA1 – data not shown).

As in our research group's previous studies (Butcher et al., 2008, Thomas et al., 2012, Yakeu et al., 2010), relative expression levels of the target genes in the mixed leukocytes were calculated via the 2<sup>-ΔΔC<sub>T</sub></sup> formula, in which Δ represents the difference between C<sub>T</sub> values between individual experimental samples, and ΔC<sub>T</sub> represents the difference between C<sub>T</sub> values for the gene of interest and the endogenous reference gene, GAPDH, in each case.
Figure 2.10 displaying dissociation curve obtained from sample reactions using MMP-9 primers. Sharp peak at ~82°C, suggesting amplification of one PCR product. Dissociation curve obtained by running MMP-9 primer RT-PCR on whole blood sample derived cDNA samples.

Figure 2.11 depicting RT-PCR relative efficiency of MMP-9 and GAPDH RT-PCR primer efficiency run using serially-diluted cDNA derived from THP-1 cells, as described in section 2.2.6.
Table 2.2 displaying primer sequences of the genes of interest and the endogenous reference gene, as designed using OligoPerfect Designer

<table>
<thead>
<tr>
<th>Gene (Accession No.)</th>
<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36 (NM_001001548.1)</td>
<td>GGAAGTGATGATGAACAGCAGC</td>
<td>GAGACTGTGTGTGTCCTCAGCG</td>
<td>117</td>
</tr>
<tr>
<td>ABCA1 (NM_005502.2)</td>
<td>GCACTGAGGAAGATGCTGAAA</td>
<td>AGTTCTGGAAGGTCTTGTTCA</td>
<td>205</td>
</tr>
<tr>
<td>MMP9 (NM_004994.2)</td>
<td>TTGACAGCGACAAGAAGTGG</td>
<td>CCCTCAGTGGAACGGGTACAT</td>
<td>148</td>
</tr>
<tr>
<td>GAPDH NM_002046.3</td>
<td>CATTGACCTCAACTACATG</td>
<td>TCTCCATGGTGGAAGAC</td>
<td>209</td>
</tr>
</tbody>
</table>

Sequence analyses and alignments were performed using DNASTAR software (Lasergene, version 7; DNASTAR Inc., WI, USA). All sequences were obtained from NCBI databases (http://www.ncbi.nlm.nih.gov/).

2.2.7 Enzyme-Linked Immunosorbent Assay (ELISA)

In addition to the above investigations into the impact of the green-exercise intervention on gene expression (or, more accurately, mRNA expression), ELISA analysis was also run on IL-6 and MMP-9 in order to determine whether there was any effect of the green-exercise intervention on the circulating protein levels of these inflammation-associated markers, in the serum and plasma, respectively. The basic principle behind ELISA analysis involves a monoclonal primary antibody specific for the protein of interest (IL-6 and MMP-9 in the current study) being pre-coated onto a microplate, samples are then pipetted into the wells and if any of the protein of interest is present, it is bound by the immobilised antibody. Unbound substances are washed away and an enzyme-linked polyclonal secondary
antibody specific for the primary antibody is added to the wells. A second wash removes any unbound antibody-enzyme reagent, and substrate solution addition to the wells develops colour in proportion to the amount of the protein of interest that was bound in the initial step. Colour development is stopped and the intensity of the colour is measured (R&D Systems, 2012, R&D Systems, 2011).

2.2.7.1 Interleukin-6 (IL-6) ELISA analysis

IL-6, as discussed in Chapter 1 (section 1.2), is a cytokine that is normally expressed at 1pg/mL (with a range of 0.447-9.996pg/mL, in serum) (R&D Systems, 2011). It is present at sites of atherosclerosis and in CVD development (Jenny et al., 2002) and is associated with psychological stress (Kiecolt-Glaser et al., 2003, Lutgendorf et al., 1999). Interestingly, IL-6 levels have been seen to increase acutely in the immediate aftermath of each individual exercise bout, but it should be stressed that this effect is due to release of IL-6 from contracting skeletal myocytes rather than inflammatory cells such as monocytes (Pedersen and Febbraio, 2012). Thus, it is important to differentiate between acute increases in myocyte-derived IL-6 and chronic ‘baseline’ (i.e. not associated with any individual exercise bout) increases in monocyte-derived IL-6. With regard to relevance to the current study, it should be noted that that latter has been reported to improve following regular frequent participation in exercise (Oberbach et al., 2008).

IL-6 levels in the serum samples (see section 2.2.4) were analysed via ELISA, using R&D Systems Human IL-6 Quantikine High Sensitivity kits (Abingdon, UK). The assay was carried out as per the manufacturer’s guidelines, as follows:

Prior to loading the ELISA plate, all reagents and working standards were prepared as directed in the manufacturer’s guidelines. Whilst the reagents and working standards were being prepared, the serum samples were left to thaw at room temperature.
Prior to preparing the reagents and working standards, all components from the kit were brought to room temperature. The wash buffer, substrate solution, amplifier solution and IL-6 working standards were prepared as per the manufacturers guidelines. Following this, the serial dilutions of the standard were prepared, as per the guidelines. To each well 100µl Assay Diluent and then 100µl of each standard and sample was pipetted in to their respective well. The plate was then covered with an adhesive strip and incubated at room temperature for 2 hours, on a horizontal microplate shaker, set to 50 revolutions per minute (RPM).

Following incubation, the plate was washed. 200µl of IL-6 Conjugate was then pipetted into each well. The plate was covered with an adhesive cover and left to incubate at room temperature, for 2 hours, on a horizontal microplate shaker, set to 50 RPM.

The wash step was then repeated. Following this wash, 50µl of Substrate Solution was pipetted in to each well. The plate was covered with an adhesive cover and left on the bench-top, at room temperature, for 60 minutes. Following the incubation, 50µl of Amplifier Solution was pipetted in to each well. The plate was covered with an adhesive cover and incubated on the bench-top at room temperature for 30 minutes. Finally, following incubation, 50µl of Stop Solution was pipetted into each well. Within 15 minutes, the optical density of each well was determined using a microplate reader set to 490nm, with wavelength correction set to 690nm (TECAN Infinite 200 PRO, Switzerland).

2.2.7.2 Matrix Metalloproteinase-9 (MMP-9) ELISA analysis

MMP-9 expression at the circulating protein level was analysed in plasma samples collected using Heparin blood tubes (as described in section 2.2.3) via ELISA, using R&D Systems Human MMP-9 Quantikine kits (Abingdon, UK); the intention was that this could then be compared to mRNA expression levels in the leukocyte samples (as discussed earlier in section 2.2.6.4). As discussed in Chapter 1 (section 1.2.3), MMP-9 is an endopeptidase that
is involved in ECM degradation, and such a comparison is important because MMP-9 can be secreted from a variety of sources (leukocytes, endothelial cells, SMCs), and therefore confirmation is needed that plasma MMP-9 levels should be attributed to MMP-9 expression within (and secretion from) leukocytes. Levels of MMP-9 have been seen to decrease following an aerobic exercise programme, but this is yet to be observed in an exercise-only intervention in healthy adults. In plasma, the detectable range of MMP-9 using the R&D kit is 13.2-105 ng/mL (R&D Systems, 2012).

The assay was carried out as per the manufacturer’s guidelines and broadly followed the same format as that described for IL-6 in the previous section, with the exceptions that all participant-derived samples were diluted 40-fold, by mixing 10µl of the sample with 390µl of Calibrator Diluent RD5-10 (as directed in the guidance notes), and there was no Amplifier Solution.

Also, following the addition of the MMP-9 Conjugate, the incubation step was just 1 hour, not two. Also, the Substrate Solution only required a 30 minute incubation, not 60, and was incubated in the dark room in order to protect it from light, at room temperature.

Within 15 minutes of addition of the Stop Solution, the optical density of each well was determined using a microplate reader set to 450nm, with wavelength correction set to 570nm (TECAN Infinite 200 PRO, Switzerland).

2.2.8 Biochemical analyses of serum lipids, plasma glucose and plasma insulin

Following the aliquoting and freezing of serum and plasma from the participant-derived blood samples, one aliquot each of the serum and plasma samples was transported to the Diabetic Research Network Wales laboratories (DRNW, Swansea, UK) for total cholesterol, HDL-cholesterol, triglyceride, glucose and insulin analysis. HTA guidelines for the transportation of the samples were adhered to. Samples were aliquoted in volumes that left no sample remaining for storage/disposal by the DRNW laboratory.
2.2.8.1 Determination of Blood Lipids.

**Total-cholesterol analysis**

Total-cholesterol was measured in serum using an IL Test Cholesterol assay on an ILab300 Plus analyser (Instrumentation Laboratory, Warrington, UK).

**HDL-cholesterol analysis**

HDL-cholesterol was measured in serum using an IL Test HDL Cholesterol assay on an ILab300 Plus analyser.

**Triglyceride analysis**

Triglycerides were measured in serum using an IL Test Triglyceride assay on an ILab300 Plus analyser.

**LDL-cholesterol analysis**

Serum LDL-cholesterol levels were not measured directly, but were calculated using the triglyceride, total cholesterol and HDL-cholesterol levels. This was achieved using the Friedewald equation (Friedwald et al., 1972):

\[
[\text{LDL-cholesterol}] = [\text{Total cholesterol}] - [\text{HDL-cholesterol}] - \left(\frac{[\text{Triglycerides}]}{2.2}\right)
\]

2.2.8.2 Glucose analysis

Glucose levels were measured in plasma using an IL Test Glucose Oxidase assay on an ILab300 Plus analyser.

2.2.8.3 Insulin analysis

Insulin levels were measured in plasma using the Invitron immunochemiluminometric assay (Monmouth, UK) on a Berthold Plate Luminometer (Bad Wildbad, Germany), which
measures the concentration of insulin by measuring the amount of light emitted from the sample.
2.3 **Anthropometric measurements - cross-sectional and intervention study**

2.3.1 Weight

Weight was measured on a hard and even surface using Seca® electronic scales (Birmingham, UK). Participants were instructed to remove shoes and excess clothing, i.e. big cardigans, jumpers and coats. Weight was measured in kilograms, and recorded to one decimal place.

2.3.2 Height

Height was measured on a hard and even surface using a Seca® electronic stadiometer (Birmingham, UK). Participants were instructed to remove shoes and to stand upright. Height was measured in centimetres, and recorded to one decimal place.

2.3.3 Body Mass Index (BMI)

BMI was calculated using the formula;

\[
\text{BMI} = \frac{\text{weight in kilograms}}{\text{height in metres} \times \text{height in metres}}
\]

BMI was calculated in \( \text{kg/m}^2 \), and recorded to two decimal places (Keys et al., 1972).

2.3.4 Waist circumference

Waist circumference was measured in centimetres using a Holtain LTD (Pembrokeshire, UK) tape measure. The measurement was made according to the National Heart Lung and Blood Institute’s (National Heart Lung and Blood Institute, 1998) clinical guidelines for the identification and evaluation of overweight and obesity in adults. As per these guidelines, the circumference was measured at the mid-point between the lower border of the costal margin and the uppermost border of the iliac crest. In overweight people it can be difficult to locate these bony sites and in such a case, the measure was taken with the tape placed at
the same level as the navel (National Heart Lung and Blood Institute, 1998). Participants were instructed not to breathe in and measurements were made underneath clothes, against the skin.
2.4 Measures of vascular haemodynamics

2.4.1 Blood pressure measurements (seated and supine)

Blood pressure measurements were performed after participants had rested in the seated and supine position, respectively, for 10 minutes, in a quiet, temperature controlled room. Participants were asked to refrain from talking during the rest periods and during the taking of the measurements in order to ensure that the blood pressure readings could be considered to yield basal data. All measurements were carried out according to British Hypertension Society guidelines (Williams et al., 2004); blood pressure was measured at the brachial artery of the dominant arm of each participant, using a validated semi-automated cuff-oscillometric sphygmomanometer (O'Brien et al., 1996) (Omron, Milton Keynes, UK). All measurements were taken in duplicate in order to rule out any discrepancies. Any measurements that differed by more than 10 mm Hg were repeated a third time. Upon obtaining two recordings in which the two systolic and the two diastolic readings were within 10 mm Hg of each other, the mean values were used for the subsequent analyses. Seated measurements were taken prior to the seated pulse wave analysis (PWA) assessment (see section 2.4.3.2 below). Supine measures were made following the PWA assessment, prior to the pulse wave velocity (PWV) assessment (see section 2.4.3.4 below).

2.4.2 Arterial stiffness

As discussed in Chapter 1, measuring arterial stiffness assesses an individual’s vascular health, and it has been shown to be associated with ageing, disease, pharmacological intervention and exercise (Mattace-Raso et al., 2006, McEniery et al., 2007, Tanaka et al., 1998, McDonnell et al., 2013, Mitchell et al., 2004, de Luca et al., 2004, Kelly et al., 2001, Wilkinson et al., 2001b, O'Rourke, 1992a).

There are several methods available for the measurement of arterial stiffness (Mackenzie et al., 2002). These methods differ by the ease with which they can be operated and
transported, how invasive they are, and their relative cost. Also, the information that can be retrieved from each of the various methods differs. Some of the methods provide data on systemic stiffness (e.g. Alx), whilst others only provide information on the local stiffness (e.g. FMD, carotid intima-media thickness (cIMT), distensibility) and regional stiffness (e.g. aPWV, bPWV) of the vessel being studied. Therefore, it is vitally important that the various methods of measuring arterial stiffness are not used interchangeably when comparing data. As mentioned in the previous chapter, in a number of arterial beds aPWV has been identified as the gold-standard method for the measurement of large arterial stiffness (Laurent et al., 2006), with PWA-derived augmentation index (Alx) being a useful surrogate marker of arterial stiffness (Laurent et al., 2006) but more specifically as a measure of wave reflection (Mackenzie et al., 2002, O'Rourke and Gallagher, 1996). Therefore, the current study employed PWV and PWA-derived Alx as indices of arterial stiffness, using the SphygmoCor® (AtCor Medical, Australia) system. In order to carry out these measures, pulse pressure waveforms were obtained by using the widely employed method of applanation tonometry.

2.4.3 The measurement of vascular haemodynamics, using a non-invasive system

- the SphygmoCor system

2.4.3.1 Applanation tonometry

Applanation tonometry allows for the measurement of pressure within an anatomical structure. Historically, the measurement of ocular pressure was the first reported use of tonometry and the technique is still used in the detection of glaucoma in the eye (Stamper, 2011). The intra-ocular pressure is measured by the application of a gentle external force which acts to flatten the cornea. The technique is based upon the Imbert-Fick Law (Goldmann and Schmidt, 1957), $P = F/A$, whereby
“the pressure within a sphere (P) is approximately equal to the external force (F) required to flatten a section of the sphere, divided by the area (A) of the sphere which has been flattened”

The equation is then adjusted to allow for the thickness of the cornea and the force of the cornea which is forcing the applanation surface away from the eye (Goldmann and Schmidt, 1957).

In 1963, the theory and technique behind the use of applanation tonometry on the eye was applied to the measurement of arterial pulse pressure waves (Pressman and Newgard, 1963). This was an important advance in the non-invasive measurement of the arterial pressure pulse. The first non-invasive method of measuring the contour of the arterial pressure pulse was first designed in the 1800’s when the sphygmograph was designed and introduced by Vierordt (1855) and improved by Marey (1860) (see Figure 2.12). These first sphygmographs worked by covering the radial artery with a base plate which was connected to a clockwork-driven smoke drum, upon which the arterial pulse fluctuations were traced. The emergence of the sphygmograph allowed for the characterisation of essential hypertension (Mahomed, 1874). Mahomed, amongst other ground-breaking work, demonstrated the differences between the old and young arterial waveforms (Mahomed, 1872) (see Figure 2.13). At the time it was not entirely appreciated that the differing waveforms indicated the existence of a relationship between the arterial waveform, arterial stiffness, and CV risk. Much later, in 1963, was the first instance of tonometer-based arterial applanation, when Pressman and Newgard built a transducer that was able to determine the arterial pulse pressure wave shape. In 1989, Kelly et al (1989a, 1989b) devised the first modern-day tonometers; a non-invasive but accurate measure of pressure pulse waves that allowed for the documentation and quantification of age-related changes and disease from the contour of the pulse.

Such tonometers are now available commercially, and multi-unit arrays as well as single-unit hand held systems have been introduced. The multi-unit arrays are controlled by the
electronic system to which they are connected, placed in a position that straddles the artery and they are operated automatically with an automatic application of pressure. The single-unit hand held systems are simple to use and pencil-like in appearance. The sensor on the tonometer is held on the skin above the most appropriate section of the arterial pulse.

Arterial applanation tonometry was employed in the current study (as described by O'Rourke and Gallagher (1996) and Wilkinson et al. (1998)) via the use of a hand-held, transcutaneous tonometer; a pencil-shaped probe with a high-fidelity micromanometer (SPC-301; Millar Instruments, Texas, Houston) at its tip. The tip is made up of a 0.5 x 1mm piezoelectric crystal with a frequency response rate > 2 kHz. The tip is co-planar with a 7mm diameter which allows for direct applanation of the superficial artery being studied. The tonometer is rested on the skin at the relevant arterial bed, with a slight pressure. Applanation tonometry relies on a small part of the arterial wall (of the artery under study) being flattened (but not fully occluded) by being gently compressed against the underlying, proximal bone (see Figures 2.14 and 2.15), thus reducing circumferential pressure through the artery and enabling accurate detection of the pressure wave transmission underneath. The micromanometer sensor needs to be applied directly above the section of the artery that has the greatest pulsatility, allowing for the continuous recording of the arterial pressure pulse waveform travelling underneath.
Figure 2.12 Sphygmographs developed by Marey (O'Rourke and Avolio, 1992)

Figure 2.13 Difference in pulse waves recorded by Fredrick Akbar Mahomed, 1874

(Mahomed, 1874, O'Rourke, 1992b)

(A) Illustrates the first waveforms measured in young individuals.
(B) Illustrates the first waveforms measured in older individuals.
For example, at the wrist, the most pulsatile section of the radial artery is pressed against the radius bone, thus causing the strain and circumferential pressures to be equalised, and only the transluminal pressure remaining; resulting in only the arterial pulsatility pressure being characterised, creating a high-fidelity pressure waveform. If the artery is not sufficiently flattened against the bone then no signal from the artery is recorded. Similarly, if the pressure applied to the tonometer is too great, then the signal achieved is not reliable. When applanating an artery, large amounts of tissue (such as that which is seen in overweight and obese individuals) overlying the arterial bed is not optimal and causes the recording of pressure waveforms to be more difficult to obtain.

2.4.3.2 Pulse Wave Analysis (PWA)

Applanation tonometry results in the output of high-fidelity waveforms. Pulse wave analysis (PWA) was carried out upon the recorded waveforms, allowing for derivation of the central aortic pressure waveform, as described by O'Rourke and Gallagher (1996). PWA involves the analysis of waveforms recorded at the peripheral arteries, including the radial artery. This is achieved by using a pressure sensor to palpate the artery at its strongest signal, or at its greatest point of pulsatility. Using Fourier analysis, the inbuilt SphygmoCor software separates the averaged radial pulse wave that was recorded and splits it into its component harmonics. A central aortic waveform is reconstructed using a generalised transfer function to give rise to the central pressure waveform (see Figures 2.16 and 2.17). Figure 2.17 illustrates a typical aortic waveform that has been constructed from a peripheral arterial waveform by the SphygmoCor software. The calculation of a central aortic waveform obtained via the use of a generalised transfer function has previously demonstrated that the absolute difference between an actual central pressure and a central pressure that was estimated by transfer function, was less than 1mm Hg (Pauca et al., 2001). The generalised transfer function (developed and evaluated by O'Rourke and colleagues (O'Rourke et al., 1999, Pauca et al., 2001)) describes the properties of the arterial tree between the peripheral
site of applanation and the ascending aorta. The function was validated in 35 anesthetised patients prior to coronary artery bypass surgery (O'Rourke et al., 1999), and in 62 anesthetised patients undergoing cardiac surgery (Pauca et al., 2001).

Figure 2.14 Principle of applanation tonometry, taken from Mackenzie et al. (2002)

Figure 2.15 The Millar hand-held tonometer demonstrating applanation of the left radial artery, with the obtained waveform being demonstrated in the background
The pressure sensor is a hand-held tonometer (previously described), applied at the radial artery (see Figure 2.15). The arterial pressure waveforms are then analysed using the SphygmoCor® software. The device is calibrated to the seated brachial blood pressure that was obtained from the upper part of the dominant arm. The radial pulse wave trace is followed for two consecutive screens (or 10 heart beats) and then recorded (see Figure 2.16). The software processes the trace, creating one average reading from the ten consecutive beats recorded. To ensure validity and reliability, an operator index (indication of the reproducibility and quality of the obtained waveform) is computed and all readings falling outside the acceptable range are repeated. Further to this, a second acceptable recording is carried out, and an average of the two recordings is used for later statistical analysis. If the two PWA-derived Alx readings differ by more than 5% (Alx is expressed as a percentage), then a third reading is taken and an average of these two is used.

All measures in the present study were taken at the wrist on the right hand side of the body, with the participants seated and their arm extended in order to allow comfortable and easy measurement at the wrist.

In conclusion, therefore, the fundamental part of PWA is the retrieval of an accurate radial pulse waveform, from which all aortic pressure wave parameters associated with CV risk can subsequently be derived. In the context of this thesis, the most relevant parameters recorded from the waveform were central blood pressure (cBP), heart rate (HR), Alx, and HR adjusted Alx (Alx@HR75), which can all be derived with further analysis of the waveform.
Figure 2.16 Data recorded by the SphygmoCor system (version 8.1), displaying results obtained from PWA.

![SphygmoCor data display](image)

Figure 2.17 representing a typical aortic pressure waveform constructed by the SphygmoCor software. Taken from Wilkinson et al. (2000b)
2.4.3.3 Augmentation Index (Alx)

As shown in Figure 2.17, Alx is a measure of wave reflection and is recorded as the difference between the aortic pressure waves first ($P_1$) and second ($P_2$) systolic peaks, which is then recorded as a percentage of the pulse pressure (PP)

$$Alx \, (\%) = \left( \frac{(P_2 - P_1)}{PP} \right) \times 100$$

(Laurent et al., 2006, Mackenzie et al., 2002)

The first systolic peak is the maximum pressure that is expressed by the forward travelling wave, whilst the second systolic peak is the peak of the pressure generated from the combined influence of the forward and backward travelling waves. The difference between the two peaks is important because the second peak is an indication of the wave reflection and the difference reflects the degree to which central pressure is augmented by this wave reflection. A major site of wave reflection occurs at the aortic bifurcation and at other sites of impedance mis-match (i.e. sites where elastic arteries become more muscular) as the pulse travels along the arterial tree. Therefore, height is a factor that needs addressing when comparing groups as difference in height affects the distance that the reflected wave is required to travel.

It should be noted that since Alx is influenced by aPWV, the sites of impedance mis-match between elastic and muscular arteries and by the magnitude of wave reflection from the peripheries; as such, it is considered an indirect measure of arterial stiffness (Laurent et al., 2006). When measuring Alx, it is important to be aware that, in addition to height, HR and mean arterial pressure also influence Alx (Laurent et al., 2006). It is important to adjust for HR due to the fact that increases in HR shift the reflected pressure wave from systole into diastole via the shortening of ejection duration, and thus lowering of the height of the second systolic peak, and reducing the augmentation of central systolic pressure and Alx (Wilkinson et al., 2002b, Wilkinson et al., 2000b). Further to this, MAP and Alx have been observed to rise linearly following infusion with vasoconstrictor pharmaceutical interventions (Wilkinson et al., 2001b).
2.4.3.4 Pulse Wave Velocity (PWV)

PWV is regarded as the gold-standard measure for the assessment of regional arterial elasticity (Laurent et al., 2006). Using the SphygmoCor system and applanation tonometry, carotid-radial (brachial) and carotid-femoral (aortic) PWV can be measured non-invasively, with participants supine. The superficial pulses at the radial, carotid and femoral arteries are palpated and a mark left (with a pen) at the optimal site of applanation (as indicated by the most prominent site of arterial pulsatility). As PWV is a measure of velocity, it is necessary to calculate the path length of the pulse wave. This is done in millimetres so that it is compatible with the SphygmoCor system and is achieved by using a tape measure across the surface of the body to determine the distance between the carotid-radial sites and the carotid-femoral sites being measured. The measurement is carried out by measuring the distance from the carotid site to the supra-sternal notch, and the distance from the supra-sternal notch to the radial or femoral site. These distances are inputted on to the SphygmoCor software and the pulse wave path length is thus calculated. A three lead electrocardiogram (ECG) is then attached to each participant, with electrodes placed as follows; one on the right clavicle, one on the left clavicle and one on the bottom rib on the left side of the body (see Figure 2.18). This ECG-electrode positioning is used as it creates a more usable and prominent R-wave from which heart rate can be gauged, using the SphygmoCor software. The electrodes are placed over the bone rather than over muscle so as to minimise interference from muscle movement.

ECG is employed in the measurement of PWV because the waveforms obtained using the tonometer need to be gated against the R-wave of the ECG output. Carotid-radial and carotid-femoral PWVs can then be measured sequentially as the time taken for the pulse to travel from the R-wave of the three lead ECG-gated signal to the first up-stroke of the pulse wave at the carotid, radial and femoral arterial site.

\[ PWV \text{ (m/s)} = \frac{\text{distance}}{\Delta \text{time (time taken)}} \]
Figure 2.18 Placement of ECG electrodes on the torso for the measurement of ECG-gated PWV
Foot-to-foot methodology is employed whereby the foot of the pulse is used as the marker of
the wave because the foot is the one section of the wave that is consistent and not
influenced by wave reflection. The foot of the wave marks the end of diastole. The
intersecting tangent algorithm is used to determine where the upstroke (and therefore point
of recording) ends.

Once again, for each set of measures (i.e. carotid-radial PWV and carotid-femoral PWV),
two separate readings are obtained with approximately a two minute delay between each
reading. The waveforms and readings obtained are assessed by the user to ensure quality.
If the two readings differ by more than 0.5 m/s then a third reading is taken. If the third
reading is within 0.5 m/s of one of the first readings, then an average of these two is used for
future statistical analyses. All measures were taken at the radial, carotid and femoral
arteries on the right hand side of the body.

Following PWV measurement, ECG wires and electrodes were removed from the chest and
torso region and the participant was slowly brought up from the supine position into the
seated position. Since PWV is affected by blood pressure, when creating the statistical
database, carotid-radial PWV and carotid-femoral PWV were both adjusted for MAP.

2.4.3.5 Reliability and quality control of data collection

When appropriate, quality control and reliability has been discussed in the current section of
Chapter 3, and this sub-section will briefly re-cap. PWA and PWV are robust measures with
good reliability, partly due to the ease with which the waveform can be obtained; all that is
required is the upstroke of each waveform. When performing PWA, it was ensured that the
derived waveform exceed an operator index score of 80 (as recommended by the
SphygmoCor manual) (AtCor Medical, 2008), as an operator index score lower than this will
occur for reasons such as low signal strength or an unacceptable degree of variance
between the individual captured waveforms. The quality control indices ensure that average
pulse height, pulse height variation, diastolic variation and shape variation are all within an acceptable range, and inform the user if they are not. The operator index is based on these indices. Finally, as discussed in sections 2.4.3.2 and 2.4.3.4, when collecting data, it was ensured that measurements were taken at least twice in order to rule out an anomalous result.

2.4.3.6 SphygmoCor system

The SphygmoCor (AtCor Medical, Australia) was the selected system for use in the current project for several reasons. It is lightweight and very portable which makes it useful for transportation, as the majority of consultations in the current project took place in the field, outside of the physiology laboratory. In addition to this, the measurements are non-invasive, relatively quick, and painless. Importantly, it allows for the determination of a wide range of vascular data as a result of the operator acquiring a peripheral pulse wave and the system computing a central pulse wave as it would exist in the ascending aorta. The device and software thus provide measures of peripheral vascular function, such as AIx and aPWV. Further to this, the use of aPWV via the SphygmoCor system was advocated in the relevant European expert consensus document for this discipline (Laurent et al., 2006) and determined to be the gold-standard non-invasive measure of arterial stiffness. This is due to the majority of non-invasive, aPWV outcome data available being related to the SphygmoCor device. Thus, using the SphygmoCor device in the current study makes it possible to compare previously published data from clinical populations with data obtained from the current cohort.

2.4.3.7 Confounding factors to consider when measuring arterial stiffness

The following section discusses the reasoning behind why participants were asked to refrain from consuming caffeine and a large meal prior to the health consultations and why they are
asked to avoid alcohol for the 12 hours preceding the consultation and strenuous exercise for 24 hours. Following the consumption of a meal, systemic vascular resistance is decreased and heart rate and cardiac output are increased (Waaler et al., 1991). Further to this, blood pressure is seen to decrease postprandially, particularly in participants older than 60 years (Imai et al., 1998), all of which are likely to decrease PWV. Waaler et al. (1991) demonstrated that these haemodynamic changes manifest for up to two hours following a small meal, and longer following a larger meal. Therefore, it was recommended that participants abstained from the consumption of food for a period of at least four hours prior to measurement, particularly in the case of repeated measurements.

Also, prior to arterial stiffmess measurement, it is also recommended that participants abstain from the consumption of caffeinated beverages due to associations between caffeine and increased aPWV (Vlachopoulos et al., 2003, Riksen et al., 2009). It is further recommended that participants are asked to refrain from smoking for a period of at least three hours prior to the taking of measurements as it has been shown that smoking acutely increase arterial stiffness and PWV, an effect that lasts for between 1.5 and 2.5 hours (Failla et al., 1997). Further to this, it has been demonstrated that chronic exposure to passive and active smoking also causes an increase in arterial stiffness (Tomiyama et al., 2010, Mayer et al., 2010, Xie et al., 2009, Malayeri et al., 2008).

Finally, participants should also be asked to refrain from taking part in vigorous physical activity for 24 hours prior to the taking of measurements, as acute bouts of aerobic exercise have been seen to decrease central DBP and Alx (Heffernan et al., 2007).
2.5 Mental health methods

2.5.1 Measures of mental health

This section will describe the questionnaire-based evaluation of the impact of exercise on the health and mental health of participants of the current study. Briefly, participants completed questionnaires that assessed demographic variables and health; food frequency/dietary variables; self-reported physical activity/exercise levels; and a series of psychometric/mental health questionnaires. The rationale and validity for inclusion of each of these questionnaires will be described below.

The psychometric questionnaires were printed in a booklet along with questionnaires assessing demography, health, typical food habits and exercise habits. The questionnaire booklet took participants approximately 30 minutes to complete. All participants of the longitudinal study were provided with the full set of questionnaires to complete. However, it should be noted that not all participants of the cross-sectional study were provided with all of the questionnaires. Specifically, \( n = 65 \) (i.e. the participants of the cross-sectional study who also participated in the intervention study) were given the demography, health, diet, exercise and psychometric questionnaires to complete, whereas \( n = 55 \) (i.e. the participants of the cross-sectional study who were recruited via the Cardiff Metropolitan University 'pedometer challenge/Health MOT' exercise) were provided only with the health questionnaire. Therefore, only the health questionnaire was provided to all participants. ‘Health MOT’ is a term used at the University, and more widely across the UK, for a health check. Health MOTs are often undertaken in workplaces as it can be difficult for employees to find the time to visit their GP for a health check.

2.5.1.2 Demographic questionnaire

The demographic questionnaire given to participants was used to record and determine their age, gender, post code, ethnicity, marital status, education status, employment status and
housing tenure. This data was collected so that it could potentially be used as a means of stratifying participants for data analysis, and also for adjusting data for potential cofounders, such as SES and level of education attainment (Bassuk et al., 2002, Avendano et al., 2006, Karp et al., 2004).

2.5.1.3 Health questionnaire

The general health questionnaire was used in order to provide an overall picture of the current health of participants, and was taken from the Anglo-Cardiff Collaborative Trial (ACCT) template (McDonnell et al., 2013). The questionnaire poses questions which enable the determination of whether respondents are current or past smokers, how many alcoholic units they consume in a typical week, how frequently and for what duration they exercise, whether they suffer from asthma, how frequently they have visited their GP in the previous 12 months, how they assess their current health (ranging between ‘good’ and ‘poor’), whether they have suffered from cancer, mental health or muscular-skeletal problems in the last two years, and whether they are on any form of medication such as lipid-altering drugs. Finally, the health questionnaire determines whether the participants or any of their immediate family have ever suffered from hypertension, hypercholesterolemia, diabetes, stroke, angina, peripheral vascular disease, or CHD.

These data were collected so that they could potentially be used as a means of stratifying the data for analysis or for understanding the data set better; for instance, to help determine whether anomalies in the data are potentially as a result of pharmaceutical intervention, for example.

2.5.1.4 Food Frequency Questionnaire

The Food Frequency Questionnaire used in the current study was an amended version of the Eating Habits questionnaire used as part of the English Health Survey (The Health
Survey for England, 2006). The original scale was amended for the current study in order to make it shorter, as the original questionnaire contained more information than was required for the current study. It was amended so that it was able to provide basic data on typical eating habits of the main food groups rather than to provide a thorough and detailed account of dietary habits. Therefore, the questionnaire in the current study is composed of 19 questions assessing the frequency at which some common food stuffs are consumed and the type of food stuffs usually consumed (i.e. skimmed, semi-skimmed or whole milk). The amendments were carried out with guidance and advice from Hilary Wickett, Registered Public Health Nutritionist, of Cardiff School of Health Sciences, Cardiff Metropolitan University.

The questionnaire poses questions such as ‘On average, how many times per week do you eat a serving of cakes, pies, puddings or pastries? (Including rice pudding, semolina)’, to which possible responses range from ‘rarely/never’ to ‘6+ times per week’ and ‘Do you usually add sugar (not including artificial sweetener) to hot drinks or to foods e.g. cereal?’ to which possible responses include ‘yes always; yes sometimes; no, usually use artificial sweetener’.

Since changes in diet were not promoted as part of the current study, analysis of this type of data allows for determination of whether any changes seen in the cohort over the period of the longitudinal study were associated with broad changes in eating habits. Hajjar et al. (2001) demonstrated in 17,030 participants that SBP was positively associated with higher sodium and alcohol intake and negatively associated with potassium intake. A lower rate of rise in SBP with age was associated with a higher intake of calcium. Further to this, a growing evidence base has demonstrated the beneficial effect of dietary factors such as n-3 fatty acids, antioxidant vitamins and L-arginine on endothelial function, and therefore CV risk (Brown and Hu, 2001). Therefore, it was deemed important to be able to determine whether any changes in diet had occurred over the period of the longitudinal intervention study.
2.5.1.5 The International Physical Activity Questionnaire – short form

The International Physical Activity Questionnaire (IPAQ) is a well-developed instrument that is used in order to determine the frequency, duration and intensity at which respondents have exercised during the last seven days (Craig et al., 2003). Its development began in Geneva in 1998 in response to a need for a nationally and internationally comparable questionnaire that could be used to measure physical activity in large groups (Craig et al., 2003). This data was collected in the current study for two reasons; it is useful as a means of quantifying baseline exercise levels and monitoring any change over the 8-week period of the intervention study, and also it could potentially be used as a means of stratifying the data of participants when running statistical analysis.

The questionnaire was developed as a method of standardising international measurements of physical activity quantification. Short and long versions (4-item and 27-item versions, respectively) of the questionnaire were developed and tested across 12 countries in 2000 (Craig et al., 2003). The short version (IPAQ-S) assesses the time spent carrying out physical activities at vigorous intensities, moderate intensities and also assesses time spent walking and in sedentary behaviour. The long version collects more detailed information by assessing time spent in domains of physical activity including housework and gardening activities and occupational activity. Both versions of the questionnaire compile the physical activity data by estimating weekly energy expenditure in metabolic equivalents (METs) minutes. Using the questionnaire and its guidance notes, weekly MET-minutes are calculated as:

\[ \text{MET.min.week}^{-1} = \text{duration} \times \text{frequency per week} \times \text{MET intensity} \]

MET intensities were obtained from the 2000 compendium of physical activities, with moderate intensity being activities between 3 and 6 METs, and vigorous intensity being activities >6 METs (Ainsworth et al., 2000). The \textit{MET intensity} value that is used in the above calculation is ‘8’ for vigorous activities, and ‘4’ for moderate activities; for walking
activities, \textit{MET intensity} is ‘3.3’. Similarly, total physical activity MET minutes/week is measured using:

\[
\text{Total physical activity MET minutes/week} = \text{sum of [Walking + Moderate + Vigorous MET-minutes/week] scores}
\]

The amount of sedentary time can also assessed by asking respondents about ‘total sitting time’; this is important as sedentary behaviour is a risk factor in itself and has been linked to health outcomes independently of physical activity levels (Balboa-Castillo et al., 2011). The IPAQ-S measures sedentary time in minutes/week.

The IPAQ-S has been extensively tested and validated in the United Kingdom and has been shown to have concurrent validity of alpha 0.69 and moderate criterion validity with objective accelerometry data in a UK sample of \( p = 0.40 \) (Craig et al., 2003). A test-retest reliability correlation of between 0.69 and 0.87 was produced using the same cohort of UK participants (Craig et al., 2003). This paper demonstrated using the IPAQ-S that 82% of the 1,974 respondents met the recommended 150 minutes per week of moderate intensity physical activity, with a median weekly MET.min of 2514. The median weekly MET.min for the Great British population is 1653, and the mean is 3238 (standard deviation ±4524) (Rütten and Abu-Omar, 2004, Rütten et al., 2003).

At this point during the current PhD project, there was no access to objective measures of physical activity levels (such as accelerometers) and therefore the study was dependent upon self-reported measures.
2.5.2 Mental health and psychometric measurements

The mental health status of the participants over the eight week period of the green-exercise intervention was assessed using the following questionnaires (each of which are standardised and well-validated psychometric experimental tools, as will be described in this section):

- the Rosenberg Self-Esteem scale
- the Cognitive Failures Questionnaire
- the State-Trait Anxiety Inventory
- the Social Provisions Scale
- the Perceived Stress Scale
- the Centre for Epidemiologic Studies-Depression scale
- the Profile of Mood States

As discussed in Chapter 1, these measures of mental health were chosen due to their use in previous and similar exercise research (and hence the fact that the results that they yield could be compared to those obtained in other research studies), and the ease with which they could be administered (Pretty et al., 2005b, Kohut et al., 2006, Starkweather, 2007, Blumenthal et al., 1999, Knubben et al., 2007, McGale et al., 2011, Froeliger et al., 2012), and therefore the results that they yield could be compared to those obtained in other research studies.

2.5.2.1 Rosenberg Self-Esteem Scale (R-SES)

The Rosenberg Self-Esteem Scale (R-SES) assesses global self-esteem, and is considered the most widely used and popular measure of self-esteem in health psychology research (Blascovich and Tomaka, 1991, Gray-Little et al., 1997, Sinclair et al., 2010). Global self-esteem is one component of the self-concept, and refers to each individual’s negative and positive attitude towards the self (Rosenberg et al., 1995).
The current study employed the R-SES questionnaire developed by Rosenberg (1965). It is composed of ten questions to which the participant responds to using a four-point Likert-type scale. The scale ranges from ‘strongly disagree’ to ‘strongly agree’ and participants state to what degree they agree with each of the statements. An example of a statement is “On the whole, I am satisfied with myself”. Scores range between 0 and 30, with lower scores suggesting poorer self-esteem. In an American adult cohort (n= 503), the average R-SES score was observed to be 22.62 (Sinclair et al., 2010).

For use in cohorts in the United Kingdom, an internal consistency alpha of 0.90 has been reported using this scale (Schmitt and Allik, 2005). Also reported in the same paper was good reliability and validity of the scale, with discriminant reliability also reported. The reliability and validity of the scale has been extensively demonstrated, and has been validated for use in both female and male populations of adolescent, adult and elderly populations. Focht et al. (2007) and Poon and Fung (2008) both demonstrated good reliability of the scale in older adults and good test-retest reliability has also been shown in an older sample (Windle et al., 2008).

### 2.5.2.2 Cognitive Failures Questionnaire (CFQ)

The MMSE is a clinical tool commonly used in diagnosing and assessing progression and severity of dementia (Alzheimer’s Society, 2012). It has been used in exercise research (Weuve et al., 2004, Lytle et al., 2004); however, it was deemed inappropriate for the current research project by the ethics committee due to it being a clinical tool.

To assess cognitive function, the Cognitive Failures Questionnaire (CFQ), as developed by Broadbent et al. (1982), was used. It is composed of 25 items that are responded to using a five-point Likert-type scale. The responses range from ‘very often’ to ‘never’. The questionnaire assesses the occurrence of self-reported failures in everyday tasks in the domains of perception, memory and motor function. An example of statements participants
respond to is “Do you find you forget why you went from one part of the house to the other?” The total scores range between 0 and 100, with a higher score indicating higher incidence of cognitive failures. The failures assessed fall within four domains, these are labelled as memory, distractibility, blunders and memory for names.

Bridger et al. (2013) recently tested the internal reliability and test-retest of the CFQ in a cohort with an average age of 41 years. The internal reliability of the CFQ was demonstrated to have a Cronbach’s alpha coefficient of 0.92 over 12 months and 0.93 over 36 months. The test-retest reliability of the questionnaire over a 24 month period was $r=0.71$. Bridger et al. also demonstrated that high CFQ scores were related to increased vulnerability to depression and anxiety. Broadbent et al. (1982) reported a CFQ test-retest score of $r=0.82$ and $r=0.80$ over a period of up to 24 months. Broadbent et al. also demonstrated a high internal consistency of the scale and therefore suggested it should be used to measure the single construct of cognitive failure, rather than breaking it down into the separate factors.

2.5.2.3 State-Trait Anxiety Inventory (STAI)

The State-Trait Anxiety Inventory (STAI) was developed by Spielberger et al. (1983). It is a commonly used tool in the measurement of state and trait anxiety. The questionnaire is composed of 40 items that are responded to using a four-point Likert-type scale. The responses are scaled from ‘not at all’ to ‘very much so’. The scale is composed of 20 questions which assess state anxiety, and 20 questions which assess trait anxiety. State anxiety refers to temporary and transient anxiety in response to a specific situation, whereas trait anxiety refers to more long-term and general anxiety. The state scale, only, of the questionnaire was employed in the current study.

The state scale poses statements such as ‘I feel strained’ and ‘I am presently worrying over possible misfortunes’. The possible scores range between 20 and 80, with higher scores
indicating greater anxiety. Knight et al. (1983) and Addolorato et al. (1999) suggest that a score of 39-40 detects clinically significant symptoms of anxiety, whereas Kvaal et al. (2005) suggest a cut off of 54-55 in older adults.

The scale has been demonstrated to be reliable and consistent, with internal consistency alpha coefficients for the scale being seen to range between 0.86 and 0.95 (Spielberger et al., 1983). Test-retest reliability coefficients ranged between 0.31 and 0.86, at time points ranging between 1 hour and 104 days. The coefficients were lower for the state scale than the trait scale, however, this would be expected due to the state scale being used to detect more transient states of anxiety (Spielberger et al., 1983). During the development of the scale, over 10,000 adults and adolescents were tested and the STAI was tested against the Taylor Manifest Anxiety Scale (Taylor, 1953) and the Cattell and Scheier’s Anxiety Scale Questionnaire (Cattell and Sheier, 1963). The overall correlations between the STAI and these two measures of anxiety were 0.73 and 0.85, respectively.

2.5.2.4 Social Provisions Scale (SPS)

The current study employed the Social Provisions Scale (SPS) which was developed by Cutrona and Russell (1987) as a measure of the perceived social support received from the respondent’s family and close personal relationships. The SPS consists of six subscales of provision, and these are; reliable alliance (assurance that others can be counted on in times of need), guidance (advice), attachment (emotional closeness), social integration (a sense of belonging), reassurance of worth (recognition of one’s competence), and opportunity for nurturance (being able to provide assistance to others). These provisions were described by Weiss (1973) and Weiss (1974) and are said to reflect what is received from personal relationships. The scale allows not only for the measurement of each of the provisions, but also for the measurement of global social support. The scale is composed of 24 items, which participants respond to using a four-point Likert-type scale. An example of a statement is ‘No-one needs me to take care of them’; a statement which participants
respond with either ‘strongly disagree, disagree, agree or strongly agree’. The possible scores range between 24 and 96, with a higher score indicating a greater degree of perceived social support. The various constructs measured using the scale cause it to be a multi-dimensional method of measuring social support which lends data to determine whether social relationships provide functions that aid in the buffering of stress (Cohen and McKay, 1984).

The SPS scale has been shown to be a reliable and valid measure, Cronbach’s alpha coefficient of internal reliability ranging between 0.83 and 0.90 (Cutrona, 1986, McAuley et al., 2000b). Further to this, it has construct validity against measures of loneliness and interpersonal relationships (Cutrona and Russell, 1987). Cutrona et al. (1984) demonstrated that in a sample of older adults, SPS has test-retest reliability correlates of $r = 0.60$, and has been used successfully with older populations, (Cutrona, 1986, Aquino et al., 1996).

2.5.2.5 Perceived Stress Scale (PSS)

The Perceived Stress Scale (PSS) scale assesses the degree to which events in an individual’s life are considered to be stressful. It was developed by Cohen and Williamson (1988) and is the most widely used psychological assessment for measuring the perception of stress. It is a shorter version of the original PSS, as it has just 10 items; as such, it was selected on the basis of its accessibility (without compromising the validity of the data obtained) for use in the current study. The questions are designed to assess how overwhelming, unpredictable and uncontrollable participants perceive their lives to be; three components that have been shown to be central to the experience of stress (Averill, 1973, Cohen, 1978, Glass, 1972). Questions posed in the scale include “In the last month, how often have you felt that things were going your way?” The items all assess thoughts and feelings over the last month. Participants respond using a five-point Likert-type scale to these items, with responses ranging between ‘never’ to ‘very often’. The overall score ranges between 0 and 40, with a higher score indicating greater levels of perceived stress.
The original PSS contained 14 items, with 10 and 4 item versions also having been developed and validated. Cohen and Williamson (1988) describe that the PSS-10 allows for the assessment of perceived stress but without losing any of psychometric quality and in fact slightly gaining some quality, when compared to the PSS-14. In a probability sample, the PSS-14 was shown to have a Cronbach’s alpha coefficient of internal reliability of 0.75, whilst the PSS-10 had an alpha coefficient of 0.78.

The PSS measures appraised stress which is influenced by daily hassles, major events and changes in coping resources. Other useful aspects of the scale are how easy it is to administer, that it only take a couple of minutes to complete, the ease of its scoring and that it is sensitive to the non-occurrence of events but also to on-going events in the participant’s life. It has been shown that the PSS significantly demonstrates a range of outcomes at follow-up despite having controlled for psychological symptoms at baseline (Cohen et al., 1993). This therefore reduces the concern that items in the scale could potentially be confounded by other psychological outcomes.

2.5.2.6 Centre for Epidemiologic Studies-Depression Scale (CES-D)

Many exercise studies have employed the BDI measurement for assessing depression (Babyak et al., 2000, Blumenthal et al., 1999, Blumenthal et al., 2005), however, as it is a clinical measure of depression it was deemed inappropriate for the current research project by the ethics committee.

The Centre for Epidemiologic Studies-Depression Scale (CES-D) was developed by Radloff (1977) as a measure of depression in community samples and was selected for use in the current project. It is a short, self-report scale which is composed of 20 items, answered using a four-point Likert-type scale, with responses ranging from ‘rarely’ to ‘most/all of the time’. The scale is designed to assess depressive symptomatology in the general population, with assessment of symptoms such as poor appetite, hopelessness, pessimism,
and fatigue occurring. Scores range between 0 and 60, with higher scores indicating greater depressive symptomatology. A score of 16 or greater on the CES-D is said to be indicative of moderate depression (Radloff, 1977).

The CES-D has four subscales; depressed affect, somatic symptoms, positive affect, and interpersonal problems. The existence of these four subscales has been validated in populations such as older adults (Hertzog et al., 1990, Knight et al., 1997) and the homeless populations (Wong, 2000). However, most research studies use the total CES-D score (Schulz et al., 2004, Gallicchio et al., 2002).

The scale measures both positive and negative affect; in a healthy population, a low negative correlation is expected to exist between the two states (Radloff, 1977). The paper demonstrated a coefficient alpha internal consistency score of 0.85 in a healthy population, and 0.90 in a patient sample. Test-retest correlations were between 0.45 and 0.70, depending on the time interval between the test and re-test. The scale also has excellent concurrent validity by self-report and clinical criteria and substantial evidence of construct validity. Lewinsohn et al. (1997) have also demonstrated the scale to have good sensitivity and specificity and to have high internal consistency.

2.5.2.7 Profile of Mood States (POMS)

The current study employed the Profile of Mood States questionnaire (POMS), which is widely used in exercise and psychology research as a measure of mood states. It was developed by McNair et al. (1971a), with its development having begun in the early 1960s. It is a measure of affective mood fluctuations and is widely used in the general population but also in clinical settings and sports settings. ‘Affect’ refers to the experience of an emotion and can be both negative and positive. High positive affect is a state of high energy, full concentration, and engagement, whereas high negative affect is a state that includes unpleasurable engagement and subjective distress which manifests in mood states such as
anger, guilt and nervousness. Low negative affect is a state of serenity and calm (Watson et al., 1988).

The questionnaire consists of 65 items, which are responded to using a five point Likert-type scale. The questionnaire measures feelings and emotions such as how cheerful or efficacious one feels and asks how the participant has felt with regard to each feeling over the past week. The possible responses range between ‘not at all’ to ‘extremely’: The constructs of mood that the questionnaire assesses are tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia and confusion-bewilderment. Further to this, total mood disturbance (TMD) is also measured by adding all five of the negative mood constructs, and then subtracting the score for vigour (positive construct). TMD is a global score of affective state, and represents an overall assessment of the respondent’s emotional state, as an indicator of overall mood (Dyrbye et al., 2006).

Anger-hostility scores range between 0 and 48, confusion-bewilderment scores range between 0 and 28, depression-dejection scores range between 0 and 60, fatigue-inertia scores range between 0 and 28, tension-anxiety scores range between 0 and 36, and vigour-activity scores range between 0 and 32. Lower scores for each subscale indicate more stable mood profiles, except for the positive affect state; vigour-activity, in which a higher score is associated with a more stable mood profile. TMD scores range between -32 and 200, with higher scores of TMD indicating a higher degree of mood disturbance.

These subscales have been shown to have excellent internal consistency, with Cronbach’s alpha coefficient scores ranging between $r= 0.63$ (for confusion-bewilderment) and 0.96 (for depression-dejection). Test-retest analyses were also carried out, with reliability coefficients ranging between 0.65 (vigour-activity), and 0.74 (depression-dejection) (McNair et al., 1971b).

The scale is well used in exercise setting; in the Profile of Mood States Bibliography 1964-2002, under the heading of Exercise and Fitness, there were 136 research articles items that had cited use of the POMS questionnaire (LeUnes and Burger, 1998) (with 315 published
articles using the questionnaire for sport and exercise psychology research between 1971 and 2000 (LeUnes, 2000)). These included studies assessing aerobic exercise, anaerobic exercise, jogging and non-competitive sports (McNair et al., 2003). The questionnaire is easily administered and is reported to take between three and five minutes, and can be understood by participants with year 8 (approximately age 12) reading skills (McNair et al 1991).

**Conclusion**

Therefore, based on their use in previous studies relevant to the current PhD study, the following questionnaires have been selected. The Rosenberg Scale will be used to measure self-esteem, the Cognitive Failures Questionnaire will be used to measure cognitive function, the State Trait Anxiety Inventory will be used to measure state anxiety, the Social Provisions Scale will be used to measure perceived social support, the Perceived Stress Scale will be used to measure perceived stress, the Centre for Epidemiologic Studies-Depression scale will be used to measure depression and the Profile of Mood States questionnaire will be used to measure mood. Further to this, health, demography, diet and exercise data will also be assessed in the current PhD project.
Chapter 3 - Cross-sectional Investigation – Exercise Levels
3.1 Introduction

As discussed in Chapter 1, the evidence base regarding regular participation in exercise upon health and CV risk is well regarded and growing. However, the particular effect of regular participation in exercise upon the specific measures of interest of this project, and whether these measures interact (and if so, how) is not definitively known. Therefore, the purpose of the current cross-sectional aspect of the current thesis was to determine whether regular participation in exercise was associated with reduced CV risk, as assessed via blood-borne markers of CV risk, vascular haemodynamics and arterial stiffness, and markers of mental health (using the range of measurements discussed in Chapter 2).

Regular participation in aerobic exercise is associated with improved glucose and lipid levels (Knowler et al., 2002, Leon and Sanchez, 2001), improved blood pressure and markers of arterial stiffness (via aPWV and AIx) (Vaitkevicius et al., 1993, Tanaka et al., 1998, Boreham et al., 2004, McDonnell et al., 2013) and improved markers of mental health (Abbott et al., 2004, Colcombe and Kramer, 2003, Laurin et al., 2001, Rovio et al., 2005, Yaffe et al., 2001, Strawbridge et al., 2002, Penedo and Dahn, 2005, Fox et al., 2007, Fox, 1999). However, missing from the literature is a multi-disciplinary investigation of these three arms of CV risk-associated health within a single study. Further to this, missing from the research literature are mental health studies that have investigated more than two or three different mental health constructs in association with aerobic exercise habits, and therefore it is difficult to compare how the different constructs of mental health respond to the same modality, intensity and frequency of an exercise habit. Accordingly, the study also aimed to determine whether regular aerobic exercise is associated with differential effects on markers of mental health.

Therefore, the study aimed to determine the impact of regular exercise on multiple markers of health that are all associated by their relationship with CV risk, and to determine whether any relationships exist between these three areas of health, which are more commonly studied individually. Additionally, the current cross-sectional study aimed to determine
whether the measures of CV risk described in Chapter 2 would be sensitive enough to determine any exercise-associated differences in CV risk in a cross-sectional study, prior to their employment in a longitudinal exercise study. The hypothesis of the current cross-sectional study was that increased physical activity levels would be associated with improved markers of CV risk, including blood pressure, arterial stiffness, and markers of mental health.

3.2 Methods

As described in Chapter 2, participants were recruited from two different sources for the current study. One source was the recruitment of those who, having declared an interest in beginning a green-exercise programme (see Chapter 4), volunteered to take part in up to two data-collection consultations - data from these baseline consultations are used in a cross-sectional manner in the current chapter. The second source of data is from participants who took part in a ‘health MOT’ health and well-being initiative undertaken at and by Cardiff Metropolitan University. These ‘health MOTs’ were one-off health checks that any member of university staff was welcome to sign up to and take part in.

The consultations and method of data collection took place as described in Chapter 2, with the exceptions that assessment of mental health via questionnaire did not take place in those who were recruited as part of the health MOT study, and that leukocyte samples were not obtained from the blood of the health MOT study participants, and therefore mRNA gene expression of ABCA1, CD36 and MMP-9 did not occur for those participants. Across the whole cross-sectional cohort, 10 participants did not provide a blood sample and two participants did not return their baseline mental health questionnaire booklets.

As mentioned above, the methodology employed in the current cross-sectional study was outlined in detail in Chapter 2. Specifically, the biomolecular and biochemical measurements were carried out as described in section 2.2, the anthropometric
measurements were carried out as described in section 2.3, the vascular haemodynamic measurements were carried out as described in section 2.4, and the measures of mental health were carried out as described in section 2.5.

Data are expressed as mean (±SD), unless otherwise stated. Analyses were performed using SPSS version 20 (SPSS Inc., Ill, USA), or GraphPad Prism 5 software (GraphPad Software Inc., CA, USA) for ELISA data; one-way ANOVA analyses allowed for comparison of the mean of three different groups that manifested upon stratifying the participants via exercise levels (see section 3.3.5). Bivariate correlation analyses were also performed using Pearson’s correlation coefficient method. Statistical significance was set to a priori $P \leq 0.05$ (unless otherwise stated), with all other results being reported as non-significant (NS).

### 3.3 Results

#### 3.3.1 General characteristics of the cohort

The general characteristics of the cohort are detailed below in Tables 3.1a-c. Briefly, the cohort ($n=120$) was composed of males ($n=31$) and females ($n=89$). The mean age of the cohort was approximately 43 (±13.23) years, with 96.7% of participants being of Caucasian race (see Table 3.1a).

When considered as a whole, the cohort had a mean weight of 74kg, a mean BMI of 27kg/m$^2$, and a mean waist circumference of 87cm. When split by gender, the males on average had a waist circumference of approximately 93cm and a BMI of 27kg/m$^2$, while the female participants in the cohort had an average waist circumference of approximately 84cm. On average, approximately 84% of males and 64% of females regularly took part in physical activity, as per self-reported duration, frequency and intensities of exercise sessions. Approximately 6% of all participants were current smokers.
### Table 3.1a General demographic, anthropometric and lifestyle risk factor data obtained from the entire cohort of participants in the cross-sectional study

<table>
<thead>
<tr>
<th>Demographic, health and lifestyle variables</th>
<th>Mean (±SD)</th>
<th>Range</th>
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<tbody>
<tr>
<td><em>n</em> = 120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.26 (±13.23)</td>
<td>20-76</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>96.7</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.34 (±10.68)</td>
<td>153.00-190.50</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.88 (±16.17)</td>
<td>46.00-135.00</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>86.63 (±12.78)</td>
<td>59.00-136.00</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.89 (±5.37)</td>
<td>18.20-47.83</td>
</tr>
<tr>
<td>Regularly exercise (%)</td>
<td>69.2</td>
<td></td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Past smoker (%)</td>
<td>26.1</td>
<td></td>
</tr>
</tbody>
</table>

Mean data of all participants of the cross-sectional study. BMI, body mass index.

### Table 3.1b General demographic, anthropometric and lifestyle risk factor data obtained from the entire cohort of male participants in the cross-sectional study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (±SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>n</em> = 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.45 (±13.11)</td>
<td>23-76</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.75 (±9.87)</td>
<td>172.51-190.50</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.30 (±13.32)</td>
<td>64-123.10</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>92.91 (±10.61)</td>
<td>76-121.50</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.19 (±3.91)</td>
<td>21.97-37.00</td>
</tr>
<tr>
<td>Regularly exercise (%)</td>
<td>83.9</td>
<td></td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Past smoker (%)</td>
<td>19.4</td>
<td></td>
</tr>
</tbody>
</table>

Males, BMI, body mass index.
Table 3.1c General demographic, anthropometric and lifestyle risk factor data obtained from the entire cohort of female participants in the cross-sectional study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (±SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.84 (±13.32)</td>
<td>20-75</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.87 (±10.95)</td>
<td>153.00-175.00</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.94 (±16.11)</td>
<td>46.00-135.00</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>84.46 (±12.80)</td>
<td>59.00-136.00</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>26.79 (±5.81)</td>
<td>18.20-47.83</td>
</tr>
<tr>
<td>Regularly exercise (%)</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Past smoker (%)</td>
<td>28.6</td>
<td></td>
</tr>
</tbody>
</table>

Females, BMI, body mass index.

3.3.2 Biomolecular and biochemical markers of CV risk in the whole cohort

Table 3.2 displays the mean fasting levels of the biomolecular and biochemical factors that were assessed. The data is representative of the whole cohort; briefly, clinical markers of CV risk including glucose levels were in the normal range (5.25 ±0.93mmol/L) (Diabetes UK, 2012) and insulin levels (32.64 ±18.12 pmol/L) were also in the normal range (Kahn et al., 1997). Circulating levels of serum IL-6 (0.98 ±0.69pg/mL) and plasma MMP-9 (46.69 ±23.82ng/mL) were also in the normal range (R&D Systems, 2012, R&D Systems, 2011). The total-cholesterol and LDL-cholesterol levels were slightly higher than the recommended levels of 5mmol/L and 3mmol/L (NHS, 2013b), respectively. However, HDL-cholesterol and triglycerides were within the recommended ranges.
3.3.3 Vascular haemodynamic markers of CV risk within the whole cohort

Tables 3.3a and 3.3b display the mean values of the seated (Table 3.3a) and supine (Table 3.3b) vascular haemodynamic variables that were assessed. The data is representative of the whole cohort, and can be summarised as follows: peripheral SBP and DBP pressures were slightly above the target guidelines of 120mm Hg (SBP) and 80mm Hg (DBP), but lower than those pressures used as guidelines in the diagnosis of hypertension, i.e.140mm Hg (SBP) and 90mm Hg (DBP) (British Heart Foundation, 2014). Briefly, seated peripheral SBP was 128 ±15mm Hg, peripheral seated DBP was 84 ±10mm Hg, central seated SBP was 118 ±16mm Hg and central seated DBP was 85 ±10mm Hg. Supine measures included the same variables as seated, but additionally include measures of PWV.

3.3.4 Markers of mental health within the whole cohort

Table 3.4 displays the mean scores of the markers of mental health that were assessed in approximately half of the cross-sectional study cohort (n= 63). The table displays the mean scores obtained for questionnaires that assessed self-esteem, cognitive functioning, state anxiety, social provisions, perceived stress, depression and mood. Lower scores are representative of better mental health for the following measures; CES-D, CFQ, PSS, STAI, all POMS constructs (except vigour-activity POMS construct) and higher scores indicate better mental health for the following measures; R-SES, SPS, and POMS vigour-activity. Therefore, observation of the data suggests that the subset of the cross-sectional cohort that completed the questionnaire had relatively low scores for CES-D, CFQ, PSS, STAI, and all POMS constructs (except vigour-activity POMS construct) and relatively high scores for R-SES, SPS, and POMS vigour-activity. Briefly, the perceived stress (as measured using the PSS, with a range of 0-40) of the cohort had a mean score of 16.82 (±6.95), depression (as measured using the CES-D scale; with a range of 0-60) had a mean score of 13.25 (±8.38) and state anxiety (as measured using the STAI; with a range of 20-80) had a mean score of 37.73 (±8.88)
Table 3.2 Fasting biomolecular and biochemical data obtained from the cohort of participants in the cross-sectional study

<table>
<thead>
<tr>
<th>Blood-borne marker</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n= 110</td>
<td></td>
</tr>
<tr>
<td>ABCA1 (cf. GAPDH)</td>
<td>1.00 (±0.09)</td>
</tr>
<tr>
<td>CD36 (cf. GAPDH)</td>
<td>1.00 (±0.09)</td>
</tr>
<tr>
<td>MMP-9 (cf. GAPDH)</td>
<td>1.00 (±0.01)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.25 (±0.93)</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>32.64 (±18.12)</td>
</tr>
<tr>
<td>Total-cholesterol (mmol/L)</td>
<td>5.42 (±1.59)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.68 (±0.65)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.21 (±1.21)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.18 (±0.89)</td>
</tr>
<tr>
<td>Serum IL-6 (pg/mL)</td>
<td>0.98 (±0.69)</td>
</tr>
<tr>
<td>Plasma MMP-9 (ng/mL)</td>
<td>46.69 (±23.82)</td>
</tr>
</tbody>
</table>

The mean leukocyte mRNA gene expression of the PPARγ target genes and the average concentration of the serum and plasma derived circulating CV risk-related markers of interest in the cross-sectional study cohort.

cf, compared to; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol
Table 3.3a Seated vascular haemodynamic data obtained from the entire cohort of participants in the cross-sectional study

<table>
<thead>
<tr>
<th>Seated variables</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>128 (±15)</td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>84 (±10)</td>
</tr>
<tr>
<td>Peripheral PP (mm Hg)</td>
<td>44 (±12)</td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>99 (±11)</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>118 (±16)</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>85 (±10)</td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>33 (±12)</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>96 (±11)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>64 (±12)</td>
</tr>
<tr>
<td>Alx (%)</td>
<td>22 (±13)</td>
</tr>
<tr>
<td>Alx @HR75 (%)</td>
<td>17 (±13)</td>
</tr>
</tbody>
</table>

Measures of vascular haemodynamics obtained from participants whilst seated. Central pressures, HR, Alx and Alx@HR75 were obtained using SphygmoCor. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; Alx, augmentation index; Alx@HR75, augmentation index at a heart rate of 75.
Table 3.3b Supine vascular haemodynamic data obtained from the entire cohort of participants in the cross-sectional study

<table>
<thead>
<tr>
<th>Supine variables</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>122 (±14)</td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>77 (±8)</td>
</tr>
<tr>
<td>Peripheral PP (mm Hg)</td>
<td>45 (±12)</td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>92 (±10)</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>113 (±13)</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>78 (±10)</td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>35 (±11)</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>90 (±10)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>59 (±9)</td>
</tr>
<tr>
<td>Alx (%)</td>
<td>26 (±13)</td>
</tr>
<tr>
<td>Alx @HR75 (%)</td>
<td>18 (±13)</td>
</tr>
<tr>
<td>CRPWW (m/s)</td>
<td>7.0 (±1.2)</td>
</tr>
<tr>
<td>MAP-adjusted CRPWW (m/s)</td>
<td>7.0 (±1.1)</td>
</tr>
<tr>
<td>CFPWW (m/s)</td>
<td>6.8 (±1.5)</td>
</tr>
<tr>
<td>MAP-adjusted CFPWW (m/s)</td>
<td>6.8 (±1.0)</td>
</tr>
</tbody>
</table>

Measures of vascular haemodynamics obtained from participants whilst supine. Central pressures, HR, Alx, Alx@HR75, CRPWW and CFPWW were obtained using SphygmoCor. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; Alx, augmentation index; Alx@HR75, augmentation index at a heart rate of 75; CRPWW, carotid radial pulse wave velocity; CFPWW, carotid femoral pulse wave velocity.
Table 3.4 Measures of mental health obtained from a subgroup of the participants in the cross-sectional study

<table>
<thead>
<tr>
<th>Measure of mental health</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Range)</td>
<td></td>
</tr>
<tr>
<td>n=63</td>
<td></td>
</tr>
<tr>
<td>R-SES (0-30)</td>
<td>16.16 (±2.02)</td>
</tr>
<tr>
<td>CFQ (0-100)</td>
<td>39.63 (±11.11)</td>
</tr>
<tr>
<td>STAI (20-80)</td>
<td>37.73 (±8.88)</td>
</tr>
<tr>
<td>SPS-attachment (4-16)</td>
<td>13.89 (±2.40)</td>
</tr>
<tr>
<td>SPS-social integration (4-16)</td>
<td>13.55 (±1.93)</td>
</tr>
<tr>
<td>SPS-reassurance of worth (4-16)</td>
<td>12.57 (±2.19)</td>
</tr>
<tr>
<td>SPS-reliable alliance (4-16)</td>
<td>14.32 (±1.87)</td>
</tr>
<tr>
<td>SPS-guidance (4-16)</td>
<td>14.00 (±2.36)</td>
</tr>
<tr>
<td>SPS-opportunity for nurturance (4-16)</td>
<td>12.46 (±2.74)</td>
</tr>
<tr>
<td>Perceived Stress Scale (0-40)</td>
<td>16.82 (±6.95)</td>
</tr>
<tr>
<td>CES-D (0-60)</td>
<td>13.25 (±8.38)</td>
</tr>
<tr>
<td>POMS-tension anxiety (0-36)</td>
<td>9.07 (±5.24)</td>
</tr>
<tr>
<td>POMS-depression dejection (0-60)</td>
<td>12.05 (±7.53)</td>
</tr>
<tr>
<td>POMS-anger hostility (0-48)</td>
<td>8.55 (±5.40)</td>
</tr>
<tr>
<td>POMS-vigour activity (0-32)</td>
<td>16.00 (±7.42)</td>
</tr>
<tr>
<td>POMS-fatigue inertia (0-28)</td>
<td>9.89 (±6.36)</td>
</tr>
<tr>
<td>POMS-confusion bewilderment (0-28)</td>
<td>7.69 (±4.43)</td>
</tr>
<tr>
<td>POMS-total mood disturbance (-32-200)</td>
<td>31.27 (±25.85)</td>
</tr>
</tbody>
</table>

Measures of mental health obtained using well-validated questionnaires. R-SES, Rosenberg Self-Esteem Scale; CFQ, Cognitive Failures Questionnaire; STAI, State-Trait Anxiety Inventory; SPS, Social Provisions Scale; CES-D, Center for Epidemiologic Studies-Depression; POMS, Profile of Mood States.
3.3.5 Stratifying the cohort on the basis of physical activity levels

Stratification, based on the frequency, duration and intensity of participants’ weekly physical activity was carried out in order to aid interpretation of the cross-sectional data obtained. These data were obtained from a health questionnaire in which participants were asked how often they undertook physical activity each week, how long each bout lasted and what each activity consisted of. Sedentary participants were defined as those taking part in no recreational physical activity (International Physical Activity Questionnaire, 2005). The physically active subset of participants within the cohort were split into a moderately physically active group (defined as 3 or more days of vigorous-intensity activity of at least 20 minutes per day; or 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day; or accumulating > 600 MET-minutes/week from a combination of walking, moderate-intensity and vigorous-intensity activities), and a highly physically active group (defined as vigorous-intensity activity on at least 3 days, accumulating >1500 MET-minutes/week; or 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities, accumulating >3000 MET-minutes/week). In order to classify the participants in this manner, data were collected on physical activity levels and expressed in MET-minutes per week using the IPAQ (International Physical Activity Questionnaire, 2005). At this point during the current PhD project, there was no access to objective measures of physical activity levels (such as accelerometers) and therefore the study was dependent upon self-reported measures.

Table 3.5a displays the mean age, height, weight, BMI, waist circumference and IPAQ MET-minutes/week of each of the three groups. The groups were significantly different for the measure of IPAQ MET-minutes/week. As expected, due to the stratification of the cohort, IPAQ MET-minutes were significantly higher in the moderately active and highly active groups, compared to sedentary (914 ±248MET-minutes/weeks; 1688 ±374MET-minutes/weeks; 205 ±201MET-minutes/weeks, respectively). The highly physically active group also had a significantly greater IPAQ score than the moderately active. Interestingly,
both the moderately active and highly physically active groups had significantly lower BMI scores than the sedentary group (26.02±4.56kg/m²; 24.88±3.82kg/m²; 29.89±6.42kg/m² respectively).

Tables 3.5b-e display the data obtained from the multi-disciplinary measures of health related to CV risk following the stratification of the participants into the three physical activity status groups.

Table 3.5b demonstrates that the measured biomolecular and biochemical markers of CV risk differed between the three groups for levels of HDL-cholesterol and circulating levels of MMP-9. The sedentary group had significantly lower levels of HDL than the moderately active group and highly active group (1.33 ±0.58mmol/L, 1.66 ±0.49mmol/L, and 2.18 ±0.78mmol/L, respectively). The moderately active group (42.75 ±19.56ng/mL) and the highly active group (40.50 ±27.09 ng/mL) both had significantly lower levels of circulating MMP-9 than the sedentary group 58.92 ±24.73ng/mL).

Table 3.5c demonstrates that peripheral seated DBP was significantly lower in the highly active group (80 ±8mm Hg) compared to the moderately active group (85 ±10mm Hg) and the sedentary group (86 ±11mm Hg). The highly active group’s central seated DBP (81 ±9 mm Hg) and seated HR (58 ±12 bpm) were also significantly lower than those of the sedentary group (88 ±11mm Hg, and 68 ±11bpm, respectively). The data also demonstrates that the highly active group had significantly lower seated AIx@HR75 than the moderately active group and the sedentary group (11 ±14%; 19 ±13%; 19 ±11%, respectively). Central seated MAP was also lower in the highly active group (92 ±9mm Hg) compared to the sedentary (98 ±12 mm Hg), but this did not reach statistical significance.

Table 3.5d demonstrates that peripheral supine DBP was significantly lower in the highly active group (73 ±7mm Hg) than in the sedentary group (80 ±11mm Hg). Peripheral supine MAP was lower in the highly active group (88 ±7mm Hg) compared to the sedentary (94 ±11mm Hg), however, this did not reach statistical significance. Central supine DBP, supine
HR and supine Alx@HR75 were all non-significantly lower in the highly active group than the sedentary.

Finally, Table 3.5e demonstrates that the moderately active group had significantly higher scores for R-SES than the sedentary group (16.75 ±1.99; 15.28 ±1.99, respectively), and had significantly lower scores for the CFQ than the sedentary group (35.68 ±10.21; 43.36 ±10.88, respectively). The POMS construct ‘anger-hostility’ was higher in the highly physically active group than the moderately active group and sedentary group; however, this did not reach statistical significance (12.00 ±5.42; 7.72 ±5.44; 7.90 ±4.93, respectively). The POMS construct ‘vigor-activity’ was also higher in the physically active groups compared to the sedentary group, but this also did not reach statistical significance.

Table 3.5a Demographic, anthropometric and lifestyle risk factors of the cross-sectional cohort based on physical activity stratification (sedentary, moderately physically active and highly physically active)

<table>
<thead>
<tr>
<th>Demographic, health and lifestyle variables</th>
<th>Sedentary</th>
<th>Moderately active</th>
<th>Highly active</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td>P value</td>
</tr>
<tr>
<td>n= 33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.06 (±15.42)</td>
<td>44.40 (±12.45)</td>
<td>39.81 (±11.67)</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.58 (±9.78)</td>
<td>164.42 (±10.15)</td>
<td>166.72 (±9.57)</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.56 (±18.46)</td>
<td>71.86 (±15.00)</td>
<td>70.85 (±14.01)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.89 (±6.42)</td>
<td>26.02 (±4.56)**</td>
<td>24.88 (±3.82)*</td>
<td>**0.001, * &lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89.19 (±13.92)</td>
<td>86.93 (±12.71)</td>
<td>82.70 (±10.81)</td>
<td>NS</td>
</tr>
<tr>
<td>IPAQ (MET-min/week)</td>
<td>205 (±201)</td>
<td>914 (±248)**</td>
<td>1688 (±374)**</td>
<td>**&lt;0.001, * &lt;0.001, ^&lt;0.001</td>
</tr>
</tbody>
</table>

One way ANOVA, Tukey’s post hoc test, ** denotes significant difference from sedentary, * denotes significant difference from sedentary, ^ denotes significant difference from moderately active.
Table 3.5b Biomolecular and biochemical data obtained from the entire cohort of participants of the cross-sectional study after stratification by physical activity status (sedentary, moderately physically active and highly physically active)

<table>
<thead>
<tr>
<th>Biomolecular and biochemical variables</th>
<th>Sedentary (Mean ±SD)</th>
<th>Moderately active (Mean ±SD)</th>
<th>Highly active (Mean ±SD)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=33</td>
<td>n=53</td>
<td>n=27</td>
<td>P value</td>
</tr>
<tr>
<td>ABCA1 (cf. GAPDH)</td>
<td>1.02 (±0.02)</td>
<td>1.05 (±0.14)</td>
<td>1.03 (±0.03)</td>
<td>NS</td>
</tr>
<tr>
<td>CD36 (cf. GAPDH)</td>
<td>1.01 (±0.01)</td>
<td>1.04 (±0.13)</td>
<td>1.03 (±0.04)</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-9 (cf. GAPDH)</td>
<td>1.01 (±0.01)</td>
<td>1.01 (±0.01)</td>
<td>1.01 (±0.01)</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.95 (±0.86)</td>
<td>5.35 (±1.03)</td>
<td>5.38 (±0.74)</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>32.86 (±14.19)</td>
<td>34.76 (±19.96)</td>
<td>28.35 (±18.96)</td>
<td>NS</td>
</tr>
<tr>
<td>Total-cholesterol (mmol/L)</td>
<td>4.79 (±1.27)</td>
<td>5.66 (±1.76)</td>
<td>5.73 (±1.42)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.33 (±0.58)</td>
<td>1.66 (±0.49)</td>
<td>2.18 (±0.78)^*</td>
<td>*&lt;0.001, ^0.011</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.98 (±1.04)</td>
<td>3.40 (±1.23)</td>
<td>3.03 (±1.38)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.02 (±0.38)</td>
<td>1.32 (±1.18)</td>
<td>1.08 (±0.50)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum IL-6 (pg/mL)</td>
<td>0.91 (±0.39)</td>
<td>0.98 (±0.73)</td>
<td>1.10 (±0.91)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma MMP-9 (ng/mL)</td>
<td>58.92 (±24.73)</td>
<td>42.75 (±19.56)^**</td>
<td>40.50 (±27.09)^*</td>
<td>**0.025, *0.035</td>
</tr>
</tbody>
</table>

One way ANOVA, Tukey’s post hoc test, ** denotes significant difference from sedentary, * denotes significant difference from sedentary, ^ denotes significant difference from moderately active. cf, compared to; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol
Table 3.5c Seated vascular measurements obtained from the entire cohort of participants of the cross-sectional study after stratification by physical activity status (sedentary, moderately physically active and highly physically active)

<table>
<thead>
<tr>
<th>Seated vascular haemodynamic variables</th>
<th>Sedentary Mean (±SD)</th>
<th>Moderately active Mean (±SD)</th>
<th>Highly active Mean (±SD)</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 33</td>
<td>n= 53</td>
<td>n= 27</td>
<td></td>
</tr>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>127 (±13)</td>
<td>130 (±17)</td>
<td>125 (±14)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>86 (±11)</td>
<td>85 (±10)</td>
<td>80 (±8)^*</td>
<td>*0.028, ^0.053</td>
</tr>
<tr>
<td>Peripheral PP (mm Hg)</td>
<td>41 (±9)</td>
<td>45 (±12)</td>
<td>45 (±13)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>100 (±11)</td>
<td>100 (±11)</td>
<td>95 (±9)</td>
<td>NS</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>118 (±14)</td>
<td>119 (±17)</td>
<td>114 (±15)</td>
<td>NS</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>88 (±11)</td>
<td>85 (±10)</td>
<td>81 (±9)*</td>
<td>*0.052</td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>30 (±9)</td>
<td>34 (±13)</td>
<td>33 (±13)</td>
<td>NS</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>98 (±12)</td>
<td>97 (±11)</td>
<td>92 (±9)</td>
<td>NS</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>68 (±11)</td>
<td>63 (±11)</td>
<td>58 (±12)^*</td>
<td>*0.007</td>
</tr>
<tr>
<td>Alx (%)</td>
<td>23 (±12)</td>
<td>23 (±14)</td>
<td>19 (±13)</td>
<td>NS</td>
</tr>
<tr>
<td>Alx @HR75 (%)</td>
<td>19 (±11)</td>
<td>19 (±13)</td>
<td>11 (±14)^*</td>
<td>*0.038, ^0.050</td>
</tr>
</tbody>
</table>

One way ANOVA, Tukey’s post hoc test, ** denotes significant difference from sedentary, * denotes significant difference from sedentary, ^ denotes significant difference from moderately active. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; Alx, augmentation index; Alx@HR75, augmentation index at a heart rate of 75.
Table 3.5d Supine vascular measurements obtained from the entire cohort of participants of the cross-sectional study after stratification by physical activity status (sedentary, moderately physically active and highly physically active).

<table>
<thead>
<tr>
<th>Supine vascular haemodynamic variables</th>
<th>Sedentary Mean (±SD) n= 33</th>
<th>Moderately active Mean (±SD) n= 53</th>
<th>Highly active Mean (±SD) n= 27</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>122 (±13)</td>
<td>123 (±16)</td>
<td>119 (±10)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>80 (±11)</td>
<td>77 (±10)</td>
<td>73 (±7)*</td>
<td>*0.015</td>
</tr>
<tr>
<td>Peripheral PP (mm Hg)</td>
<td>42 (±10)</td>
<td>46 (±14)</td>
<td>46 (±9)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>94 (±11)</td>
<td>92 (±10)</td>
<td>88 (±7)</td>
<td>NS</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>115 (±13)</td>
<td>114 (±14)</td>
<td>110 (±11)</td>
<td>NS</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>82 (±11)</td>
<td>77 (±9)</td>
<td>76 (±7)</td>
<td>NS</td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>33 (±10)</td>
<td>37 (±13)</td>
<td>34 (±8)</td>
<td>NS</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>93 (±11)</td>
<td>90 (±9)</td>
<td>87 (±7)</td>
<td>NS</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>61 (±9)</td>
<td>57 (±9)</td>
<td>56 (±7)</td>
<td>NS</td>
</tr>
<tr>
<td>Alx (%)</td>
<td>26 (±13)</td>
<td>28 (±11)</td>
<td>21 (±16)</td>
<td>NS</td>
</tr>
<tr>
<td>Alx @HR75 (%)</td>
<td>20 (±13)</td>
<td>20 (±11)</td>
<td>12 (±16)</td>
<td>NS</td>
</tr>
<tr>
<td>CRPWV (m/s)</td>
<td>7.1 (±1.3)</td>
<td>6.8 (±1.1)</td>
<td>7.2 (±1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>MAP-adjusted CRPWV (m/s)</td>
<td>7.0 (±1.2)</td>
<td>6.9 (±1.0)</td>
<td>7.3 (±1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>CFPWV (m/s)</td>
<td>7.0 (±1.4)</td>
<td>6.8 (±1.5)</td>
<td>6.9 (±1.5)</td>
<td>NS</td>
</tr>
<tr>
<td>MAP-adjusted CFPWV (m/s)</td>
<td>6.7 (±1.0)</td>
<td>6.8 (±1.1)</td>
<td>6.7 (±1.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

One way ANOVA, Tukey’s post hoc test, ** denotes significant difference from sedentary, * denotes significant difference from sedentary, ^ denotes significant difference from moderately active. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; Alx, augmentation index; Alx@HR75, augmentation index at a heart rate of 75; CRPWV, carotid radial pulse wave velocity; CFPWV, carotid femoral pulse wave velocity.
Table 3.5e Measures of mental health obtained from a subgroup of the participants of the cross-sectional study after stratification by physical activity status (sedentary, moderately physically active and highly physically active)

<table>
<thead>
<tr>
<th>Measure of mental health (Range)</th>
<th>Sedentary Mean (±SD, n=25)</th>
<th>Moderately active Mean (±SD, n=28)</th>
<th>Highly active Mean (±SD, n=10)</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-SES (0-30)</td>
<td>15.28 (±1.99)</td>
<td>16.75 (±1.99)**</td>
<td>16.70 (±1.42)</td>
<td>**0.019</td>
</tr>
<tr>
<td>CFQ (0-100)</td>
<td>43.36 (±10.88)</td>
<td>35.68 (±10.21)**</td>
<td>41.30 (±11.52)</td>
<td>**0.045</td>
</tr>
<tr>
<td>STAI (20-80)</td>
<td>39.00 (±9.80)</td>
<td>35.92 (±8.25)</td>
<td>39.60 (±8.37)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-attachment (4-16)</td>
<td>13.38 (±2.67)</td>
<td>14.04 (±2.42)</td>
<td>14.60 (±1.58)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-social integration (4-16)</td>
<td>13.14 (±1.80)</td>
<td>14.20 (±1.98)</td>
<td>12.80 (±1.75)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-reassurance of worth (4-16)</td>
<td>12.48 (±2.18)</td>
<td>12.88 (±2.13)</td>
<td>12.00 (±2.45)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-reliable alliance (4-16)</td>
<td>13.81 (±1.99)</td>
<td>14.56 (±1.92)</td>
<td>14.80 (±1.32)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-guidance (4-16)</td>
<td>13.24 (±2.72)</td>
<td>14.36 (±2.10)</td>
<td>14.70 (±1.89)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-opportunity for nurturance (4-16)</td>
<td>12.52 (±2.96)</td>
<td>12.32 (±2.91)</td>
<td>12.70 (±1.95)</td>
<td>NS</td>
</tr>
<tr>
<td>Perceived Stress Scale (0-40)</td>
<td>18.10 (±6.58)</td>
<td>15.56 (±8.02)</td>
<td>17.30 (±4.32)</td>
<td>NS</td>
</tr>
<tr>
<td>CES-D (0-60)</td>
<td>15.29 (±9.05)</td>
<td>12.75 (±9.07)</td>
<td>11.00 (±4.47)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-tension anxiety (0-36)</td>
<td>9.19 (±5.68)</td>
<td>8.32 (±4.96)</td>
<td>10.70 (±5.08)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-depression dejection (0-60)</td>
<td>12.38 (±6.43)</td>
<td>12.52 (±8.90)</td>
<td>10.20 (±6.21)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-anger hostility (0-48)</td>
<td>7.90 (±4.93)</td>
<td>7.72 (±5.44)</td>
<td>12.00 (±5.42)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-vigour activity (0-32)</td>
<td>13.76 (±8.06)</td>
<td>17.28 (±7.01)</td>
<td>17.50 (±6.49)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-fatigue inertia (0-28)</td>
<td>9.76 (±6.31)</td>
<td>9.56 (±7.04)</td>
<td>11.00 (±4.99)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-confusion bewilderment (0-28)</td>
<td>8.00 (±4.76)</td>
<td>7.20 (±4.74)</td>
<td>8.30 (±2.87)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-total mood disturbance (-32-200)</td>
<td>33.48 (±26.73)</td>
<td>28.04 (±27.58)</td>
<td>34.70 (±20.32)</td>
<td>NS</td>
</tr>
</tbody>
</table>

One way ANOVA, Tukey’s post hoc test, ** denotes significant difference from sedentary, * denotes significant difference from sedentary, ^ denotes significant difference from moderately active.  R-SES, Rosenberg Self-Esteem Scale; CFQ, Cognitive Failures Questionnaire; STAI, State-Trait Anxiety Inventory; SPS, Social Provisions Scale; CES-D, Center for Epidemiologic Studies-Depression; POMS, Profile of Mood States.
3.3.6 Correlational analyses

Bivariate correlations were run to investigate whether any associations existed between level of exercise (as determined using IPAQ MET-minutes) and the measured parameters within the three areas of interest of the current study, or between the individual parameters measured within each area of interest. The correlations involving the IPAQ are confirmatory analyses, as the associations were predicted based on the evidence discussed in Chapter 1, therefore, despite a large number of correlation analyses being conducted, the level of significance remains at $P<0.05$.

A large number of analyses were conducted investigating the mental health measures with i) the blood-borne markers of CV risk, and ii) the vascular haemodynamic markers of risk. The associations between the mental health markers and blood-borne markers were exploratory as opposed to confirmatory (apart from any mental health associations with IL-6, due to the data associating stress and IL-6), therefore levels of significance were adjusted so that $P<0.05$ signifies a trend in the data, $P<0.005$ signifies significant associations, and $P<0.001$ signifies highly significant associations. The associations were exploratory as they weren’t predicted in the literature. Due to the literature and hypothesis regarding mental health, SNS activity and blood pressure; associations between mental health and vascular health were confirmatory, and therefore, $P$ values were not adjusted.

3.3.6.1 Correlations involving biomolecular/biochemical data

There was a significant correlation between IPAQ MET-minutes and HDL-cholesterol; ($r=0.406$, $P<0.001$). This was the only significant correlation observed between IPAQ MET-minutes and the biomolecular/biochemical markers. (Data not presented)

However, it should be noted that significant correlations were observed within the biomolecular/biochemical dataset: leukocyte expression levels of ABCA1 and CD36 were significantly and positively correlated ($r=0.964$, $P<0.001$), as were blood-borne levels of glucose and insulin ($r=0.411$, $P<0.001$), and IL-6 and insulin ($r=0.234$, $P=0.053$).
Circulating levels of MMP-9 were significantly associated with total-cholesterol ($r = 0.295$, $P = 0.010$) and LDL-cholesterol ($r = 0.260$, $P = 0.026$). Triglyceride levels were significantly associated with total-cholesterol ($r = 0.557$, $P < 0.001$), LDL-cholesterol ($r = 0.421$, $P < 0.001$), glucose ($r = 0.299$, $P = 0.009$) and insulin ($r = 0.404$, $P < 0.001$). Glucose was also significantly associated with LDL-cholesterol ($r = 0.463$, $P < 0.001$). (Data not presented)

Significant correlations were observed within the vascular haemodynamic data set and within the mental health markers data set, however, these correlations were expected due to co-linearity within both sets of measures (Wilkinson et al., 2002b, Wilkinson et al., 2000b, Bovier et al., 2004, Bridger et al., 2013).

Significant correlations were observed between biomolecular/biochemical parameters and i) mental health measures, and ii) several vascular haemodynamic measures (see Table 3.6a and 3.6b, respectively). For example, plasma levels of MMP-9 had a significant and positive association with STAI (state anxiety measure); $r = 0.313$, $P = 0.015$, and also with the measure of total mood disturbance (POMS TMD); $r = 0.432$, $P = 0.002$.

Table 3.6a Selected correlations between biomolecular/biochemical and mental health measures

<table>
<thead>
<tr>
<th>Plasma MMP-9 (ng/mL)</th>
<th>Mental health measure</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAI</td>
<td></td>
<td>0.313</td>
<td>0.015</td>
</tr>
<tr>
<td>POMS confusion-bewilderment</td>
<td></td>
<td>0.281</td>
<td>0.046</td>
</tr>
<tr>
<td>POMS vigour-activity</td>
<td></td>
<td>-0.317</td>
<td>0.024</td>
</tr>
<tr>
<td>POMS tension-anxiety</td>
<td></td>
<td>0.338</td>
<td>0.015</td>
</tr>
<tr>
<td>POMS TMD</td>
<td></td>
<td>0.432</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma glucose (mmol/L)</th>
<th>R-SES</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.287</td>
<td>0.037</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma insulin (pmol/L)</th>
<th>R-SES</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-SES</td>
<td>-0.276</td>
<td>0.053</td>
</tr>
<tr>
<td>POMS vigour-activity</td>
<td>-0.311</td>
<td>0.040</td>
</tr>
<tr>
<td>POMS fatigue-inertia</td>
<td>0.359</td>
<td>0.017</td>
</tr>
<tr>
<td>POMS TMD</td>
<td>0.326</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Bivariate correlations using Pearson’s method observed between fasting levels of plasma MMP-9, fasting levels of serum IL-6, and fasting levels of plasma glucose, with markers of mental health.
Table 3.6b Selected correlations between biomolecular/biochemical markers and vascular measures of CV risk

<table>
<thead>
<tr>
<th>Vascular haemodynamic measure</th>
<th>Serum IL-6 (pg/mL)</th>
<th>Plasma MMP-9 (ng/mL)</th>
<th>Plasma glucose (mmol/L)</th>
<th>Total-cholesterol (mmol/L)</th>
<th>HDL-cholesterol (mmol/L)</th>
<th>LDL-cholesterol (mmol/L)</th>
<th>Triglycerides (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seated Alx (%)</td>
<td>0.403</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seated HR (bpm)</td>
<td>0.267</td>
<td>0.039</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine HR (bpm)</td>
<td>0.425</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placed Alx@HR75 (%)</td>
<td>0.421</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral seated SBP (mm Hg)</td>
<td>0.248</td>
<td>0.035</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral seated PP (mm Hg)</td>
<td>0.298</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seated Alx (%)</td>
<td>0.241</td>
<td>0.051</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral supine SBP (mm Hg)</td>
<td>0.339</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral supine PP (mm Hg)</td>
<td>0.261</td>
<td>0.029</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central supine SBP (mm Hg)</td>
<td>0.264</td>
<td>0.054</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral seated SBP (mm Hg)</td>
<td>0.248</td>
<td>0.036</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral seated PP (mm Hg)</td>
<td>0.349</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral supine PP (mm Hg)</td>
<td>0.265</td>
<td>0.026</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seated HR (bpm)</td>
<td>-0.349</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central seated PP (mm Hg)</td>
<td>0.309</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central supine SBP (mm Hg)</td>
<td>0.326</td>
<td>0.016</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral supine DBP (mm Hg)</td>
<td>-0.236</td>
<td>0.053</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seated HR (bpm)</td>
<td>-0.327</td>
<td>0.008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral seated SBP (mm Hg)</td>
<td>0.269</td>
<td>0.025</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral seated PP (mm Hg)</td>
<td>0.328</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral supine SBP (mm Hg)</td>
<td>0.241</td>
<td>0.050</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral supine PP (mm Hg)</td>
<td>0.299</td>
<td>0.014</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seated HR (bpm)</td>
<td>-0.286</td>
<td>0.022</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central seated PP (mm Hg)</td>
<td>0.282</td>
<td>0.024</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central supine SBP (mm Hg)</td>
<td>0.340</td>
<td>0.014</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central supine PP (mm Hg)</td>
<td>0.332</td>
<td>0.016</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral seated SBP (mm Hg)</td>
<td>0.279</td>
<td>0.017</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral seated MAP (mm Hg)</td>
<td>0.258</td>
<td>0.029</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral supine SBP (mm Hg)</td>
<td>0.298</td>
<td>0.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral supine MAP (mm Hg)</td>
<td>0.277</td>
<td>0.020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central seated SBP (mm Hg)</td>
<td>0.273</td>
<td>0.026</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central seated DBP (mm Hg)</td>
<td>0.247</td>
<td>0.044</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central seated MAP (mm Hg)</td>
<td>0.276</td>
<td>0.024</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central supine SBP (mm Hg)</td>
<td>0.321</td>
<td>0.018</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson’s method observed between fasting levels of serum IL-6 and fasting levels of plasma glucose, with vascular markers of CV risk. Alx; augmentation index; HR, heart rate; PP, pulse pressure; SBP; systolic blood pressure; DBP, diastolic blood pressure;
3.3.6.2 Correlations involving vascular haemodynamic data

Table 3.7a displays significant inverse correlations between IPAQ MET-minutes and seated peripheral DBP, Alx, Alx@HR75, central DBP and central MAP. Similarly, Table 3.7b displays significant inverse correlations between IPAQ MET-minutes and supine peripheral DBP, peripheral MAP, heart rate, central DBP and Alx@HR75.

Also, as shown in Tables 3.6b and 3.7c, significant correlations were observed between vascular haemodynamic measures, i) with several biomolecular/biochemical, and ii) with mental health measures, respectively. For example, seated Alx@HR75 was significantly correlated with circulating levels of MMP-9 \( (r = 0.421, \ P = 0.003) \), the inflammatory marker IL-6 and glucose both had a significant and positive association with seated Alx \( (r = 0.403, \ P = 0.001, \text{ and } r = 0.241, \ P = 0.051, \text{ respectively}) \). An association was observed between state anxiety (as measured using the STAI) and MAP adjusted peripheral PWV \( (r = 0.298, \ P = 0.035) \), and MAP adjusted aortic PWV was observed to have an association with the anger-hostility construct of mood (measured using the POMS questionnaire); \( r = 0.303, \ P = 0.036 \).

Table 3.7a Selected correlations between IPAQ MET-minutes and seated vascular haemodynamics

<table>
<thead>
<tr>
<th>Vascular haemodynamic measure (seated)</th>
<th>( r )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>-0.252</td>
<td>0.007</td>
</tr>
<tr>
<td>Alx@HR75 (%)</td>
<td>-0.256</td>
<td>0.010</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>-0.333</td>
<td>0.001</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>-0.272</td>
<td>0.006</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>-0.212</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson’s method observed between MET-minute/week and seated vascular markers of CV risk. DBP, diastolic blood pressure; Alx@HR75, augmentation index corrected for heart rate of 75 bpm; HR, heart rate; MAP, mean arterial pressure
Table 3.7b Selected correlations between IPAQ MET-minutes and supine vascular haemodynamics

<table>
<thead>
<tr>
<th>Vascular haemodynamic measure (supine)</th>
<th>( r )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>-0.246</td>
<td>0.011</td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>-0.207</td>
<td>0.033</td>
</tr>
<tr>
<td>AIx@HR75 (%)</td>
<td>-0.222</td>
<td>0.043</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>-0.276</td>
<td>0.013</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>-0.216</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson's method observed between MET-minute/week and supine vascular markers of CV risk. DBP, diastolic blood pressure; MAP, mean arterial pressure; AIx@HR75, augmentation index corrected for heart rate of 75 bpm; HR, heart rate
Table 3.7c Selected correlations between measures of vascular haemodynamics and markers of mental health

<table>
<thead>
<tr>
<th>Bivariate correlations</th>
<th>CFQ</th>
<th>STAI</th>
<th>CES-D</th>
<th>POMS depression-dejection</th>
<th>POMS anger-hostility</th>
<th>POMS vigour-activity</th>
<th>POMS confusion-bewilderment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral supine DBP (mm Hg)</td>
<td>NS</td>
<td>NS</td>
<td>0.300 (0.036)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Central supine DBP (mm Hg)</td>
<td>0.274 (0.049)</td>
<td>NS</td>
<td>0.312 (0.033)</td>
<td>NS</td>
<td>NS</td>
<td>-0.272 (0.054)</td>
<td>NS</td>
</tr>
<tr>
<td>Seated Alx (%)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.267 (0.047)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Seated Alx@HR75 (%)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.277 (0.039)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Supine Alx (%)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.342 (0.011)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Supine Alx@HR75 (%)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.330 (0.015)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MAP adjusted CRPWV (m/s)</td>
<td>NS</td>
<td>0.298 (0.035)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MAP adjusted CFPWV (m/s)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.303 (0.036)</td>
<td>NS</td>
<td>0.318 (0.028)</td>
</tr>
</tbody>
</table>

Bivariate correlations using Pearson’s method observed between vascular measures of CV risk and markers of mental health. NS denotes correlations which did not reach statistical significance. CFQ; Cognitive Failures Questionnaire; STAI, State Trait Anxiety Inventory; CES-D, Centre for Epidemiologic Studies-Depression; POMS, Profile of Mood States; DBP, diastolic blood pressure; Alx, augmentation index; Alx@HR75, augmentation index corrected for heart rate of 75 bpm; MAP, mean arterial pressure; CRPWV, carotid radial pulse wave velocity; CFPWV, carotid femoral pulse wave velocity.
3.3.6.3 IPAQ MET-minutes and mental health

As shown in Table 3.8a, significant and positive correlations were observed between IPAQ MET-minutes and the Rosenberg Self-Esteem Scale, and between IPAQ MET-minutes and the vigour-activity construct of the POMS questionnaire.

Moreover, as stated above, it should be noted that correlations were also observed between certain measures of mental health and biomolecular/biochemical measures (see section 3.3.6.1 and Table 3.6a), and between mental health and measures of vascular haemodynamic measures (see section 3.3.6.2 and Table 3.7c).

Table 3.8a Selected correlations between IPAQ MET-minutes and markers of mental health

<table>
<thead>
<tr>
<th>IPAQ MET-minute/week score</th>
<th>Mental health measure</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-SES</td>
<td>0.324</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>POMS vigour-activity</td>
<td>0.288</td>
<td>0.031</td>
<td></td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson’s method observed between MET-minutes/week and markers of mental health. R-SES, Rosenberg Self Esteem Scale; POMS, Profile of Mood States
3.4 Discussion

The major findings of interest of the current study were the lower levels of MMP-9 in the regularly physically active groups, and also the fact that i) the marker of wave reflection, AIx, was also lower in the regularly physically active groups compared to the sedentary group, and ii) that the levels of MMP-9 and AIx were significantly associated. These were considered the major findings of interest due to their novelty; as far as the current author is aware, no investigations previously have demonstrated the association between levels of exercise and levels of MMP-9 in a healthy, mixed gender cohort, or demonstrated a significant association between MMP-9 and wave reflection.

AIx has previously been observed to be lower in regularly active participants (< 50 years) in a large cross-sectional study (McDonnell et al., 2013). The findings of the current cross-sectional study support those of McDonnell and colleagues, and further to this, the associated reduction in MMP-9 may explain part of the mechanism behind this exercise-associated reduction in markers of arterial stiffness, and hence CV risk. MMP-9 is present at sites of arterial inflammation, and inflammation has been observed to be associated with increased AIx (Booth et al., 2004, Kampus et al., 2006) and therefore the association may be due to an exercise-associated improvement in inflammation and hence wave reflection. However, IL-6, more frequently used as a measure of inflammation than MMP-9, did not differ between the three groups of the current study. However, IL-6 did exhibit a significant correlation with AIx (HR-uncorrected). A second mechanism by which MMP-9 may be associated with exercise-associated improvements in wave reflection should be borne in mind: MMP-9 may play a role in delaying sites of impedance mis-match in the arterial tree from becoming more proximal, i.e. delaying the progression of elastic arteries into becoming more muscular due to an MMP-9 mediated elastin breakdown. Hence, a down-regulation of MMP-9 may result in less elastin degradation and hence slower age-related stiffening of the arterial wall, and therefore a slowing in the age-associated early return of the reflected wave which contributes to increased CV risk. In addition to demonstrating an exercise-associated
reduction in AIx in physically active, relatively young participants, the current study also supported similar data by McDonnell et al. (2013) by demonstrating reduced peripheral DBP in the relatively young, physically active participants.

These data were collected from a cohort with a mean age of 43 years, a quarter of whom were male. The males on average had a waist circumference of approximately 93cm and a BMI of 27kg/m². In this population, the recommended waist circumference for males is no more than 94cm, and a BMI of no more than 25. Therefore, the males of the current study are just within the healthy range for abdominal obesity (NHS choices, 2012b), but outside the healthy range for BMI (NHS choices, 2014). The female participants in the cohort had an average waist circumference of approximately 84cm, which is 4cm greater than the recommended waist circumference for females, and a BMI of 27kg/m²; also above the recommended BMI of 25. Therefore, in terms of the BMI measure of obesity, both the males and females in the cohort had increased CV risk. On average, approximately 84% of male participants reported regularly exercising, whilst 64% of females reported regular exercise (an overall 69.2% of males and females reported being regularly physically active). These self-report exercise frequencies are relatively in keeping with the levels reported by the Welsh Health Survey (NatCen Social Research, 2012). The survey demonstrated that 13% of Welsh adults reported doing no physical activity and that 21% had done no more than light activity. Therefore, when considering the cohort as a whole, the participants were fairly representative of the general population in terms of exercise levels.

This cross-sectional study illustrated that there were significant differences in biomolecular and biochemical parameters of CV risk between the sedentary participants and the physically active participants for the levels of HDL-cholesterol, with HDL-C levels being higher in the highly physically active group compared to the moderately active group and the sedentary group. The levels of circulating MMP-9 also significantly differed across the groups. Both the moderately active and highly active groups had significantly lower levels than participants in the sedentary cohort. Based on these data, it is possible that reduced
expression of MMP-9 in the physically active groups may be associated with slower elastin degradation in the arterial walls of the physically active cohorts than in those of the sedentary cohort (Yasmin et al., 2005, Lau et al., 2008). Surprisingly, increases in HDL-cholesterol levels were not associated with the expression of ABCA1, which plays a key role in the formation of HDL. However, serum samples (and hence HDL-cholesterol samples) were obtained from both methods of participant recruitment, whereas, mRNA samples (and hence ABCA1 expression) were not obtained from the health MOT method of recruitment. Therefore, the lack of association of HDL-cholesterol levels with the HDL-C transporter (ABCA1) may be due to insufficient participant numbers. Data regarding lipid profile and exercise in the literature is contrasting, with no single study appearing to demonstrate an exercise associated improvement in total-cholesterol, HDL-C LDL-C and triglyceride levels, and therefore, the current study is comparable to existing literature (Durstine et al., 2002, Durstine et al., 2001). Specifically, Butcher et al. (2008) only demonstrated an exercise-associated increase in HDL-cholesterol levels and decrease in total-cholesterol levels.

Blood-borne levels of insulin in the highly active group were lower than in the sedentary and moderately active group, however, this effect did not prove to be significant. As the cohort size was relatively small for a cross-sectional investigation observing the effect of exercise intensities on a diverse range of CV risk factors, the difference may have reached significance in a larger cohort with less error. Further, high-intensity exercise in young females has been shown to reduce fasting insulin levels following a 15-week intervention, with no change in the moderate-intensity, steady state exercise group, despite both groups demonstrating improvements in CV fitness (Trapp et al., 2008). Trends were also observed in levels of glucose and IL-6 as both factors were higher in the physically active group than the other two groups, however, these differences did not prove to be statistically significant either.

Broadly, the seated vascular data, i.e. peripheral DBP, central DBP, HR and HR corrected AIx illustrated significantly lower levels in the physically active groups compared to the
sedentary group. Central and peripheral MAPs were lowest in the highly active group, but differences in these parameters did not reach statistical significance. Supine vascular data displayed similar findings; peripheral DBP was significantly lower in the highly active group and peripheral MAP was nearing statistical significant difference from the sedentary group. Central SBP, DBP and MAP and HR were lowest in in the highly active group, but these differences did not reach statistical significance. The same is true of Alx, and of Alx@HR75. Collectively, these data suggest that being regularly physically active has beneficial effects on vascular haemodynamics that are related to CV risk. MAP estimates the steady component of blood pressure, which is a function of vascular resistance and elasticity over time (Sesso et al., 2000, Safar, 1989, Benetos et al., 1997a), while Alx provides surrogate data for arterial stiffness; hence, lower levels of MAP and Alx suggest that physical activity in these participants is associated with improved functioning of the peripheral and resistance arteries, and hence a reduction in CV risk. It is suggested that these improvements occurred as a result of exercise-associated improved endothelial function and/or an exercise associated slowing of stiffening of the arterial wall, possibly due to an exercise-mediated reduction in MMP-9.

Interestingly, regular exercise illustrated beneficial associations with constructs of mental health. In the moderately active group, the Rosenberg Self-Esteem Scale was significantly higher than in the sedentary group and these data suggest that the moderately active group had more favourable levels of self-esteem compared to the sedentary group. Improved exercise-associated self-esteem may be mediated by an improvement in self-efficacy as a result of participants feeling like they are able to achieve goals and tasks as a result of meeting exercise-associated goals. The CFQ was also lower in the moderately active group than the sedentary group, suggesting fewer cognitive failures in the moderately active group (and, hence, better cognitive functioning), perhaps as a result of exercise-associated improved blood flow to the brain (Brown et al., 2010). These two results were the only statistically significant results; however, trends in the data that did not reach significance.
include lower levels of anxiety and lower levels of stress in the moderately active group. Depression and POMS depression-dejection were non-significantly lower (and therefore also improved) in the highly active group compared to the sedentary. The vigour-activity mood construct of POMS was higher in both of the physically active groups compared to the sedentary group, suggesting regular exercise has positive effects on this positive subscale of mood. However, for the mental health measures, the cohort size of the highly physically active group was $n=10$. This creates difficulties when attempting to infer much from the data and it also creates difficulties when attempting to compare the data from this group with the sedentary group and the moderately active group.

Bivariate correlations were conducted to determine whether any significant associations existed between exercise levels and the CV risk-associated markers of interest. Correlation analyses were also conducted to investigate whether any significant associations existed between the three areas of interest. Caution must be used when concluding from the correlations due to a relatively small cohort size and relatively low effect sizes for many of the associations. IPAQ MET-minutes correlated significantly and negatively with peripheral seated DBP, seated Alx@HR75, heart rate and central seated DBP and MAP. It also significantly correlated with peripheral supine DBP and MAP, heart rate, central supine DBP and supine Alx@HR75. These data suggest that those with higher levels of physical activity have lower values of vascular haemodynamic measures that are related to CV risk. Possibly linked to these observations is the significant positive correlation between IPAQ MET-minutes and HDL-cholesterol, which may suggest that participation in exercise exerts anti-atherogenic effects on the vascular system, and hence impacts favourably upon vascular haemodynamics.

Similarly, IPAQ MET-minutes were significantly and positively correlated with self-esteem and the vigour-activity construct of mood. These data suggest that those with higher levels of physical activity have better self-esteem and higher levels of vigour-activity. Thus, suggesting that regular exercise is associated with a greater sense of vitality and energy.
There were a large number of correlations observed between the three different areas of health that were assessed. With regard to biomolecular/biochemical measures, ABCA1 and CD36 were very strongly associated, suggesting evidence of the key role they both play in the RCT system and that they are both regulated at the transcription level by activation of PPARγ, as discussed in Chapter 1. Similarly, blood-borne levels of glucose and IL-6 were also related to a number of vascular measures, suggesting a relationship between biochemical measures of CV risk and vascular measures of CV risk. Finally, MMP-9 expression exhibited associations with several measures of mental health (see data in Table 3.6a). These data are interesting as MMP-9 is a marker of inflammation (as previously discussed in Chapter 1), and the inflammatory marker IL-6 has previously been observed to be associated with psychological stress (as discussed in Chapter 1). It would be interesting to investigate the levels of MMP-9 in response to psychological stress-inducing tasks in order to determine whether there is a relationship between the two.

Measures of vascular haemodynamic and markers of mental health also exhibited numerous associations, suggesting that improved mental health is related to improved vascular function and therefore reduced CV risk. For example, it was interesting to observe a significant and positive relationship between MAP adjusted CF-PWV and the POMS mood construct ‘anger-hostility’, which suggested that lower levels of this mood construct was associated with lower levels of aPWV and therefore improved arterial stiffness. These data could tenuously suggest that over time, prolonged feelings such as anger and hostility could be associated with increases in pressure in the arteries and hence contribute to shear stress and large artery stiffness (possibly mediated by SNS activation), thereby suggesting a mechanism between poor mental health and CV risk. However, further work is clearly needed before this speculative suggestion can be confirmed.

These data appear to enable discrimination between being physically active versus sedentary in the measures of CV risk associated health that were assessed. Specifically, it appears that there is an important distinction between being moderately active and highly
active, in that highly active participants had greater improvements than moderately active participants with regard to vascular measures of CV risk (compared to sedentary in both cases). Conversely, being a moderately physically active participant appeared to convey more significant beneficial effects on markers of mental health than in the highly active participants. The observed association between the POMS mood construct of anger-hostility with arterial stiffness (aPWV) could suggest that increased anger-hostility levels could be associated with increased sympathetic activity and hence link mental health with vascular measures of CV risk. However, further investigation would be required in order to clarify this tenuous suggestion.

It should be noted that the data reported in this chapter are in agreement with much of the exercise-associated literature that exists regarding the measures employed in the current study. For example, exercise programmes have previously been observed to be associated with a reduction in circulating levels of MMP-9 (Roberts et al., 2007, Roberts et al., 2006). However, these two studies were carried out in children with atherosclerotic risk factors and men with metabolic syndrome, and further to this, the studies were both 'exercise plus diet' interventions. Therefore, the current study appears to be the first to show an exercise-associated improvement in levels of MMP-9 in a mixed gender, middle-aged, healthy population. McDonnell et al. (2013) demonstrated in a cohort aged < 50 years that regular exercise was associated with lower levels of AIx. The mean age of the current cohort was 43 years, the age at which AIx is the appropriate marker of stiffness, as opposed to aPWV (McEniery et al., 2005). Further to this, aPWV did not differ between the regularly physically active groups and the sedentary group, also in agreement with data on the younger participants of the McDonnell et al. study.

Importantly, however, the sample size was relatively small, and it may have been possible to draw more robust conclusions and to have observed more significant differences in the data had there been a larger cohort. The observed correlations in general are quite weak, and therefore also need to be approached with caution when drawing conclusions. Further to
this, the study was cross-sectional in design and therefore it is difficult to conclude any cause and effect regarding the exercise and the markers of CV risk assessed. In addition, there are limitations in the current study with regard to the exercise data that was collected from the participants and was consequently used to split participants in to exercise groups. More accurate representation of the exercise undertaken by each participant (and hence more accurate grouping of the participants) would have occurred had it been possible to fit each participant with an accelerometer for a period of three days. This would have enabled the accurate quantification of the average amount, intensity and duration of the exercise that each participant undertook.

Despite these limitations, the data presented in this chapter are broadly suggestive of an effect of exercise participation on the measures selected and, based on the literature discussed in Chapter 1, it would be interesting to use these measures in order to assess the synergistic impact of green-exercise on systemic indices of health. Specifically, there is a need to determine whether a relatively unstructured and low intensity green-exercise programme is a strong enough stimulus to evoke changes in the exercise-associated parameters that were investigated in the current cross-sectional study, and perhaps also to enable greater identification of cause and effect phenomena than the current cross-sectional study allows. Given that correlations were seen to exist between the different arms of the research project, it would also be interesting to see if these relationships manifest when sedentary participants take up a green-exercise programme.

Hence, the fourth chapter of the current thesis will provide an account of a similar multi-disciplinary study to that described in the current chapter, but with the important distinction that the study described in the subsequent chapter was undertaken on a longitudinal rather than cross-sectional basis – i.e. it aimed to evaluate the impact of a specific 8-week 'green-exercise' intervention on the parameters under investigation.
Chapter 4 -

Green-Exercise Intervention Study
4.1 Introduction

As discussed in Chapter 1, there is a large and growing body of evidence that demonstrates the beneficial impact of exercise interventions upon markers of mental health and CV risk in previously sedentary participants (Manson et al., 2002, Penedo and Dahn, 2005, Sigal et al., 2006, McDonnell et al., 2013). Furthermore, the data obtained in the cross-sectional study (Chapter 3 of the current PhD project) further supports existing literature within these subject areas. Chapter 1 also discussed evidence that suggests a synergistic effect of exercising whilst in a natural environment (Pretty et al., 2005a, Pretty et al., 2005b). This evidence-base is somewhat limited, and is largely based upon measuring the impact of a single, acute bout of green-exercise on mental health and blood pressure; importantly, however, the impact of an extended (e.g. 8-week) green-exercise programme upon these parameters and other health-related measures (specifically, CV risk associated measures) has not before been investigated.

As discussed in Chapter 1, the south Wales valleys presents an area of low SES where inhabitants have little disposable income to spend on costs associated with exercise, such as trainers, clothing, equipment, and travel to the place of exercise such as the gym or swimming pool (The Office for National Statistics, 2011). The area is composed of much green space and therefore presents a potential salutogenic environment (Caerphilly County Borough Council, 2011), but it is an area with low physical activity levels and poor health statistics (The Office for National Statistics, 2013b, The Office for National Statistics, 2013a), and therefore the access to green space may be a useful tool to combat both. It is thus important to determine whether increasing physical activity levels via green-exercise in these areas has a significant impact upon the aspects of health under investigation in this thesis, and therefore whether green-exercise interventions may have potential as tools for alleviating the poor health experienced by the inhabitants of these areas.

As the data from the cross-sectional study demonstrated, regular physical activity is associated with improved markers of CV risk and mental health, compared to data obtained from participants who did not regularly partake in exercise. Therefore the current chapter
aimed to determine whether participation in an 8-week green-exercise programme would elicit similar changes to those differences observed to exist between sedentary and physically active participants in the cross-sectional study of Chapter 3; i.e. the study aimed to determine whether adherence to an 8-week green-exercise programme alters the markers of CV risk that were observed to be improved with regular exercise in Chapter 3.

Thus, the hypothesis to be tested in the current chapter was that in a previously sedentary cohort, a low/moderate intensity green-exercise walking programme would be associated with improvements in the markers associated with CV risk employed in the current study.

### 4.2 Methods

The methodology employed in the current intervention study was outlined in detail in Chapter 2. Specifically, the biomolecular and biochemical measurements were carried out as described in section 2.2, the anthropometric measurements were carried out as described in section 2.3, the vascular haemodynamic measurements were carried out as described in section 2.4, and the measures of mental health were carried out as described in section 2.5. Briefly, as described in Chapter 2, sedentary participants were recruited and underwent a data collection consultation at baseline and again following the 8-week green-exercise programme that they had registered with at baseline. The measures of health assessed in the data collection consultations were all associated with CV risk.

Participants only included those who had registered to begin a green-exercise walking programme or challenge within the regions of south Wales and the south Wales valleys. As described in detail in section 2.1, the green-exercise intervention that was employed involved recruiting participants who indicated that they undertook no structured exercise at baseline but were about to embark on a green-exercise programme. These programmes took the form of community-based and work-place based outdoor walking groups that took place in natural, green environments. The specific sites of recruitment were based at Pontlottyn, Llanhilleth, Pontypridd, Cardiff Metropolitan University, and three Cardiff Council sites. All participants arrived at the group meeting place via a very short walk from their
home or workplace, and all walks took place in rural, natural and green settings. Each walking group had a walk leader who was involved in keeping a registration of each walk and planning the walking routes. The intensity of the walks wasn't controlled as it was an evaluation of a 'real-life' exercise programme and the project aimed not to influence the walks undertaken.

In addition to what has previously been described, a subgroup of participants wore a BodyMedia SenseWear advanced accelerometer arm band (BodyMedia SenseWear PA, USA) which allowed for quantification of variables such as MET intensity and steps walked. This was undertaken in order to determine the intensity of the walks.

### 4.2.1 Power calculation

In order to test the study hypothesis, a power calculation was performed in order to ensure that an appropriate number of participants were recruited on to the current 8-week exercise study. Due to its highly significant clinical relevance and usefulness in predicting CV risk (as discussed in Chapter 1), aPWV was used as the primary variable in the power calculation:

\[
10.5 \times 1.17^2 \times 2
\]

\[
0.7^2
\]

\[
10.5 = \text{Constant used to obtain a significance level of 0.05 with a power of 0.90},
\]

\[
1.17 = \text{SD from repeated measure aortic PWV},
\]

\[
0.7 = \text{a clinically significant difference in aPWV}.
\]

Based on a standard deviation of differences between repeated measurements of aortic pulse wave velocity of 1.17 m/s (taken from the Anglo Cardiff Collaboration Trial (ACCT) study database of > 2, 500 participants), the power calculation demonstrates that 59
participants will provide a 90% chance of detecting a 0.7 m/s difference in aPWV, at the 0.05 significance level. A change in aPWV below 0.7 m/s is unlikely to be clinically or physiologically relevant.

4.2.2 Statistics

Data are expressed as mean (±SD), unless otherwise stated. All analyses were performed using SPSS version 20 (SPSS Inc., Ill, USA), except analysis of ELISA data, which was carried out using GraphPad Prism 5 software (GraphPad Software Inc., CA, USA). Independent sample t-tests were used to compare delta (Δ) changes (from baseline to 8-weeks) between the two groups that emerged following stratification of the cohort (see section 4.3.2.1). Bivariate correlation analyses were also performed using Pearson’s method. Statistical significance was set to a priori $P \leq 0.05$ (unless otherwise stated); all other results were reported as non-significant (NS).

4.3 Results

4.3.1 General demographic data obtained from the cohort of participants from the intervention study

Table 4.1 displays demographic data obtained from the whole cohort; $n=65$ participants. The mean age was 44.5 years, with the majority of participants being female (~85%) and Caucasian (~99%). 3.3% of participants were current smokers (below the England and Wales average, according to the Office for National Statistics), and on average participants had visited their GP twice in the last 12 months (below England and Wales average for this age group). The majority of participants were married (above the average for England and Wales), educated to at least further education level (above the Welsh average), in full time employment (below the Welsh average) and owners of their own home (above the England and Wales average) (The Office for National Statistics, 2013b).
Table 4.1 Demographic data obtained from the whole cohort of the intervention study

<table>
<thead>
<tr>
<th>Variable (n= 65)</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.52 (±13.50)</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>84.6</td>
</tr>
<tr>
<td>Smoker (%) [Past smoker (%)]</td>
<td>3.3 [18.3]</td>
</tr>
<tr>
<td>GP visits last 12 months</td>
<td>2.07 (±1.95)</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>98.5</td>
</tr>
<tr>
<td>Area reside in (%)</td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>40.7</td>
</tr>
<tr>
<td>Suburban</td>
<td>42.4</td>
</tr>
<tr>
<td>Rural</td>
<td>16.9</td>
</tr>
<tr>
<td>Marital Status (%)</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>50</td>
</tr>
<tr>
<td>Living with partner</td>
<td>10</td>
</tr>
<tr>
<td>Widowed</td>
<td>6.7</td>
</tr>
<tr>
<td>Divorced</td>
<td>5</td>
</tr>
<tr>
<td>Separated</td>
<td>1.7</td>
</tr>
<tr>
<td>Never married</td>
<td>26.7</td>
</tr>
<tr>
<td>Highest education attainment (%)</td>
<td></td>
</tr>
<tr>
<td>Some primary</td>
<td>1.6</td>
</tr>
<tr>
<td>Some secondary</td>
<td>6.6</td>
</tr>
<tr>
<td>GCSEs</td>
<td>8.2</td>
</tr>
<tr>
<td>A-levels</td>
<td>0</td>
</tr>
<tr>
<td>Further education college</td>
<td>29.5</td>
</tr>
<tr>
<td>Undergraduate degree</td>
<td>29.5</td>
</tr>
<tr>
<td>Masters/PhD</td>
<td>24.6</td>
</tr>
<tr>
<td>Employment status (%)</td>
<td></td>
</tr>
<tr>
<td>Full time</td>
<td>56.5</td>
</tr>
<tr>
<td>Part time</td>
<td>19.3</td>
</tr>
<tr>
<td>Self-employed</td>
<td>0</td>
</tr>
<tr>
<td>Unemployed</td>
<td>1.6</td>
</tr>
<tr>
<td>Retired</td>
<td>14.5</td>
</tr>
<tr>
<td>Student</td>
<td>4.8</td>
</tr>
<tr>
<td>Volunteering</td>
<td>1.6</td>
</tr>
<tr>
<td>Other</td>
<td>1.6</td>
</tr>
<tr>
<td>Housing tenure (%)</td>
<td></td>
</tr>
<tr>
<td>Owner of own home</td>
<td>75</td>
</tr>
<tr>
<td>Renting</td>
<td>16.7</td>
</tr>
<tr>
<td>Living with and supported by others</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Demographic data of the cohort recruited on to the intervention study; displaying age, gender, smoking status, GP visits/year, ethnicity, area resident in, marital status, education status, employment status and housing tenure.
4.3.2 The impact of an 8-week green-exercise programme on markers of CV risk

4.3.2.1 Adherence and non-adherence

On average, the green-exercise programme involved two walks per week, each lasting for approximately 45 minutes. Table 4.3 displays the average length of each green-exercise walk, as characterised using a BodyMedia SenseWear advanced accelerometer arm band (BodyMedia SenseWear PA, USA). The intensity of the walks was considered moderate based on the MET data. Registers kept by walk leaders demonstrated that not all participants adhered to the green-exercise programme by not regularly attending the walks. Based on this, the cohort was split into two subgroups; those who did not adhere to the green-exercise programme and those who did adhere to the green-exercise programme.

Adherence to the green-exercise programme was defined as attendance of at least 70% of the sessions (Fox et al., 1997). On average, participants of the non-adherent group attended 28.5% of the walks.

Of the 65 participant cohort, 33 participants were not adherent to the exercise programme and 32 participants were adherent. Of those 33 participants that did not adhere, 4 of the participants did not return for the 8-week consultation. 8 participants did not provide all blood sample samples, 6 participants did not have the full set of vascular measures completed on them, and 8 participants did not contribute full sets of mental health data.

‘Δ-change’ data (i.e. differences between baseline and 8-weeks) with regard to the change in MET-minutes per week (as determined using the IPAQ) between baseline and 8-weeks were calculated following splitting the cohort into the two groups, i.e. the non-adherent group who did not increase their structured exercise by attending the green-exercise programme, and the adherent group who did increase their structured exercise by attending the walks. Table 4.2 demonstrates that there was a statistically significant, adherent group-specific increase in weekly IPAQ MET-minutes from baseline to 8-weeks.
Table 4.2 Differences in activity levels at baseline and the difference in change from baseline to 8-weeks in the non-adherent group and the adherent group of the intervention study, as determined using IPAQ data

<table>
<thead>
<tr>
<th>Measure of exercise</th>
<th>Non-adherent group</th>
<th>Adherent group</th>
<th>T-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n = 33 )</td>
<td>( n = 32 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPAQ at baseline (MET-min/week)</td>
<td>560 (±311)</td>
<td>507 (±299)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>( \Delta ) change in IPAQ score from baseline to 8-weeks (MET-mins/week)</td>
<td>-51 (±199)</td>
<td>+992 (±518)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

IPAQ, International Physical Activity Questionnaire, MET, metabolic equivalent

Table 4.3 Characteristics of an average green-exercise walk undertaken during the intervention study

<table>
<thead>
<tr>
<th>Measure of exercise</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n = 10 )</td>
</tr>
<tr>
<td>Duration (minutes)</td>
<td>45 (±8)</td>
</tr>
<tr>
<td>METs</td>
<td>3.58 (±0.34)</td>
</tr>
<tr>
<td>Time spent at a moderate intensity (minutes)</td>
<td>34 (±3)</td>
</tr>
<tr>
<td>Steps</td>
<td>4,342 (±118)</td>
</tr>
<tr>
<td>Calories</td>
<td>220 (±15)</td>
</tr>
</tbody>
</table>

Data obtained from the BodyMedia SenseWear armbands displaying the average duration of the walks, the MET intensity of the average walk, the time spent walking at a moderate intensity, the average number of steps taken and the estimated number of calories burnt. MET, metabolic equivalent
4.3.2.2 Quantifiable anthropometric characteristics of the cohort of participants from the intervention study at baseline, and following the undertaking of the green-exercise programme

An independent sample t-test was performed and illustrated that there were no significant differences in age, weight, waist circumference or BMI between the two groups at baseline (see Table 4.4). Furthermore, an additional independent sample t-test was performed in order to investigate whether there was any significant change between groups in the anthropometric measures assessed. Table 4.5 demonstrates that adhering to the green-exercise programme had no significant effect on anthropometric measures such as weight, waist circumference and BMI.

Using the Food Frequency Questionnaire, it was observed that diet did not change between baseline and 8-weeks in either of the groups (data not shown).

4.3.2.3 Biomolecular and biochemical markers of CV risk

Table 4.6 displays baseline data with regard to the biomolecular and biochemical markers of CV risk that were investigated. The data demonstrates that at baseline the level of total-cholesterol and HDL-cholesterol was significantly higher in the adherent group compared to the non-adherent group (total-cholesterol 5.20 ±1.90mmol/L and 4.39 ±1.01mmol/L and HDL-C; 1.71 ±0.61mmol/L and 1.34 ±0.62mmol/L, respectively).

Importantly, the data displayed in Table 4.7 demonstrates that ‘Δ-change’ data (i.e. differences between baseline and 8-weeks) were significantly different between the adherent group compared to the non-adherent group for a number of the biomolecular and biochemical markers of CV risk. For example, following the green-exercise intervention, there were adherent group-specific, statistically significant increases in the expression of ABCA1 and CD36 and significant reductions in leukocyte expression of MMP-9 and in the levels of circulating MMP-9. Further to this, there were adherent group-specific, non-significant reductions in LDL cholesterol and insulin, and non-significant increases in HDL cholesterol.
Table 4.4 Differences in baseline anthropometric characteristics between non-adherent and adherent groups of the intervention study

<table>
<thead>
<tr>
<th>Health and lifestyle variables</th>
<th>Non-adherent group</th>
<th>Adherent group</th>
<th>T-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 33</td>
<td>n= 32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.73 (±12.79)</td>
<td>44.29 (±14.42)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.45 (±10.01)</td>
<td>162.27 (±9.87)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.57 (±16.78)</td>
<td>75.02 (±19.12)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.57 (±5.77)</td>
<td>27.70 (±6.29)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>88.97 (±14.63)</td>
<td>85.00 (±13.82)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Differences in the mean age, weight, waist circumference and BMI of non-adherent and adherent groups prior to green-exercise programme, using independent sample t-tests. BMI, body mass index

Table 4.5 Δ-changes from baseline to 8-weeks in anthropometric characteristics obtained from the cohort of participants from the intervention study: non-adherent and adherent groups

<table>
<thead>
<tr>
<th>Δ change baseline-to-8weeks</th>
<th>Non-adherent group</th>
<th>Adherent group</th>
<th>T-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 29</td>
<td>n= 32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.03 (±1.11)</td>
<td>0.23 (±1.34)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.34 (±0.44)</td>
<td>0.05 (±0.62)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-0.79 (±1.85)</td>
<td>-0.75 (±2.55)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Differences in the mean change, from baseline, in the weight, waist circumference and BMI of the non-adherent group and the adherent group following the 8-week green-exercise programme, using independent sample t-tests. BMI, body mass index
Table 4.6 Differences in baseline biomolecular and biochemical markers of CV risk obtained from the cohort of participants from the intervention study; non-adherent and adherent groups

<table>
<thead>
<tr>
<th>Biomolecular/ biochemical variable</th>
<th>Non-adherent group Mean (±SD) n= 28</th>
<th>Adherent group Mean (±SD) n= 29</th>
<th>T-test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1 (cf. GAPDH)</td>
<td>1.03 (±0.05)</td>
<td>1.04 (±0.12)</td>
<td>NS</td>
</tr>
<tr>
<td>CD36 (cf. GAPDH)</td>
<td>1.02 (±0.05)</td>
<td>1.03 (±0.11)</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-9 (cf. GAPDH)</td>
<td>1.01 (±0.01)</td>
<td>1.01 (±0.01)</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.81 (±0.61)</td>
<td>4.81 (±0.51)</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>31.85 (±16.26)</td>
<td>30.21 (±14.97)</td>
<td>NS</td>
</tr>
<tr>
<td>Total-cholesterol (mmol/L)</td>
<td>4.39 (±1.01)</td>
<td>5.20 (±1.90)</td>
<td>0.012</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.34 (±0.62)</td>
<td>1.71 (±0.61)</td>
<td>0.040</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.59 (±0.99)</td>
<td>3.07 (±1.07)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.00 (±0.57)</td>
<td>1.01 (±0.47)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma IL-6 (pg/mL)</td>
<td>0.79 (±0.21)</td>
<td>1.03 (±0.52)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma MMP-9 (ng/mL)</td>
<td>53.80 (±24.81)</td>
<td>50.58 (±24.05)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Differences in the mean leukocyte mRNA gene expression of the PPARγ target genes and the average concentration of the serum and plasma derived circulating CV risk-related markers of interest in the non-adherent group and adherent group prior to the green-exercise programme, using independent sample t-tests. cf, compared to; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.
Table 4.7 Δ-changes from baseline to 8-weeks in biomolecular and biochemical markers of CV risk obtained from the cohort of participants from the intervention study: non-adherent and adherent groups

<table>
<thead>
<tr>
<th>Δ change baseline-to-8weeks</th>
<th>Non-adherent group</th>
<th>Adherent group</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (±SD)</td>
<td>n= 28</td>
<td>n= 29</td>
<td></td>
</tr>
<tr>
<td>ABCA1 (cf. GAPDH)</td>
<td>0.47 (±0.75)</td>
<td>1.78 (±2.41)</td>
<td>0.013</td>
</tr>
<tr>
<td>CD36 (cf. GAPDH)</td>
<td>0.15 (±1.26)</td>
<td>0.88 (±0.85)</td>
<td>0.018</td>
</tr>
<tr>
<td>MMP-9 (cf. GAPDH)</td>
<td>0.14 (±0.35)</td>
<td>-0.31 (±0.43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.03 (±0.21)</td>
<td>0.14 (±0.52)</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>0.34 (±4.56)</td>
<td>-0.15 (±4.13)</td>
<td>NS</td>
</tr>
<tr>
<td>Total-cholesterol (mmol/L)</td>
<td>-0.01 (±0.45)</td>
<td>0.01 (±0.40)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.01 (±0.26)</td>
<td>0.05 (±0.25)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.05 (±0.53)</td>
<td>-0.12 (±0.94)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>-0.17 (±0.57)</td>
<td>-0.10 (±0.37)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma IL-6 (pg/mL)</td>
<td>-0.03 (±0.13)</td>
<td>-0.02 (±0.31)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma MMP-9 (ng/mL)</td>
<td>-1.49 (±6.18)</td>
<td>-17.28 (±15.29)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Differences in the mean change in the blood-borne markers of CV risk in the non-adherent group and the adherent group following the 8-week green-exercise programme, using independent sample t-tests. cf, compared to; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.
4.3.2.4 Vascular haemodynamic markers of CV risk

Table 4.8 displays baseline seated vascular haemodynamic measurement data. These data demonstrate that at baseline there were no significant differences in these markers. Similarly, Table 4.9 displays baseline supine vascular haemodynamics measurement data that were obtained. Once again, these data demonstrate that at baseline there were no significant differences between groups for these markers.

The data displayed in Table 4.10 demonstrate that ‘Δ-change’ data (i.e. differences between baseline and 8-weeks) were significantly different in the adherent group compared to the non-adherent group for some of the seated vascular haemodynamic measurements. Specifically, statistically significant adherent group-specific decreases were seen with regard to peripheral and central SBP, DBP and MAP and in AIx and AIx@HR75.

The data displayed in Table 4.11 demonstrate broadly similar trends in the supine vascular haemodynamic measurements to the data shown in Table 4.10 – i.e. ‘Δ-change’ data (i.e. differences between baseline and 8-weeks) were significantly different in the adherent group compared to the non-adherent group for several of the markers of vascular haemodynamics. Specifically, statistically significant, adherent group-specific decreases were seen with regard to peripheral and central SBP, DBP and MAP and in AIx and AIx@HR75. There was also a statistically significant, adherent group-specific decrease with regard to CFPWV; however, this effect did not remain upon correcting for MAP.
Table 4.8 Differences in baseline vascular haemodynamic (seated) data obtained from the cohort of participants from the intervention study; non-adherent and adherent groups

<table>
<thead>
<tr>
<th>Seated vascular haemodynamic variables</th>
<th>Non-adherent group</th>
<th>Adherent group</th>
<th>T-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n= 28</td>
<td>n= 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>127 (±16)</td>
<td>126 (±14)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>85 (±11)</td>
<td>84 (±10)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Peripheral PP (mm Hg)</td>
<td>42 (±10)</td>
<td>42 (±8)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>99 (±12)</td>
<td>98 (±11)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>118 (±16)</td>
<td>116 (±14)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>86 (±11)</td>
<td>85 (±10)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>32 (±11)</td>
<td>31 (±7)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>97 (±12)</td>
<td>96 (±11)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>67 (±11)</td>
<td>66 (±11)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>AIx (%)</td>
<td>24 (±12)</td>
<td>21 (±15)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>AIx @HR75 (%)</td>
<td>20 (±10)</td>
<td>17 (±16)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Differences in the mean seated vascular haemodynamics readings in the non-adherent group and adherent group prior to the green-exercise programme, using independent sample t-tests. Central pressures, HR, AIx and AIx@HR75 were obtained using SphygmoCor. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; AIx, augmentation index; AIx@HR75, augmentation index at a heart rate of 75.
Table 4.9 Differences in baseline vascular haemodynamic (supine) data obtained from the cohort of participants from the intervention study; non-adherent and adherent groups

<table>
<thead>
<tr>
<th>Supine vascular haemodynamic variables</th>
<th>Non-adherent group</th>
<th>Adherent group</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>n= 28</td>
<td>n= 31</td>
<td></td>
</tr>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>121 (±14)</td>
<td>121 (±14)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>79 (±12)</td>
<td>77 (±10)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral PP (mm Hg)</td>
<td>42 (±11)</td>
<td>44 (±14)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>93 (±12)</td>
<td>92 (±10)</td>
<td>NS</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>113 (±13)</td>
<td>113 (±13)</td>
<td>NS</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>79 (±11)</td>
<td>78 (±10)</td>
<td>NS</td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>34 (±11)</td>
<td>35 (±12)</td>
<td>NS</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>90 (±11)</td>
<td>90 (±10)</td>
<td>NS</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>58 (±8)</td>
<td>60 (±8)</td>
<td>NS</td>
</tr>
<tr>
<td>AIx (%)</td>
<td>28 (±10)</td>
<td>27 (±12)</td>
<td>NS</td>
</tr>
<tr>
<td>AIx @HR75 (%)</td>
<td>21 (±11)</td>
<td>20 (±13)</td>
<td>NS</td>
</tr>
<tr>
<td>CRPWV (m/s)</td>
<td>6.8 (±1.2)</td>
<td>7.3 (±1.1)</td>
<td>NS</td>
</tr>
<tr>
<td>MAP-adjusted CRPWV (m/s)</td>
<td>6.8 (±1.2)</td>
<td>7.2 (±1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>CFPWV (m/s)</td>
<td>7.0 (±1.3)</td>
<td>6.9 (±1.3)</td>
<td>NS</td>
</tr>
<tr>
<td>MAP-adjusted CFPWV (m/s)</td>
<td>7.0 (±1.0)</td>
<td>6.9 (±0.8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Differences in the mean supine vascular haemodynamics readings between the non-adherent group and adherent group prior to the green-exercise programme, using independent sample t-tests. Central pressures, HR, AIx, AIx@HR75, CRPWV and CFPWV were obtained using SphygmoCor. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; AIx, augmentation index; AIx@HR75, augmentation index at a heart rate of 75; CRPWV, carotid radial pulse wave velocity; CFPWV, carotid femoral pulse wave velocity.
Table 4.10 Δ-changes from baseline to 8-weeks in vascular haemodynamic (seated) data obtained from the cohort of participants from the intervention study; non-adherent and adherent groups

<table>
<thead>
<tr>
<th>Δ change baseline-to-8weeks</th>
<th>Non-adherent group Mean change (±SD) n=28</th>
<th>Adherent group Mean change (±SD) n=31</th>
<th>T-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>-1 (±8)</td>
<td>-8 (±6)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>-0 (±4)</td>
<td>-5 (±4)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peripheral PP (mm Hg)</td>
<td>-1 (±5)</td>
<td>-3 (±5)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>-0 (±5)</td>
<td>-6 (±4)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>1 (±11)</td>
<td>-8 (±8)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>-1 (±5)</td>
<td>-4 (±5)</td>
<td></td>
<td>0.024</td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>2 (±9)</td>
<td>-4 (±6)</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>0 (±6)</td>
<td>-5 (±6)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>2 (±9)</td>
<td>-1 (±8)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Alx (%)</td>
<td>0 (±6)</td>
<td>-3 (±9)</td>
<td></td>
<td>0.040</td>
</tr>
<tr>
<td>Alx @HR75 (%)</td>
<td>0 (±4)</td>
<td>-3 (±8)</td>
<td></td>
<td>0.030</td>
</tr>
</tbody>
</table>

Differences in the mean change in seated vascular haemodynamics readings in the non-adherent group and adherent group following the 8-week green-exercise programme, using independent sample t-tests. Central pressures, HR, Alx and Alx@HR75 were obtained using SphygmoCor. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; Alx, augmentation index; Alx@HR75, augmentation index at a heart rate of 75.
Table 4.11 Δ-changes from baseline to 8-weeks in vascular haemodynamic (supine) data obtained from the intervention study participants; non-adherent and adherent groups

<table>
<thead>
<tr>
<th>Δ change baseline-to-8weeks</th>
<th>Non-adherent group Mean change (±SD) n= 28</th>
<th>Adherent group Mean change (±SD) n= 31</th>
<th>T-test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>-1 (±5)</td>
<td>-6 (±9)</td>
<td>0.009</td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>1 (±4)</td>
<td>-3 (±6)</td>
<td>0.003</td>
</tr>
<tr>
<td>Peripheral PP (mm Hg)</td>
<td>-2 (±6)</td>
<td>-3 (±9)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>0 (±4)</td>
<td>-4 (±6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>0 (±5)</td>
<td>-5 (±7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>2 (±3)</td>
<td>-3 (±7)</td>
<td>0.002</td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>-1 (±5)</td>
<td>-2 (±7)</td>
<td>NS</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>1 (±4)</td>
<td>-4 (±6)</td>
<td>0.001</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>1 (±6)</td>
<td>0 (±7)</td>
<td>NS</td>
</tr>
<tr>
<td>AIx (%)</td>
<td>1 (±7)</td>
<td>-5 (±7)</td>
<td>0.002</td>
</tr>
<tr>
<td>AIx @HR75 (%)</td>
<td>1 (±7)</td>
<td>-4 (±6)</td>
<td>0.003</td>
</tr>
<tr>
<td>CRPWV (m/s)</td>
<td>0.5 (±1.5)</td>
<td>0.1 (±1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>MAP-adjusted CRPWV (m/s)</td>
<td>0.4 (±1.5)</td>
<td>0.2 (±1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>CFPWV (m/s)</td>
<td>0.2 (±0.4)</td>
<td>-0.1 (±0.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>MAP-adjusted CFPWV (m/s)</td>
<td>0.1 (±0.4)</td>
<td>0.1 (±0.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Differences in the mean change in the supine vascular haemodynamics readings in the non-adherent group and adherent group following the 8-week green-exercise programme, using independent sample t-tests. Central pressures, HR, AIx, AIx@HR75, CRPWV and CFPWV were obtained using SphygmoCor. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; AIx, augmentation index; AIx@HR75, augmentation index at a heart rate of 75; CRPWV, carotid radial pulse wave velocity; CFPWV, carotid femoral pulse wave velocity.
4.3.2.5 Markers of mental health

Table 4.12 displays the baseline data of the markers of mental health that were investigated. These data demonstrate that at baseline there was a significant difference between the non-adherent group and the adherent group for the measures of SPS-attachment, and SPS reassurance of worth, but that none of the other measures differed significantly at baseline.

The data displayed in Table 4.13 demonstrates that ‘Δ-change’ data (i.e. differences between baseline and 8-weeks) were significantly different in the adherent group compared to the non-adherent group for a number of the markers of mental health. Specifically, statistically significant, adherent group-specific decreases were observed with regard to STAI, PSS, CES-D, POMS tension-anxiety and POMS total mood disturbance, whilst a statistically significant, adherent group-specific increase was seen with regard to the R-SES.
Table 4.12 Differences in baseline mental health marker data obtained from the cohort of participants from the intervention study; non-adherent and adherent groups

<table>
<thead>
<tr>
<th>Mental health variables (Range)</th>
<th>Non-adherent group Mean (±SD) n= 29</th>
<th>Adherent group Mean (±SD) n= 31</th>
<th>T-test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-SES (0-30)</td>
<td>16.34 (±2.04)</td>
<td>15.97 (±2.01)</td>
<td>NS</td>
</tr>
<tr>
<td>CFQ (0-100)</td>
<td>40.35 (±11.93)</td>
<td>39.03 (±10.53)</td>
<td>NS</td>
</tr>
<tr>
<td>STAI (20-80)</td>
<td>38.52 (±7.79)</td>
<td>37.10 (±9.75)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-attachment (4-16)</td>
<td>13.16 (±2.76)</td>
<td>14.48 (±1.91)</td>
<td>0.039</td>
</tr>
<tr>
<td>SPS-social integration (4-16)</td>
<td>13.12 (±2.13)</td>
<td>13.90 (±1.72)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-reassurance of worth (4-16)</td>
<td>11.72 (±2.23)</td>
<td>13.26 (±1.93)</td>
<td>0.008</td>
</tr>
<tr>
<td>SPS-reliable alliance (4-16)</td>
<td>13.84 (±2.03)</td>
<td>14.71 (±1.66)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-guidance (4-16)</td>
<td>13.64 (±2.56)</td>
<td>14.29 (±2.18)</td>
<td>NS</td>
</tr>
<tr>
<td>SPS-opportunity for nurturance (4-16)</td>
<td>11.88 (±3.29)</td>
<td>12.67 (±2.32)</td>
<td>NS</td>
</tr>
<tr>
<td>Perceived Stress Scale (0-40)</td>
<td>17.08 (±6.48)</td>
<td>16.61 (±7.40)</td>
<td>NS</td>
</tr>
<tr>
<td>CES-D (0-60)</td>
<td>14.17 (±9.01)</td>
<td>12.44 (±7.86)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-tension anxiety (0-36)</td>
<td>9.76 (±5.76)</td>
<td>8.52 (±4.79)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-depression dejection (0-60)</td>
<td>12.72 (±7.17)</td>
<td>11.52 (±7.88)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-anger hostility (0-48)</td>
<td>8.68 (±5.97)</td>
<td>8.45 (±5.00)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-vigour activity (0-32)</td>
<td>15.00 (±7.01)</td>
<td>16.81 (±7.75)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-fatigue inertia (0-28)</td>
<td>10.28 (±7.28)</td>
<td>9.58 (±5.62)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-confusion bewilderment (0-28)</td>
<td>8.12 (±5.34)</td>
<td>7.35 (±3.59)</td>
<td>NS</td>
</tr>
<tr>
<td>POMS-total mood disturbance (-32-200)</td>
<td>34.56 (±30.74)</td>
<td>28.61 (±21.27)</td>
<td>NS</td>
</tr>
</tbody>
</table>

The mean scores derived from the measures of mental health in the non-adherent group and adherent group prior to the green-exercise programme. Measures of mental health obtained using well-validated questionnaires. R-SES, Rosenberg Self-Esteem Scale; CFQ, Cognitive Failures Questionnaire; STAI, State-Trait Anxiety Inventory; SPS, Social Provisions Scale; CES-D, Center for Epidemiologic Studies-Depression; POMS, Profile of Mood States.
Table 4.13 Δ-changes from baseline to 8-weeks in mental health marker data obtained from the cohort of participants from the intervention study; non-adherent and adherent groups

<table>
<thead>
<tr>
<th>Δ change baseline-to-8weeks</th>
<th>Non-adherent group Mean change (±SD)</th>
<th>Adherent group Mean change (±SD)</th>
<th>T-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 26</td>
<td>n= 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-SES</td>
<td>-0.17 (±1.09)</td>
<td>1.61 (±1.82)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>CFQ</td>
<td>-0.23 (±2.87)</td>
<td>-1.94 (±4.34)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>STAI</td>
<td>-0.16 (±1.57)</td>
<td>-1.58 (±3.04)</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>SPS-attachment</td>
<td>0.48 (±0.92)</td>
<td>0.16 (±0.93)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SPS-social integration</td>
<td>0.60 (±1.38)</td>
<td>0.45 (±1.31)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SPS-reassurance of worth</td>
<td>0.48 (±1.42)</td>
<td>0.35 (±1.84)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SPS-reliable alliance</td>
<td>0.32 (±1.18)</td>
<td>-0.06 (±1.26)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SPS-guidance</td>
<td>0.56 (±1.33)</td>
<td>0.19 (±1.82)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SPS-opportunity for nurturance</td>
<td>0.20 (±1.83)</td>
<td>0.26 (±1.12)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Perceived Stress Scale</td>
<td>-0.16 (±0.99)</td>
<td>-3.35 (±1.94)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>CES-D</td>
<td>0.38 (±2.10)</td>
<td>-2.00 (±3.81)</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>POMS-tension anxiety</td>
<td>-0.24 (±1.05)</td>
<td>-2.00 (±1.59)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>POMS-depression dejection</td>
<td>-0.80 (±1.15)</td>
<td>-1.29 (±2.36)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>POMS-anger hostility</td>
<td>-0.64 (±1.82)</td>
<td>-1.23 (±2.04)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>POMS-vigour activity</td>
<td>0.04 (±1.31)</td>
<td>0.81 (±2.34)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>POMS-fatigue inertia</td>
<td>0.00 (±1.26)</td>
<td>-0.42 (±2.77)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>POMS-confusion bewilderment</td>
<td>-0.28 (±1.21)</td>
<td>-0.71 (±2.08)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>POMS-total mood disturbance</td>
<td>-2.00 (±4.11)</td>
<td>-6.45 (±5.63)</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

The mean change in the scores derived from the measures of mental health in the non-adherent group and adherent group following the 8-week green-exercise programme. Measures of mental health obtained using well-validated questionnaires. R-SES, Rosenberg Self-Esteem Scale; CFQ, Cognitive Failures Questionnaire; STAI, State-Trait Anxiety Inventory; SPS, Social Provisions Scale; CES-D, Center for Epidemiologic Studies-Depression; POMS, Profile of Mood States.
4.3.3 Correlation analyses regarding Δ-changes from baseline to 8-weeks obtained from the cohort of participants from the intervention study

4.3.3.1 Correlation analyses relating changes in IPAQ to changes in other measures

Bivariate correlations were performed in order to investigate whether any associations existed between the Δ-change of the exercise data (as measured by IPAQ MET-minutes) and the Δ-change data with regard to the various variables of health that were measured within the three areas of interest. Only those correlations that were statistically significant are presented in the current section. The correlations are confirmatory, as they were predicted based on the evidence discussed in Chapter 1, therefore, despite a large number of correlations analyses being conducted, the level of significance remains at $P = 0.05$.

As shown in Table 4.14a, significant positive correlations were seen between the Δ-changes in IPAQ MET-minutes and the Δ-changes in the expression of CD36 and ABCA1, and significant inverse correlations between the Δ-changes in IPAQ MET-minutes with the Δ-changes in the mRNA expression of MMP-9, and in the levels of circulating MMP-9.

As shown in Table 4.14b, significant inverse correlations were seen between the Δ-changes in IPAQ MET-minutes with the Δ-changes in peripheral and central measures of seated SBP, DBP and MAP. Similarly, Table 4.14c displays significant inverse correlations between the Δ-changes in IPAQ MET-minutes with the Δ-changes in peripheral and central measures of supine SBP, DBP, MAP, AIX, Alx@HR75 and CFPWV (uncorrected). Further, Figure 4.1 displays the correlation between Δ-change in IPAQ MET-minutes and Δ-change in central supine MAP, while Figure 4.2 displays the correlation between Δ-change in IPAQ MET-minutes and Δ-change in supine AIX.

Table 4.14d displays significant associations between Δ-changes in IPAQ MET-minutes and Δ-changes in markers of mental health. Briefly, these included correlations with R-SES (self-esteem measure), STAI (state anxiety measure), PSS (perceived stress measure) and POMS tension-anxiety and POMS vigour-activity. Further, Figure 4.3 displays the correlation between Δ-change in IPAQ MET-minutes and Δ-change in STAI, while Figure 4.4 displays the correlation between Δ-change in IPAQ MET-minutes and Δ-change in PSS.
Table 4.14a Selected associations between Δ-changes from baseline to 8-weeks in IPAQ data versus biomolecular and biochemical markers of CV risk obtained from the cohort of participants from the intervention study

<table>
<thead>
<tr>
<th>Δ change in blood-borne markers of CV risk</th>
<th>Δ change in IPAQ (MET-minutes/week)</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36 (cf. GAPDH)</td>
<td>0.343</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>ABCA1 (cf. GAPDH)</td>
<td>0.356</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>MMP-9 (cf. GAPDH)</td>
<td>-0.502</td>
<td>≤ 0.001</td>
<td></td>
</tr>
<tr>
<td>Plasma MMP-9 (ng/mL)</td>
<td>-0.381</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson’s method observed between Δ change in IPAQ MET-minutes and Δ change in blood-borne markers of CV risk. cf., compared to

Table 4.14b Selected associations between Δ-changes from baseline to 8-weeks in IPAQ data versus measures of vascular haemodynamics (seated) obtained from the cohort of participants from the intervention study

<table>
<thead>
<tr>
<th>Δ change in vascular haemodynamic measures (seated)</th>
<th>Δ change in IPAQ MET-minutes/week</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>-0.371</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>-0.475</td>
<td>≤0.001</td>
<td></td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>-0.459</td>
<td>≤0.001</td>
<td></td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>-0.323</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>-0.327</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>-0.359</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson’s method observed between Δ change in IPAQ MET-minutes and Δ change in seated haemodynamic measures of vascular health. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure;
Table 4.14c Selected associations between Δ-changes from baseline to 8-weeks in IPAQ data versus measures of vascular haemodynamics (supine) obtained from the cohort of participants from the intervention study

<table>
<thead>
<tr>
<th>Δ change in vascular haemodynamic measures (supine)</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral SBP (mm Hg)</td>
<td>-0.326</td>
<td>0.014</td>
</tr>
<tr>
<td>Peripheral DBP (mm Hg)</td>
<td>-0.400</td>
<td>0.002</td>
</tr>
<tr>
<td>Peripheral MAP (mm Hg)</td>
<td>-0.433</td>
<td>0.001</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>-0.366</td>
<td>0.008</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>-0.425</td>
<td>0.001</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>-0.447</td>
<td>0.001</td>
</tr>
<tr>
<td>AIx (%)</td>
<td>-0.310</td>
<td>0.019</td>
</tr>
<tr>
<td>AIx@HR75 (%)</td>
<td>-0.293</td>
<td>0.029</td>
</tr>
<tr>
<td>CFPWV (m/s)</td>
<td>-0.340</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson’s method observed between Δ change in IPAQ MET-minutes and Δ change in supine haemodynamic measures of vascular health: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; AIx, augmentation index; AIx@HR75, augmentation index at a heart rate of 75; CFPWV, carotid femoral pulse wave velocity

Table 4.14d Selected associations between Δ-changes from baseline to 8-weeks in IPAQ data versus markers of mental health obtained from the cohort of participants from the intervention study

<table>
<thead>
<tr>
<th>Δ change in markers of mental health</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-SES</td>
<td>0.276</td>
<td>0.031</td>
</tr>
<tr>
<td>STAI</td>
<td>-0.497</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSS</td>
<td>-0.563</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>POMS tension-anxiety</td>
<td>-0.328</td>
<td>0.014</td>
</tr>
<tr>
<td>POMS vigour-activity</td>
<td>0.300</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson’s method observed between Δ change in IPAQ MET-minutes and Δ change in markers of mental health. R-SES, Rosenberg Self-Esteem Scale; STAI, State-Trait Anxiety Inventory; PSS, Perceived Stress Scale; POMS, Profile of Mood States
Figure 4.1 The baseline to 8-weeks Δ-change in IPAQ MET-minutes correlated against the Δ-change in central supine MAP

Figure 4.2 The baseline to 8-weeks Δ-change in IPAQ MET-minutes correlated against the Δ-change in supine AIx
Figure 4.3 The baseline to 8-weeks Δ-change in IPAQ MET-minutes correlated against the Δ-change in STAI (measuring state anxiety)

Figure 4.4 The baseline to 8-weeks Δ-change in IPAQ MET-minutes correlated against the Δ-change in PSS (measuring perceived stress)
4.3.3.2 Within-area-of-interest correlations between Δ-change (from baseline to 8-weeks) data obtained from the cohort of participants from the intervention study

Once again, bivariate correlation analyses were carried out – in this case in order to investigate whether any associations existed between the green-exercise-associated Δ-changes observed within the various biomolecular/biochemical variables under investigation.

Significant correlations were observed within the vascular haemodynamic data set and within the mental health markers data set, however, these correlations were expected due to co-linearity within both sets of measures (Wilkinson et al., 2002b, Wilkinson et al., 2000b, Bovier et al., 2004, Bridger et al., 2013).

**Biomolecular/Biochemical Measures**

Interestingly, a number of significant associations were observed within the biomolecular and biochemical data set (as displayed in Table 4.15). For example, Δ-changes in glucose and insulin were positively correlated; \( r = 0.422, P = 0.004 \), while Δ-changes in mRNA expression of MMP-9 and ABCA1 were inversely associated; \( r = -0.293, P = 0.039 \).

4.3.3.3 Correlations between exercise-associated Δ-changes from baseline to 8-weeks in anthropometric measures versus Δ-changes in vascular haemodynamic measures

As displayed in Table 4.16a, Δ-changes in weight and BMI were significantly and positively associated with Δ-changes in peripheral supine SBP, and Δ-changes in BMI were significantly and positively associated with Δ-changes in peripheral supine PP.

4.3.4.1 Correlations between exercise-associated Δ-changes from baseline to 8-weeks, between the three areas of interest

A final set of bivariate correlation analyses were conducted in order to identify associations between the green-exercise-associated Δ-changes observed with regard to biomolecular/biochemical variables versus the corresponding Δ-changes in vascular
haemodynamic measures, and versus Δ-changes in markers of mental health; and between Δ-changes in vascular haemodynamic measures versus Δ-changes in markers of mental health.

A large number of analyses were conducted investigating the mental health measures with i) the blood-borne markers of CV risk, and ii) the vascular haemodynamic markers of risk. The associations between the mental health markers and blood-borne markers were exploratory as opposed to confirmatory (apart from any mental health associations with IL-6, due to the data associating stress and IL-6), therefore levels of significance were adjusted so that $P=\leq 0.05$ signifies a trend in the data, $P=\leq 0.005$ signifies significant associations, and $P=\leq 0.001$ signifies highly significant associations. The associations were exploratory as they weren’t predicted in the literature. Due to the literature and hypothesis regarding mental health, SNS activity and blood pressure; associations between mental health and vascular health were confirmatory, and therefore, $P$ values were not adjusted.

4.3.4.2 Selected correlations between exercise-associated Δ-changes in vascular haemodynamic markers and in markers of mental health

As shown in Table 4.16b, a number of significant associations were seen to exist between markers of vascular health and markers of mental health. For example, Δ-changes in PSS (as a measure of perceived stress) was associated with Δ-changes in central seated SBP ($r=0.288$, $P=0.031$); central seated PP ($r=0.394$, $P=0.003$); and supine AIx@HR75 ($r=0.397$, $P=0.002$).

4.3.4.3 Selected correlations between exercise-associated Δ-changes in vascular haemodynamic markers and in biomolecular/biochemical markers

As shown in Table 4.16c, a number of significant associations were seen to exist between markers of vascular health and blood-borne markers of CV risk. For example, Δ-changes in leukocyte MMP-9 mRNA expression was associated with Δ-changes in supine AIx@HR75
(r = 0.282, P = 0.053). Δ-changes in central supine PP and IL-6 levels were also correlated (r = 0.349, P = 0.037), and Table 4.16c displays a significant correlation between Δ-changes in supine Alx@HR75 and Δ-changes in plasma levels of MMP-9.

4.3.4.4 Selected correlations between exercise-associated Δ-changes in biomolecular/biochemical markers and in markers of mental health

As shown in Table 4.16d, a number of associations were seen to exist between blood-borne markers of CV risk and markers of mental health. For example, Δ-changes in plasma MMP-9 levels were seen to correlate with Δ-changes in PSS (measure of perceived stress); r = 0.359, P = 0.005.

Table 4.15 Significant associations between Δ-changes from baseline to 8-weeks within the biomolecular/biochemical CV risk associated data set obtained from the cohort of participants from the intervention study

<table>
<thead>
<tr>
<th>Δ change in biomolecular/biochemical markers of CV risk</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36 (cf. GAPDH) v. IL6 (pg/mL)</td>
<td>0.328</td>
<td>0.044</td>
</tr>
<tr>
<td>IL6 (pg/mL) v. glucose (mmol/L)</td>
<td>0.353</td>
<td>0.027</td>
</tr>
<tr>
<td>Glucose (mmol/L) v. insulin (pmol/L)</td>
<td>0.422</td>
<td>0.004</td>
</tr>
<tr>
<td>MMP9 plasma (ng/mL) v. MMP9 mRNA (cf. GAPDH)</td>
<td>0.573</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMP9 (cf. GAPDH) v. ABCA1 (cf. GAPDH)</td>
<td>-0.293</td>
<td>0.039</td>
</tr>
<tr>
<td>ABCA1 (cf. GAPDH) v. plasma MMP9 (ng/mL)</td>
<td>-0.272</td>
<td>0.059</td>
</tr>
<tr>
<td>ABCA1 (cf. GAPDH) v. glucose (mmol/L)</td>
<td>-0.329</td>
<td>0.028</td>
</tr>
<tr>
<td>Total-cholesterol (mmol/L) v. MMP9 (cf. GAPDH)</td>
<td>0.398</td>
<td>0.015</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L) v. MMP9 (cf. GAPDH)</td>
<td>0.363</td>
<td>0.027</td>
</tr>
<tr>
<td>Triglycerides (mmol/L) v. CD36 (cf. GAPDH)</td>
<td>-0.325</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson’s method observed within the biomolecular and biochemical data set. Displaying within-variable associations between the Δ change of fasting levels of blood-borne markers of CV risk. cf. compared to
Table 4.16a Selected correlations between Δ-changes from baseline to 8-weeks in anthropometric measures versus vascular haemodynamic markers of CV risk obtained from the cohort of participants from the intervention study

<table>
<thead>
<tr>
<th>Δ change</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight v. peripheral supine SBP</td>
<td>0.289</td>
<td>0.031</td>
</tr>
<tr>
<td>BMI v. peripheral supine SBP</td>
<td>0.262</td>
<td>0.051</td>
</tr>
<tr>
<td>BMI v. peripheral supine PP</td>
<td>0.266</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Significant bivariate correlations using Pearson’s method observed between anthropometric measures of obesity and measures of vascular health related to CV risk. BMI, body mass index; SBP, systolic blood pressure; PP, pulse pressure.
Table 4.16b Selected associations between $\Delta$-changes from baseline to 8-weeks in mental health measures versus vascular haemodynamic markers of CV risk obtained from the cohort of participants from the intervention study

<table>
<thead>
<tr>
<th>$\Delta$ change in R-SES</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral seated SBP (mm Hg)</td>
<td>-0.333</td>
<td>0.011</td>
</tr>
<tr>
<td>Peripheral seated DBP (mm Hg)</td>
<td>-0.258</td>
<td>0.051</td>
</tr>
<tr>
<td>Peripheral seated MAP (mm Hg)</td>
<td>-0.311</td>
<td>0.006</td>
</tr>
<tr>
<td>Peripheral seated PP (mm Hg)</td>
<td>-0.254</td>
<td>0.054</td>
</tr>
<tr>
<td>Central seated SBP (mm Hg)</td>
<td>-0.300</td>
<td>0.022</td>
</tr>
<tr>
<td>Central seated MAP (mm Hg)</td>
<td>-0.287</td>
<td>0.029</td>
</tr>
<tr>
<td>Peripheral supine SBP (mm Hg)</td>
<td>-0.363</td>
<td>0.006</td>
</tr>
<tr>
<td>Peripheral supine DBP (mm Hg)</td>
<td>-0.269</td>
<td>0.045</td>
</tr>
<tr>
<td>Peripheral supine MAP (mm Hg)</td>
<td>-0.360</td>
<td>0.006</td>
</tr>
<tr>
<td>Supine AIx (%)</td>
<td>-0.319</td>
<td>0.017</td>
</tr>
<tr>
<td>Supine AIx@HR75 (%)</td>
<td>-0.316</td>
<td>0.019</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\Delta$ change in PSS</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central seated SBP (mm Hg)</td>
<td>0.288</td>
<td>0.031</td>
</tr>
<tr>
<td>Central seated PP (mm Hg)</td>
<td>0.394</td>
<td>0.003</td>
</tr>
<tr>
<td>Peripheral supine DBP (mm Hg)</td>
<td>0.277</td>
<td>0.044</td>
</tr>
<tr>
<td>Central supine SBP (mm Hg)</td>
<td>0.311</td>
<td>0.028</td>
</tr>
<tr>
<td>Central supine MAP (mm Hg)</td>
<td>0.286</td>
<td>0.044</td>
</tr>
<tr>
<td>Seated AIx (%)</td>
<td>0.359</td>
<td>0.007</td>
</tr>
<tr>
<td>Seated AIx@HR75 (%)</td>
<td>0.397</td>
<td>0.002</td>
</tr>
<tr>
<td>Supine AIx (%)</td>
<td>0.270</td>
<td>0.048</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\Delta$ change in CES-D</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral supine DBP (mm Hg)</td>
<td>0.316</td>
<td>0.054</td>
</tr>
<tr>
<td>Peripheral seated DBP (mm Hg)</td>
<td>0.325</td>
<td>0.046</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\Delta$ change in POMS tension-anxiety</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seated HR (bpm)</td>
<td>-0.434</td>
<td>0.002</td>
</tr>
<tr>
<td>Central supine SBP (mm Hg)</td>
<td>0.291</td>
<td>0.040</td>
</tr>
<tr>
<td>Supine HR (bpm)</td>
<td>-0.314</td>
<td>0.043</td>
</tr>
<tr>
<td>Supine AIx (%)</td>
<td>0.353</td>
<td>0.009</td>
</tr>
<tr>
<td>Supine AIx@HR75 (%)</td>
<td>0.301</td>
<td>0.028</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\Delta$ change in POMS vigour-activity</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral seated SBP (mm Hg)</td>
<td>-0.391</td>
<td>0.003</td>
</tr>
<tr>
<td>Peripheral seated DBP (mm Hg)</td>
<td>-0.438</td>
<td>0.001</td>
</tr>
<tr>
<td>Peripheral seated MAP (mm Hg)</td>
<td>-0.447</td>
<td>0.001</td>
</tr>
<tr>
<td>Central seated SBP (mm Hg)</td>
<td>-0.261</td>
<td>0.052</td>
</tr>
<tr>
<td>Central seated DBP (mm Hg)</td>
<td>-0.441</td>
<td>0.001</td>
</tr>
<tr>
<td>Central seated MAP (mm Hg)</td>
<td>-0.388</td>
<td>0.003</td>
</tr>
<tr>
<td>Seated AIx@HR75 (%)</td>
<td>-0.268</td>
<td>0.046</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\Delta$ change in POMS TMD</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral seated SBP (mm Hg)</td>
<td>0.350</td>
<td>0.008</td>
</tr>
<tr>
<td>Peripheral seated DBP (mm Hg)</td>
<td>0.285</td>
<td>0.033</td>
</tr>
<tr>
<td>Peripheral seated MAP (mm Hg)</td>
<td>0.336</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Bivariate correlations using Pearson’s method observed between markers of mental health and measures of vascular health related to CV risk. R-SES, Rosenberg Self-Esteem Scale; PSS, Perceived Stress Scale; CES-D, centre for Epidemiologic Studies-Depression; POMS, Profile of Mood States; TMD, Total Mood Disturbance
Table 4.16c Selected associations between Δ-changes from baseline to 8-weeks in biomolecular/biochemical versus vascular haemodynamic markers of CV risk obtained from the cohort of participants from the intervention study

| Δ change in MMP-9 (cf.GAPDH) |  | 
|------------------------------|---|---|
| Δ change in vascular haemodynamic measures |  | 
| Peripheral supine SBP (mm Hg) | 0.420 | 0.014 |
| Peripheral supine PP (mm Hg) | 0.354 | 0.003 |
| Supine Alx@HR75 (%) | 0.282 | 0.053 |

| Δ change in plasma insulin (pmol/L) |  | 
|-----------------------------------|---|---|
| Supine HR (bpm) | 0.379 | 0.021 |
| Supine Alx (%) | 0.303 | 0.043 |
| Supine Alx@HR75 (%) | 0.379 | 0.021 |

| Δ change in plasma glucose (mmol/L) |  | 
|-----------------------------------|---|---|
| Peripheral supine SBP (mm Hg) | 0.363 | 0.015 |
| Peripheral supine PP (mm Hg) | 0.390 | 0.009 |

| Δ change in serum IL-6 (pg/mL) |  | 
|--------------------------------|---|---|
| Weight (kg) | 0.455 | 0.002 |
| Waist circumference (cm) | 0.466 | 0.002 |
| Peripheral supine DBP (mm Hg) | -0.379 | 0.019 |
| Peripheral supine PP (mm Hg) | 0.341 | 0.036 |
| Central supine DBP (mm Hg) | -0.337 | 0.038 |
| Central supine PP (mm Hg) | 0.349 | 0.037 |

| Δ change in plasma MMP-9 (ng/mL) |  | 
|---------------------------------|---|---|
| Peripheral seated SBP (mm Hg) | 0.360 | 0.009 |
| Peripheral seated DBP (mm Hg) | 0.397 | 0.004 |
| Peripheral seated MAP (mm Hg) | 0.405 | 0.003 |
| Central seated SBP (mm Hg) | 0.366 | 0.008 |
| Central seated DBP (mm Hg) | 0.335 | 0.015 |
| Central seated MAP (mm Hg) | 0.388 | 0.004 |
| Peripheral supine SBP (mm Hg) | 0.652 | <0.001 |
| Peripheral supine DBP (mm Hg) | 0.352 | 0.012 |
| Peripheral supine PP (mm Hg) | 0.428 | 0.002 |
| Central supine SBP (mm Hg) | 0.483 | 0.001 |
| Central supine DBP (mm Hg) | 0.297 | 0.038 |
| Central supine MAP (mm Hg) | 0.405 | 0.005 |
| Supine Alx (%) | 0.426 | 0.002 |
| Supine Alx@HR75 (%) | 0.477 | <0.001 |

Significant bivariate correlations using Pearson’s method observed between blood-borne markers of CV risk and measures of vascular health related to CV risk. cf. compared to; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; Alx, augmentation index; Alx@HR75, augmentation index at a heart rate of 75
Figure 4.5 The baseline to 8-weeks Δ-change in plasma levels of MMP-9 correlated against the Δ-change in supine AIx@HR75.

Table 4.16d Selected associations between Δ-changes from baseline to 8-weeks in biomolecular/biochemical markers of CV risk versus measure of mental health obtained from the cohort of participants from the intervention study.

<table>
<thead>
<tr>
<th>Δ change in ABCA1 (cf.GAPDH)</th>
<th>Δ change in markers of mental health</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSS</td>
<td>-0.333</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>STAI</td>
<td>-0.298</td>
<td>0.035</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Δ change in plasma MMP-9 (ng/mL)</th>
<th>Δ change in markers of mental health</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-SES</td>
<td></td>
<td>-0.455</td>
<td>0.001</td>
</tr>
<tr>
<td>PSS</td>
<td></td>
<td>0.359</td>
<td>0.005</td>
</tr>
<tr>
<td>POMS tension-anxiety</td>
<td></td>
<td>0.415</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Bivariate correlations using Pearson’s method observed between blood-borne markers of CV risk and markers of mental health. PSS, Perceived Stress Scale; STAI, State Trait Anxiety Inventory; R-SES, Rosenberg Self-Esteem Scale; POMS, Profile of Mood States.
4.3.5 Bioinformatics analysis

As an additional analysis, bioinformatics screens of the human MMP-9 promoter (accession code: NG_011468.1) for peroxisome proliferator response elements (PPRE), NFkB, and AP-1 consensus sequences (Butcher et al., 2008, Thomas et al., 2012, Shu et al., 2000, Hetzel et al., 2003, Schroen and Brinckerhoff, 1996) were carried out. As demonstrated in Figure 4.6, no PPREs were found within ~5kbp of the MMP-9 start site, but sequences were found resembling the reported consensus sequences for NFkB and AP-1 at positions -4076bp, -637bp, -408bp, -349bp; and positions -1680bp, -556bp, respectively (Novak et al., 1991, Lee et al., 1987).

As demonstra...
4.4 Discussion
The data presented in the current chapter broadly demonstrates that adhering to an 8-week green-exercise programme is associated with improvements across a number of indices of health, specifically with regard to CV risk. The main findings of interest were with regard to the exercise-associated reduction in MMP-9 expression and an exercise-associated reduction in AIx. Importantly, there was also a significant association between these two measures. This is the first time an exercise intervention study has demonstrated these data and it suggests a possible mechanism that may contribute to how exercise is beneficial in reducing CV risk and in delaying the age-associated augmentation of wave reflection, central systolic pressure and aPWV. Additionally, it should be noted that all of the findings were novel, in the sense that this is the first time a multi-disciplinary investigation has been carried out into the impact of an 8-week green-exercise programme on health, and specifically upon the measures associated with CV risk that were employed in the current study.

The current study was conducted in a cohort with a mean age of 44.5 years. At baseline participants had indicated that they did not regularly take part in any structured exercise. Independently of the research project, they had elected to begin participation in an outdoor walking group and following this they were provided with the details of the research project. Prior to data analysis, the cohort was stratified by whether or not the participants had adhered to the green-exercise programme. This was carried out as it became clear from observation of the walking registers that a number of participants had signed up to the walking programmes and the research project but had not regularly taken part in the green-exercise, despite remaining a participant of the research project. Importantly, at baseline there were no significant differences between the non-adherent group and the adherent group across any of the vascular measures (and also with regard to the vast majority of the biomolecular/biochemical and mental health measures). It can therefore be assumed that any changes that occurred in the adherent group were associated with the increase in physical activity that occurred as a result of adhering to the green-exercise programme.
No changes in lipids occurred following the 8-week exercise programme. HDL-C levels in the adherent group at baseline were near the higher end of the recommended range and therefore perhaps were not able to increase any further with a low/moderate-intensity exercise stimulus. However, a significant increase in the mRNA gene expression of ABCA1 and CD36 was observed in the adherent group following the 8-week green-exercise programme. This suggests that an increase in exercise via participation in a green-exercise walking programme increases the expression of two of the genes involved in the RCT system. As discussed in Chapter 1, CD36 and ABCA1 are regulated by the nuclear transcription factor PPARγ. Thomas et al. (2012) demonstrated that participation in an 8-week cycling intervention was associated with increased generation of PPARγ-activating factors in the plasma, while Butcher et al. (2008) demonstrated that an 8-week treadmill-based walking programme was associated with increased activation of PPARγ within leukocytes, and therefore it is likely that the changes in expression of these genes observed in the current study is as a result of increased activation of this nuclear transcription factor. It should be noted that the increase in the expression of these genes is a novel observation in the specific context of a green-exercise programme. Further to this, the decreased expression of MMP-9 is possibly a downstream, indirect effect of PPARγ activation, albeit an indirect transrepressive effect in this case (see below) (Novak et al., 1991, Lee et al., 1987).

However, despite the presumed parallel increases in the expression of the encoded proteins (a lipid-importing scavenger receptor that internalises oxidised LDL, and a lipid-exporting transporter which exports lipids to HDL particles, respectively (Endemann et al., 1993, Collot-Teixeira et al., 2007, Brewer et al., 2004)) there was no change observed for any of the measured lipids. The reason for this might be that an increase in the expression of the genes did not translate to an increase in the levels of these genes at a protein level, and therefore lipid levels were not affected. However, the demonstration in section 2.2.2 that PPARγ-mediated changes in CD36 mRNA expression were mirrored by comparable changes at the protein level argues against this. Alternatively, as there was also no change
in levels of glucose, insulin or the inflammatory marker IL-6, it is possible that the exercise programme was not a large enough stimulus to reduce these systemic markers, despite there being a 178% increase in the leukocyte mRNA expression of ABCA1 and an 88% increase in the leukocyte mRNA expression of CD36. However, the increases in the expression of ABCA1 and CD36 are relatively modest when compared to the increases observed in Butcher et al. (2008). Following 8-weeks of treadmill-based walking, there was a 246% increase in ABCA1 expression and a 172% increase in the expression of CD36, with a significant reduction in total-cholesterol and a significant increase in HDL-cholesterol also observed. Following a similar argument, in Chapter 2 (section 2.2.2), it was demonstrated that RSG, as an exercise mimetic, was capable of inducing an increase in the expression of CD36 (as seen in the current study). The increase in mRNA expression was correlated with an increase in the surface level expression of CD36, however, the magnitude of change in the RSG-induced expression of CD36 was far greater than that seen in the current study, and thus may explain why an RSG-induced increase at a functional level was also observed in vitro but not in vivo.

A (possibly PPARγ-mediated) decrease in the leukocyte mRNA expression of MMP-9 in the adherent group was observed, as was a decrease in the levels of circulating MMP-9. This suggests that in the case of MMP-9, a down-regulation in the expression of MMP-9 mRNA did translate to a down-regulation in the activation of MMP-9 at its protein level, although it should be noted that circulating MMP-9 is derived from MMP-9 secreted from a variety of different cell-types, not just leukocytes/monocytes. Once again, this is a novel observation - reduction in gene expression of MMP-9 and circulating levels of the MMP-9 protein has not before been demonstrated following an aerobic exercise programme (or specifically, a green-exercise programme) in a healthy population.

Due to MMP-9’s role in degrading the elastic properties of the arterial wall, exercise-associated reductions in MMP-9 may result in reduced degradation of the elastin. As will be discussed later on in this section, it is possibly via this mechanism that regular exercise is
beneficial in slowing age-related changes in large artery stiffness (McDonnell et al., 2013). With regard to the means by which MMP-9 levels are reduced, one of the main regulators, specifically inhibitors, of MMP-9 levels is thought to be tissue inhibitors of metalloproteinase (TIMPs) (Nagase et al., 2006, Marchesi et al., 2012), however, TIMPs were not assessed in the current study. Instead, the current study posits that another potential mechanism by which exercise impacts upon MMP-9 expression is via the activation of PPARγ, which functions as a transcription factor regulating the expression of PPRE-bearing target genes in a ligand-dependent manner. Interestingly, the synthetic PPARγ ligand RSG has been shown to decrease both expression and activity of MMP-9 (Hetzel et al., 2003). It has been reported that RSG-activated PPARγ may bring about this effect by antagonistic transrepression of a second transcription factor, namely NFkB (Hetzel et al., 2003), and bioinformatics analyses in section 4.3.5 support this view, in that several NFkB-REs, but not PPREs, were identified in the MMP-9 promoter.

Many of the measures of vascular haemodynamics were improved following the green-exercise programme. However, the gold-standard measure of large artery stiffness, aPWV, did not change following adherence to the green-exercise programme. Similarly to the cross-sectional study (see Chapter 3), no associations were observed between MMP-9 and aPWV, but this was likely due to the age of the cohort being too young to present structural changes of the large artery. Based on the current data, it is suggested that the lower levels of stiffness observed in older, physically active individuals compared to older, sedentary individuals seen in McDonnell et al. (2013) may occur as a result of continuous exercise-induced reductions in MMP-9, and therefore reductions in age-related increases in arterial stiffness. Clearly however, further experiments are required to confirm this suggestion. In contrast, AIx, a surrogate, indirect measure of arterial stiffness (Laurent et al., 2006), did undergo a statistically significant decrease. This was expected for a number of reasons; firstly, data from McEniery et al. (2005) demonstrated that in those aged < 50 years (as in the present study), AIx is a more sensitive marker or arterial stiffness. Secondly, an 8-week
long programme of moderate-intensity exercise is likely to not be a long enough period of
time to elicit a change in the structure of the large arteries, with previous studies
demonstrating that a moderate-intensity programme of at least four months or an 8-week
programme of high-intensity exercise is required in order to observe a reduction in aPWV
(Hayashi et al., 2005, Kakiyama et al., 2005).

Similarly, reductions were observed in SBP, DBP and MAP for both seated and supine, and
peripheral and central measures. These slightly varying methods of measuring blood
pressure are important as seated measures of peripheral blood pressure are the blood
pressure measurements that are carried out in a GP surgery, and the diagnosis of
hypertension is currently based on seated, peripheral blood pressure values. However,
central blood pressure is an important measure as it represents the pressure to which the
organs are subjected, and it has been demonstrated that central blood pressure is a better
predictor of CV outcome than peripheral blood pressure that is traditionally measured at the
brachial artery (Roman et al., 2007).

A reduction in these measurements of BP suggests an improvement in the functioning of the
resistance arteries and an overall reduction in CV risk. The mean age of the cohort fits with
the age at which AIx has been shown to be a more appropriate measure of stiffness than
PWV (McEniery et al., 2005), and, as stated above, significant reductions in AIx in the
adherent group were observed following adherence to the exercise programme. Such
reductions demonstrate improvements in systemic arterial stiffness and the functioning of the
peripheral and resistance arteries, and also suggest an improvement in endothelial function.
Once again, these reductions in CV risk are novel observations in the context of a green-
exercise programme.

The actions of TZDs such as RSG in regulating PPAR\(\gamma\) target gene expression have been
shown to be responsible for beneficial effects in the context of CVD and type-2 diabetes,
including decreasing arterial stiffness over a 12-week period, in a diabetic population
(Gerstein et al., 2006, Yu et al., 2007). Further to this, Ryan et al. (2007) and Hetzel et al.
(2005) have reported reductions in PWV and improvements in endothelial function in response to TZD treatment in non-diabetic cohorts. This suggests that the improvements observed in Alx in the current study (which suggest an improvement in endothelial function) may also occur as a result of exercise-induced activation of PPARγ. This is further supported by a significant and positive correlation between mRNA gene expression of MMP-9 (or circulating levels of the MMP-9 protein) with Alx, since it is suggested that MMP-9 may be regulated via PPARγ (see above).

Importantly, therefore, while due to an association with increased risk of death from CV causes (Nissen and Wolski, 2007) the prescription of RSG has fallen dramatically in recent years (Leal et al., 2013), exercise may provide a safe alternative to RSG as a method of inducing beneficial PPARγ-dependent effects (Butcher et al., 2008, Thomas et al., 2012, Nissen and Wolski, 2007, Leal et al., 2013). With regard to the current study’s findings, it is proposed that an exercise-induced activation of the nuclear receptor PPARγ may cause the observed down-regulation in the expression of the elastin-degrading gene MMP-9 (and also possibly improve endothelial function), which in turn may ameliorate physical inactivity-related vascular remodelling. Change in MMP-9 levels was positively associated with change in LDL-cholesterol and total-cholesterol, interesting as both are associated with arterial wall inflammation and vascular remodelling.

At baseline, there were significant differences between the non-adherent group and the adherent group with regard to the following measures of mental health; SPS attachment and SPS reassurance of worth. These two constructs relate to emotional closeness and recognition of one’s own worth, respectively. Both of these measures were higher in the adherent group at baseline which might suggest that these two variables are important in participants who are aiming to improve physical activity levels via adherence to a community-based green-exercise programme.

Additionally, a number of green-exercise-associated improvements in markers of mental health occurred in the adherent group. A significant increase in the Rosenberg Self-Esteem
Scale suggests that an improvement occurred in the self-esteem of the green-exercise adherent group whilst a reduction was observed in the STAI which demonstrates an improvement in the participant's levels of state anxiety. A significant reduction in the PSS suggests an improvement in the perceived stress of those participants who adhered to the green-exercise programme, whilst a significant reduction was also seen in the CES-D scale which is suggestive of an improvement in depression levels. The construct ‘tension-anxiety’ of the POMS questionnaire was also significantly reduced in the exercise adherent group, suggesting that the tension-anxiety mood construct was improved following adherence to the green-exercise programme. Due to the various changes observed, the data suggests an improvement in the overall mental health of the adherent cohort, which is further supported by a significant reduction in the POMS total mood disturbance measure which suggests an overall improvement in the overall mood state of the adherent group.

Improvements in measures of mental health including in mood and self-esteem have been observed previously following acute bouts of green-exercise (Pretty et al., 2005b), but it is thought that this is the first time such improvements in mental health have been observed following an 8-week green-exercise programme. As discussed in Chapter 1, the literature regarding exercise and mental health suggests that different modes and intensities of exercise affect markers of mental health differentially (Scully et al., 1998). However, the current author would suggest, based on the current data, that adherence to a moderate intensity, green-exercise programme is a useful tool in improving several markers of mental health in the general population.

Interestingly, the lack of effect on anthropometric parameters such as waist circumference, BMI and body weight imply that the mechanism(s) triggered by the current intervention are distinct from weight loss per se. This suggests that the observed changes in the markers of CV risk that were measured such as MMP-9 levels, blood pressure, and self-esteem, did not occur as a result of a decrease in measures of obesity, but as an exercise-associated mechanism independent of change in obesity state. There was also no change in diet.
These data are interesting as exercise-induced weight loss is often the motivation for people to take up exercise and it can be very demotivating when changes in weight do not occur. Thus an important conclusion from the current study, whose dissemination to the wider public the current author would very much support, is that these data demonstrate that there are very important changes in CV risk factors occurring independently of reductions in body mass.

Many significant correlations were observed between and within the three areas of interest, however, it should be stressed that in general the correlations were quite weak and the cohort size was relatively small, and therefore these correlations should be interpreted with caution. Since the exercise intervention and its effect were the main focus of this project, the associations observed between the change in IPAQ MET-minutes and the other investigated parameters will be covered first. The biomolecular and biochemical data demonstrated that those participants with the greatest change in MET-minutes had the greatest change in the leukocyte mRNA expression of CD36, ABCA1 and MMP-9, and in the levels of circulating MMP-9. The association with CD36 and ABCA1 was a positive one, demonstrating that greater increases in MET-minutes was associated with greater increases in the mRNA expression of these two proteins involved in the RCT system. The MET-minutes associations with MMP-9 were inverse; demonstrating that those with the greatest increases in MET-minutes had the greatest reductions in the expression of MMP-9 and in the levels of circulating MMP-9.

The change in MET-minutes was also significantly and inversely correlated with change in peripheral and central measures of SBP, DBP and MAP and also with Alx (heart-rate-corrected and uncorrected). Finally, changes in MET-minutes also correlated with CFPWV; however, this association disappeared after correcting for MAP. Thus, the greatest increases in exercise levels correlated with the greatest improvements in vascular haemodynamics, and hence CV risk.
IPAQ MET-minutes delta change also demonstrated associations with changes in markers of mental health. The greatest changes in MET-minutes was associated with the greatest improvements in self-esteem, cognitive failures, anxiety, perceived stress, tension-anxiety (construct of mood) and vigour-activity (construct of mood). It is interesting to consider that increased physical activity levels may be associated with a reduction in cognitive failures due to an increase in cerebral blood flow (Alosco et al., 2014), and such an association would be interesting to investigate using transcranial Doppler ultrasound. However, in the current study, adherence to the green-exercise programme did not result in a significant reduction in cognitive failures from baseline to 8-weeks compared to the non-adherent group.

With regard to associations between green-exercise-associated changes in biomolecular/biochemical variables versus vascular/haemodynamic changes, the levels of circulating MMP-9 had a number of significant and positive correlations with changes in vascular measures of CV risk, including change in central and peripheral SBP, DBP and MAP, and with wave reflection as assessed by Alx. As mentioned previously, there was also a significant correlation between Alx and the expression of MMP-9. Importantly, by identifying an association between MMP-9 expression and Alx, these data may provide a biomolecular mechanism to underpin our previous observational data, in which our group have illustrated that Alx was significantly lower in highly physically active young individuals compared to sedentary controls (McDonnell et al., 2013). The observed association between decreases in MMP-9 and wave reflection (as measured via Alx) suggest that MMP-9 may have a role to play in the progression of the early return of the reflected wave, and therefore in increases in central systolic pressure. Thus, if exercise (possibly via PPARγ – see above) decreases the expression of the enzymes that are involved in age-related increases in vascular remodelling and large artery stiffness, then progression of increased systolic hypertension may be significantly reduced as a consequence.

Interestingly, there was no significant change in the levels of glucose or insulin following adherence to the green-exercise programme and therefore it is interesting to observe that
there was a significant and positive correlation between them. This suggests that those participants with the greatest changes in insulin levels also had the greatest changes in glucose levels (although each individual change did not attain statistical significance). These data are reassuring in the context of CV associated type-2 diabetes risk as the two blood-borne factors have been shown previously to be related, i.e. insulin secretion stimulates glucose uptake in healthy individuals (Wilcox, 2005, Denton and Tavaré, 1997). IL-6 did not decrease following the exercise programme, however change in IL-6 levels was associated with changes in the measures of obesity; weight and waist circumference, as has been reported previously (Kern et al., 2001). However, it was also interesting to observe that change in IL-6 was also associated with change in glucose. This observation is interesting as IL-6 has been seen to have a relationship with insulin sensitivity (Fernandez-Real et al., 2001). It is interesting that reductions in AIx occurred independently of reductions in these blood-borne markers associated with diabetes, as increased AIx is observed in diabetes patients (Wilkinson et al., 2000a).

MMP-9 (in terms of both mRNA expression and protein levels) had a significant and negative correlation with ABCA1, thus suggesting that they are both regulated by the same mechanism, i.e. PPARγ, as posited further above. As discussed in Chapter 1, MMP-9 is present in atherosclerosis as a pro-inflammatory cytokine that further promotes lipid retention (Libby et al., 2009) and its negative association with ABCA1 suggests that down-regulation of MMP-9 might be beneficial in additional contexts such as atherogenesis, alongside its role in elastin degradation and vascular remodelling.

Interestingly, there were significant correlations between the change in PSS measure of perceived stress and the change in many measures of vascular haemodynamics. This adds weight to the suggestion that was made in Chapter 1 regarding an association existing between mental health and vascular health via the SNS (Sherwood et al., 1995, Sherwood et al., 1999). A reduction in perceived stress might lead to a dampening of the SNS and therefore a reduction in blood pressure. Significant correlations between measures of
vascular haemodynamics and mental health were not limited to the PSS. Other observed correlations indicated that those with the greatest improvements in self-esteem, depression and POMS vigour-activity had the greatest reductions (and hence improvements) in vascular measures of CV risk. There were a number of significant correlations between mental health and vascular haemodynamics suggesting that either there is a relationship between the two different indices of health, or that regular exercise participation does not have differential effects on these systemic markers of health.

The current study has several limitations: for example, the current investigation needs to be replicated in a larger cohort (the correlation analyses in particular should be interpreted with caution due to the small sample size and some correlations being relatively weak), and over a longer period of time in order to confirm the above findings. Further to this, the total number of participants recruited on to the intervention study surpassed the recommendation created by the power calculation, however, due to the post-hoc splitting of the group (i.e. into adherent and non-adherent groups), there was insufficient power based on that recommended via the power calculation. Thus, highlighting the need for a larger cohort in future studies. Furthermore, a longer longitudinal study would aid determination as to whether a long-term down-regulation of MMP-9 is associated with reduced age-associated changes in aPWV. Such future studies may provide additional evidence supporting the concept that exercise mediates a slowing in age-related arterial stiffness due to a reduction in MMP-9-catalysed elastin-degradation and therefore vascular remodelling. Moreover, further investigation of the role of PPARγ and NFκB signalling in the effects investigated in the current study should be carried out in order to confirm or disprove the suggestion that the PPARγ signalling pathway has contributed to the effects observed in the current study. The IPAQ was used to quantify physical activity levels at both baseline and at 8-weeks, and it is reassuring that the adherent group demonstrated a significant increase in physical activity levels, whereas the non-adherent group demonstrated no change. This is reassuring as the groups were split based on their attendance on the walking programme, and therefore the
IPAQ data is in line with attendance. However, the IPAQ is a subjective measure and using a more objective measures of physical activity (i.e. accelerometers such as the BodyMedia SenseWear devices used on a sub-cohort of the current study) from baseline through to 8-weeks would have been a more reliable method of quantifying the level of physical activity carried out and for splitting the cohort into the two groups. Nevertheless, despite these limitations, the current study was successful in meeting its aims, and the present study provides strong evidence that beneficial improvements in markers of CV risk and improvements in mental health can be triggered in previously sedentary participants who significantly increase their physical activity levels via means of a low/moderate-intensity, aerobic, green-exercise programme. As stated in Chapter 1, most barriers reported as reasons not to exercise apply to a much lesser extent with regard to green-exercise, making it a potential route by which participation in exercise may be increased, and therefore possibly a useful tool by which physical activity levels can be increased and CV risk decreased within the general population.

In conclusion, the findings presented in the current study may provide a biomolecular mechanism (namely, exercise-associated MMP-9 down-regulation, possibly mediated via a PPARγ-dependent cell signalling effect within leukocytes) that contributes towards aerobic exercise’s ability to delay age-related increases in wave reflection, large artery stiffness and therefore in CV risk. Furthermore, data from the current moderate intensity, 8-week green-exercise intervention suggests that increasing physical activity levels in those who previously participated in no structured exercise has diverse and beneficial health benefits including beneficial impacts upon; blood-borne markers, such as genes involved in atherosclerosis and arteriosclerosis prevention, markers of arterial stiffness and central blood pressures, and mental health. Green-exercise is free and more accessible to the general public than more traditional exercise modalities such as swimming, the gym and keep-fit classes. Therefore, the data suggests that green-exercise may be a useful mechanism by which physical
inactivity related disease such as CVD and poor mental health could be alleviated and even combated, particularly in low SES areas.
Chapter 5 - General Discussion
5.1 General discussion of the project’s findings

Green-exercise is thought of as having synergistic effects due to the supposed beneficial effect of not only carrying out exercise, but also of carrying it out in a natural, green environment - which has been demonstrated to be associated with its own health benefits independent from exercise, perhaps as a result of its salutogenesis. The current project aimed to evaluate the impact of green-exercise programmes upon indices of health from three distinct but related health disciplines, which have in common their usefulness in reducing CV risk. The project aimed to carry out this investigative evaluation as the impact of a green-exercise programme upon health had not before been investigated. The project adopted a multi-disciplinary approach, as it was deemed important to achieve an understanding of the systemic effect of this synergistic mode of exercise, rather than just insight into one effect.

The author would contend that the findings that arose from the current PhD project (see Chapter 4, specifically) are very important from a public health point of view, due to the broad beneficial health effects that were accrued from a simple, accessible and cost-effective intervention. The study was successful in demonstrating that adhering to a green-exercise programme (that involved walking for approximately 45 minutes, twice a week in a natural, green environment) was associated with an up-regulation in the expression of genes that are involved in the removal of cholesterol from the endothelium of the arterial wall and therefore in reducing the risk of foam cell development, the related inflammatory response and the eventual fatty streak formation. The study also demonstrated that it was successful in reducing the surface level expression and the mRNA expression, and hence the secretion into the circulation, of MMP-9, an enzyme involved in inflammation and elastin degradation, and that this reduction was positively correlated with a reduction in vascular haemodynamic markers of CV risk including central blood pressures and arterial stiffness (via measurement of wave reflection). These data are of great interest as they suggest that the exercise-associated reductions in CV risk may occur as a result of a reduction in the degradation of
the elastin in the arterial wall, and therefore a slowing in the vascular remodelling and hence the stiffening of the arterial wall.

That peripheral and central BP and wave reflection (measured via AIx) were reduced following adherence to the green-exercise programme further highlights the beneficial impact of the green-exercise programme as a tool in reducing CV risk, with emphasis on how it might be useful as a means of reducing physical-inactivity related CV risk in areas that are of low SES but with easy access to green space, such as the south Wales valleys.

Similarly, the improvements observed in mental health suggest that adhering to the green-exercise programme was associated with improvements in the following constructs of mental health; self-esteem, anxiety, depression, perceived stress and mood. In the cross-sectional study, it was observed that moderately active participants had improved cognitive functioning (as assessed using CFQ) compared to the sedentary participants, however, a reduction in cognitive failures was not observed following adherence to an 8-week low/moderate-intensity green-exercise programme. It would be interesting to observe whether this is a change that requires a long period of regular exercise to be induced. Especially when considering that it may be mediated by an increase in cerebral blood flow (Brown et al., 2010, Alosco et al., 2014).

As these green-exercise programmes were low/moderate-intensity, community-based and work-place based walking programmes, they therefore had an element of social interaction and due to the intensity and unstructured nature of the walks, they provided an environment in which conversation would have been possible. Due to this, it is possible that some, all or none of the effect on mental health was as a result of increased social interaction. The current author would suggest that the improvements were as a result of an interaction between the increased exercise levels and the increased sociability, and that some participants will have responded more strongly to the increased green-exercise, whilst others will have responded more strongly to the increased social contact. However, it is worth noting that no significant changes in the Social Provisions Scale occurred. It would be
interesting to discern to what extent each of the two variables affect the measures of mental health by having an associated intervention whereby a cohort of individuals walk alone at the same intensity, frequency, duration and in the same environment. In the same vein, it would be interesting to be able to determine to what extent the improvements observed in the measures of mental health (and to some extent the improvement in BP) occurred as a result of the psychologically restorative benefits of the green space in which the participants exercised. In order to do this, a second study would need to be employed whereby participants are recruited to take part in a community-based or work-place based walking programme of equal length, but that is set either indoors or in an urban, grey environment.

5.2 **Wider clinical/public health relevance**

Clearly, these data have strong implications for public health policy. Based on the evidence, the current author would contend that green-exercise provides a realistic opportunity for individuals who have previously believed that exercise must involve a gym, swimming pool or participation in a sport, or have been too busy, not had enough disposable income, or been too embarrassed to take part in any of the traditionally promoted forms of exercise. Using the current study as evidence, the use of local green space as a means of beneficially improving health should be advocated, and those who may particularly benefit from such a mode of exercise should be made aware of its systemic benefit.

On the back of this evidence, more emphasis and promotion of community-based and work-place based walking programmes should be imposed as a means of combating CVD. These data presented are also interesting from a clinical point of view as they may provide a mechanism that explains how physically active lifestyles are associated with a slowing in the progression of age-related large artery stiffening (Tanaka et al., 1998, McDonnell et al., 2013). Increased levels of MMP-9 in the serum are present in isolated systolic hypertension and are associated with increased large artery stiffness, as determined using aPWV (Yasmin
et al., 2005). Therefore, as exercise in the current study was associated with a decrease in
the expression of MMP-9, it is suggested that an exercise-associated down-regulation of
MMP-9 results in a reduction in the elastin breakdown of the arterial wall and therefore a
slowing in the natural, age-associated, stiffening of the large arteries. Further to this, the
decrease in the expression of MMP-9 was correlated with a reduction in wave reflection
(measured via Alx), and this suggests that MMP-9 may play a role in the early return of the
reflected wave and therefore in increases in central systolic pressure. Therefore,
importantly, an exercise-associated decrease in MMP-9 expression may also play a role in
slowing the progression of isolated systolic hypertension. These data therefore have
important consequences for reducing CV risk.

However, it should be noted that green-exercise is not without its disadvantages, especially
in the context of exercise promotion amongst sedentary participants. Even in people
motivated to maintain regular exercise, seasonal variations draw people indoors (Hug et al.,
2008, Prochaska and Marcus, 1994). However, it was noted in the current study that those
walking groups who had a very engaging, encouraging and active walk leader had better
attendance, in all weathers. It is interesting to also note that the expectation of social benefit
is a strong determinant of participation in indoor exercise (Hug et al., 2008, Hug et al., 2009),
but community-based and work-place based green-exercise programmes provide the same
social opportunities.

Recently, much focus has been placed on the effect of walking upon health and disease
prevention. For instance, Hildebrand et al. (2013) demonstrated a significant inverse
association between physical activity levels and post-menopausal cancer risk. Interestingly,
Naci and Ioannidis (2013) demonstrated that in the secondary prevention of CHD, stroke
rehabilitation, diabetes prevention and heart failure treatment; exercise interventions and
pharmaceutical drug therapies offer similar benefits in their effects on mortality of these
conditions. Further to this, a report conducted by Ramblers and MacMillan Cancer Support
suggests that increasing levels of walking (a free activity) in England could save 37,000 lives
per year, 6,700 cases of breast cancer, and lead to up to 300,000 fewer diagnoses of type-2 diabetes (Ramblers and MacMillan Cancer Support, 2013). The data of the current PhD project support these reports by further demonstrating the beneficial effect of walking on systemic health and CV risk. Figure 5.1 demonstrates the systemic effect of green-exercise on markers associated with CV risk, and how these seemingly separate entities of health have been observed to be associated in the current exercise intervention.

Finally, in addition to the directly relevant matters discussed above, several noteworthy, but only tangentially-related, issues arise from consideration of the project’s findings. For example, the data demonstrated that there was a significant inverse correlation between ABCA1 expression and glucose levels. In the current study this is of interest, as exercise is currently of much interest as a means of finding alternatives to pharmaceutical treatment, particularly with regard to type-2 diabetes. Until 2010 (in the UK), RSG was commonly prescribed as an anti-diabetic drug to aid the lowering of fasting glucose levels (however, prescription has fallen dramatically as a result of its harmful effects (Leal et al., 2013)). Glucose is a systemic factor, 80% of which is used by the skeletal muscle. RSG’s mechanistic effect occurs via its targeting of PPARɣ in a variety of cell-types, including skeletal myocytes. In the current study, ABCA1 (which is also a target of PPARɣ) was investigated at an mRNA level, obtained from the mixed leukocytes acquired via phlebotomy/blood fractionation. The correlation between systemic glucose levels and the leukocyte PPARɣ target gene (i.e. ABCA1) expression suggests that PPARɣ is globally active and that leukocyte PPARɣ target gene expression may in fact be a systemic biomarker (i.e. an entity that can be conveniently measured) for global PPARɣ activation, systemic glucose utilisation and hence ultimately clinical type-2 diabetes risk. These data provide further evidence for the importance of promoting non-pharmaceutical therapies for combating disease burdens such as type-2 diabetes as not only have they been observed to be more beneficial in some cases, but their positive effect does not stop at the target factor,
as in the case of pharmaceuticals. On the contrary, as demonstrated in the two study chapters of the current thesis, exercise is a systemic, multi-factorial phenomenon.

Another issue of interest is that recent data suggests that one mechanism responsible for the supposed synergistic effect of green-exercise may be as a result of increased exposure to sunlight (Baker et al., 2014). The very recent publication demonstrated that following abstinence from day light for 85 days, 49 submariners had decreased levels of vitamin-D and increased levels of MMP-9 compared to baseline. An inverse association between MMP-9 levels and vitamin-D was also observed (Baker et al., 2014). These data suggest that the reduction in MMP-9 levels observed in the current study may not solely be as a result of the increase in exercise levels, but may also be due to increased exposure to daylight as a result of exercising regularly in an outdoor setting, thus adding to the hypothesis regarding a synergistic effect of exercising outdoors. Further to this, a recent study was highlighted in the press by the Royal College of Physicians president; Sir Richard Thompson. The study reported had investigated the effect of sunlight on blood pressure, demonstrating that exposure to sunlight reduces blood pressure; thus further highlighting the synergistic effect of exercising in an outdoor setting and its effect on markers of CV risk (Kinver, 2014). However, in order to confirm this suggestion, the current study would need to be replicated in order to assess vitamin-D levels, and to recruit a comparator group of participants partaking in an indoor walking group.

5.3 Study limitations and future directions (including suggestions for future work)

The specific limitations of the two studies covered in Chapters 3 and 4 were discussed within the respective ‘discussion’ sections of those chapters; in this section, more general consideration of these limitations will be used as starting-points for the drawing-up of recommendations for further study within this subject area.
As stated in Chapter 4, the intervention study was of relatively short duration, and involved a cohort of only moderate size ($n = 65$). While this was appropriate for the time and scale available for a single PhD study, in order to be able to draw stronger conclusion from the data, it would be beneficial to replicate the intervention study over a longer period of time and in a larger population. A larger cohort being observed over a longer period of time would allow for stratification into age groups, and therefore an effect of exercise adherence on aPWV in the older cohort might be observed. Further, it would be interesting to determine whether a change in MMP-9 was correlated with a change in aPWV, and even whether MMP-9 levels contributed to the change in aPWV, via regression analysis. Further to this, a longer intervention would be beneficial as a number of the markers investigated have been observed to change with an exercise programme, but the changes were observed following a longer time frame than that which was conducted in the current study. A larger cohort might also strengthen many of the correlations observed in the data set following adherence to the green-exercise, allowing for more summative conclusions to be drawn regarding the interplay between some of the variables.

Despite a 'non-adherent' group being representative of ‘real-life’, it would be beneficial to also recruit a non-exercising control group. Due to the significant differences observed between the non-adherent group and the adherent group at baseline for two of the Social Provisions Scale constructs, it would be interesting to compare non-adherence participants and non-exercise control participants at baseline in terms of mental health, to determine whether any differences exist even between people who are not taking up exercise and those who planned to, yet did not adhere. It would also be interesting to observe whether the act of signing up to the green-exercise programme yet not adhering has any effect, positive or negative, in the non-adherent compared to the non-exercising control group by week eight. Further to this, comparing the impact of the green-exercise programme to an indoor or urban exercise programme of the same length and intensity would be useful to not only discern how much of an impact the green space had upon the changes observed, but
also this may allow for determination of how much a green space-mediated reduction in stress has upon the dampening of the SNS and therefore reduction in arterial pressures. If an urban-exercise programme, which would lack the supposed rejuvenating effect of a green space, was associated with less of an improvement in stress, then it would be interesting to observe whether this was also associated with less of an improvement in blood pressure.

Based on that which was discussed above with regards to limitations and future directions section, it is suggested that a hypothetical similar research project (to be undertaken in the near future, should funding be gained to support it) would compare the markers of CV risk investigated in the current study but in a more long-term green-exercise programme, and compare the effects to participants in a concurrently run non green-exercise programme and a non-exercising control group. This may also aid in determining whether the supposed rejuvenating effect of nature is effective in dampening stress and SNS activation and therefore reducing BP to a greater extent than the impact of exercise on BP in a more traditional or gym-based/indoor setting. The investigation would likely benefit from recruiting more participants per group than the current study, and would likely benefit from running for a longer period of time, with a consultation half way through to determine which changes have and have not yet occurred. Importantly, future studies should aim to recruit an equal mix of males and females and across a broad age range in order to eliminate any confounding effects such as pre- and post-menopausal hormonal changes, with regards to the results observed.

In the future, it would also be advisable to counter-balance the order of the mental health questionnaires within the questionnaire booklet, as it is possible that the responses to the data could have been influenced by order effects. It would also be interesting to collect data regarding the ‘greenness’ or naturalness of the walks taken as part of the walking programme. This would aid in determining whether it was viable to compare the effects of green-exercise between different groups (whom use different routes and different areas). The data could also aid in determining the amount of greenness that is most beneficial.
Leslie et al. (2010) demonstrated that there is disagreement between perceived measures of greenness and objective measures of greenness (i.e. using satellite images to measure ‘normalised difference vegetation index’), and therefore it would be advisable to collect both types of data. As mentioned previously, the current study aimed to evaluate the impact of a ‘real-life’ green-exercise programme that was running independently of the research project – just as would occur in real life. Consequently factors such as heterogeneity introduced by walk leaders and exercise intensities were not controlled for; this creates difficulties if wanting to state at what intensity green-exercise is most beneficial and if wanting to compare it to another mode of exercise, such as treadmill based walking. In the future, it would be advisable to investigate the use of tri-axial accelerometers or heart rate monitors in order to be able to quantitatively determine the intensity of the walking programmes.

5.4 Final conclusion

For the first time (to the author’s knowledge), the current project has demonstrated that adherence to a low/moderate-intensity green-exercise programme, via the mode of walking, is associated with improvements in markers of health at a molecular, physiological and psychological level; thus resulting in an improvement in the overall systemic health of the participants. As displayed in Figure 5.1 a number of relationships appear to have emerged from the data, and these may help explain how the complex effects of exercise are manifested as a systemic entity. In conclusion, adherence to a green-exercise programme appears to have beneficial effects on three relatively diverse areas of health which are all associated with CV risk, and therefore green-exercise may be a useful tool for combating physical-inactivity related and age-related cardiovascular disease, especially in areas of low socio-economic status.
Figure 5.1 displaying the effects and relationships observed between the three arms of the project, with regular participation in green-exercise as a stimulus.
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