PHYSICAL ACTIVITY, ADIPOSITY, STRESS-INDUCED INFLAMMATION, AND CARDIOVASCULAR DISEASE RISK

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STUDENT DECLARATION:

I, Romano Endrighi confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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ABSTRACT

Physical inactivity and adiposity are independent risk factors for several chronic conditions including coronary heart disease. Activity and adiposity also modulate psychophysiological responses to psychosocial stress. Since heightened cardiovascular and inflammatory responses to mental stress predict cardiovascular risk, these two factors may influence cardiovascular risk through modulation of autonomic reactivity to stress. However, experimental evidence to support this hypothesis is scarce.

The aim of this project is to investigate the associations between physical activity, adiposity, mental stress and mood and physiological reactivity using naturalistic and controlled laboratory methods. Study one examined the association between self-reported physical activity participation, diurnal cortisol rhythm and mood symptoms in everyday life. Study two used an experimental design to examine the effect of physical activity on mood symptoms and on cardiovascular and inflammatory responses to acute mental stress. Exercise withdrawal was used as a model of physical inactivity to induce mood disturbances in healthy, active participants. Several stress-induced markers relevant in cardiovascular disease were examined including pro-inflammatory factors and cortisol.

Study three examined the effect of adiposity on physiological responses to acute mental stress and mood. Weight loss was experimentally induced through caloric restriction in overweight or obese women. Responses to acute stress were compared before and after weight loss. Cardiovascular and inflammatory responses

to acute stress were evaluated to establish whether adiposity is associated with a heightened or blunted response.

The combination of studies presented in this thesis provides insight into the complex relationships that links behavioural factors such as physical activity with mood and stress. An understanding of the mechanisms involved in the association between adiposity, physical activity and cardiovascular risk is invaluable in informing preventive strategies and health related programmes.

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophin hormone
ANS	Autonomic nervous system
AUC	Area under the curve
BHF	British Heart Foundation
BMI	Body mass index
BMR	Basic metabolic rate
BP	Blood pressure
Bpm	Beats per minute
CAR	Cortisol awakening response
CES-D	Centre for Epidemiological Study Depression Scale
CHD	Coronary heart disease
CNS	Central nervous system
CO	Cardiac output
CPM	Counts per minute
CRF	Corticotrophin releasing factor
CRP	C-reactive protein
CVD	Cardiovascular disease
CV	Cardiovascular
DBP	Diastolic blood pressure
DEXA	Dual-energy X-ray absorptiometry
EDTA	Ethylene-diamine-tetra-acetic acid
ELISA	Enzyme-linked Immunosorbent Assay
e-NOS	Endothelial nitric oxide synthase

EMA	Ecological momentary assessment
EWP	Exercise withdrawal paradigm
GHQ-28	General Health Questionnaire (28 items)
HDL	high-density-lipoprotein
HPA	Hypothalamic pituitary adrenal
HR	Heart rate
HRV	Heart rate variability
HSE	Health survey for England
ICAM-1	Intracellular adhesion molecule-1
IHD	Ischemic heart disease
IL-1ra	Interleukin-1 receptor antagonist
IL^n	Interleukin-(1,6,10,18)
IL-1β	Interleukin-1 beta
i-NOS	Inducible nitric oxide synthase
IPAQ	International Physical Activity Questionnaire
LDL	low-density-lipoprotein
MAP	Mean arterial pressure
MCP-1	Moncyte chemoattractant protein-1
MET	Metabolic equivalent of task
MHPG	3-methoxy-4-hydroxyphenylglycol
MT	Mirror tracing task
MVPA	Moderate to vigorous physical activity
Ox-LDL	Oxidized low density lipoprotein
PA	Physical activity
PAWS	Physical activity withdrawal and stress study

POMS-SF	Profile of Mood Scale-Short Form
PS	Public speaking scenario task
PSS	Perceived Stress Scale
RCT	Randomized controlled trial
rMSSD	Root mean square of the successive differences in inter-beat intervals
RPM	Revolutions per minute
SAM	Sympathetic adrenal medulla
SBP	Systolic blood pressure
SES	Socioeconomic status
SMC	Smooth muscle cells
sTNF-r	Soluble tumour necrosis factor receptor
TNFα	Tumour necrosis factor alpha
TPR	Total peripheral resistance
TSST	Trier Social Stress Test
WHR	Waist to hip ratio
WLSR	Weight loss and stress responses study
15-LO	15-lipoxygenase

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CHAPTER 1: LITERATURE REVIEW

In this review the aetiology and disease process of coronary heart disease (CHD) will be briefly described. In addition, the main risk factors including traditional and psychosocial factors will be discussed. I will then summarise observational research on the role of stress and inflammatory processes in cardiovascular disease (CVD) and cardiovascular (CV) risk factors. This chapter will then discuss the evidence for an association of physical activity with mood and stress and its relevance in CVD with particular attention to the inflammatory pathways involved.

This chapter will also address the literature relating obesity and adiposity with inflammation and CVD, and summarise studies addressing the association between adiposity and stress-induced autonomic and inflammatory responses. I will draw on observational and experimental research, and identify the gaps this PhD project sets out to address.

1.1 Cardiovascular Disease

CVD is the leading cause of death in developed countries accounting for 35 per cent of deaths each year. Coronary heart disease, or ischemic heart disease (IHD), and stroke are the most common form of CVD accounting for nearly 28 per cent of deaths in men and 25 per cent in women in the United Kingdom (UK) in 2008 (BHF). Mortality due to CVD in the year 2008 was 191,000 nearly half of whom were for CHD. Death rates in the UK have been falling but are higher compared to other Western European country. The prevalence of CHD in the most recent national

survey (2008) was estimated to be 2.7 million. The total economic cost of CVD in the UK in 2006 was estimated to be about 30 billion pounds per year. This cost is mainly derived from health care costs, loss of productivity and outpatient care (Luengo-Fernandez, Leal, Gray, Petersen, & Rayner, 2006). In addition to an economic cost, CVD has a profound negative impact on quality of life and often leads to psychological impairments including psychological distress, depression and cardiac fatalism in sufferers, but also in family members who in the majority of cases act as carers.

1.2 Standard Risk Factors.

The term risk factors was first used half a century ago in a publication from the Framingham study (Kannel, Dawber, & Kagan, 1961). Standard, or traditional, CV risk factors include modifiable factors such as hypertension, dyslipidemia, cigarette smoking, diabetes mellitus, obesity and physical inactivity, and non-modifiable ones such as age, male gender and family history of CVD. Traditional risk factors may explain less than half of total CVD mortality (Greenland et al., 2003; Hackam & Anand, 2003; Pasternak et al., 2003) prompting the need to identify other factors or markers that may be causally related to CVD or to the development of cardiac risk factors. In recent years the role of systemic, low-level inflammation in the development and progression of CHD (Libby, Ridker, & Hansson, 2011; Dandona, Aljada, & Bandyopadhyay, 2004) has received increase attention and will be reviewed in this chapter.

1.3 Psychosocial Factors.

1.3.1 Psychosocial Stress and CVD.

It is well established that psychosocial and behavioural factors are aetiologically important in the development and course of CVD and related metabolic disorders. Behavioural and lifestyle risk factors for CVD include excess weight and obesity, cigarette smoking, lack of physical activity and sedentary lifestyle and a diet rich in fat and lacking in fruit and vegetables. Evidence has emerged for associations of psychosocial characteristics such as low socioeconomic status (SES), negative affect, depression and anxiety, major negative life events, chronic stress at work and chronic strain and cardiovascular morbidity and mortality (Yusuf et al., 2011; Dimsdale, 2008; Rosengren et al., 2004; Frasure-Smith & Lesperance, 2010; Brotman, Golden, & Wittstein, 2007; Rozanski, Blumenthal, Davidson, Saab, & Kubzansky, 2005). Therefore, it has been estimated that psychosocial factors may explain about half of the risk for CVD.

Psychosocial, stress-related risk factors in CVD can be divided into affective, personality related and sociodemographic factors. Affective factors include depression, anxiety, burnout and negative wellbeing. Personality factors include traits such as neuroticism, anger proneness, pessimism and hostility, while sociodemographic include low social position, lack of social support or lack of a social network, negative life events and work stress. These factors may therefore influence CHD directly, indirectly, or impact on prognosis in patients with established cardiovascular disease (Rozanski et al., 2005). For example, stress may impact CV healthy through its direct effect on vascular mechanisms such as blood pressure,

insulin sensitivity or endothelial function. An indirect effect of stress may be the impact of psychosocial stress such as low social support or work stress for instance on established CV risk factors including smoking, poor diet habits or sleep problems (Steptoe & Kivimaki, 2012). In acute coronary patients, acute episode of stress such as intense anger or physical exertion may act as a trigger to initiate further cardiac events such as a myocardial infarction or cardiac death. Likewise, survivors of acute CV events may deal or cope poorly with stress related events and as a result impact negatively on quality of life or other factors such as return to work. In addition, several psychosocial risk factors do not occur in isolation but often co-exist in the same individual. As an example, depression or work stress are more prevalent in individuals of low SES (Wamala, Mittleman, Schenck-Gustafsson, & Orth-Gomer, 1999).

A great deal of research has examined the association of depressive symptoms and mood with cardiovascular outcomes. The strongest evidence to date is from two meta-analysis of prospective epidemiological studies showing that the relative risk for future CHD attributable to depression was 1.81 (95% CI 1.53 – 2.15) (Nicholson, Kuper, & Hemingway, 2006), and 1.34 (95% CI 1.17 – 1.54) for risk of future stroke (Dong, Zhang, Tong, & Qin, 2012). Depression is also important as a prognostic factor in individuals with CHD. A meta-analysis has shown that depression is associated with nearly double the risk of death (OR 1.78 95% CI 1.12 – 2.83) in patients with CHD (Barth, Schumacher, & Herrmann-Lingen, 2004). Interestingly, somatic symptom of depression including fatigue, bodily pain, sleep problems and poor appetite have been shown to predict cardiovascular mortality (Bekke-Hansen, Trockel, Burg, & Taylor, 2012) twelve months after an acute coronary event independently of the cognitive dimension of depression. This suggests that the

depression phenotype characterized by somatic or affective symptoms may be particularly damaging or toxic. This phenotype closely resembles a cluster of symptoms termed sickness behaviour in which inflammatory and immune factors are involved. Depression also conferred a higher risk of death or of a secondary event in heart failure patients (risk ration 2.1, 95% CI 1.7 – 2.6) (Rutledge, Reis, Linke, Greenberg, & Mills, 2006).

Work stress defined as a combination of high employee's effort and low reward or low control over the work environment coupled with high psychological demand is also a predictor of CHD risk. A meta-analysis of prospective observational studies has found a fifty per cent excess risk for CHD among workers with work stress operationalized as low reward – high effort, high demand – low control as well as perceived organisational injustice at work (Kivimaki et al., 2006). High job demands and lack of social support at work were also found to be risk factors for ischemic heart disease in men only (Eller et al., 2009).

Low social position or SES operationalized as low income, low educational attainment or occupational position has been found to be strongly related to CHD risk (Van Rossum, Shipley, Van De Mheen, Grobbee, & Marmot, 2000; Marmot et al., 1991). The most compelling evidence has shown that low SES was associated with risk of CHD mortality conferring an increased relative risk of between 1.3 to 2.0 (Stringhini et al., 2010; Albert, Glynn, Buring, & Ridker, 2006). Low social position and other behavioural and psychosocial factors contribute to CHD through bio-behavioural and psychobiological pathways. Stress activates autonomic responses that may become maladaptive if heightened or sustained. Therefore, it is important to understand the mechanism that mediate or moderate the association between

factors such as depression or physical activity and cardiovascular risk factors. In order to achieve this, several observational and experimental methods are available.

1.3.2 Methods of Investigation.

In order to understand the role of stress and other psychosocial factors in cardiovascular disease, different methodologies of investigation need to be applied. I will briefly outline three study methodologies in this section: The observational epidemiological method, the naturalistic, ambulatory monitoring method, and the laboratory mental stress model. I will also outline studies that have used ambulatory monitoring and mental stress testing to investigate association of psychosocial and behavioural factors with CV risk factors as these two methods have been employed in the thesis.

1.3.2.1 Epidemiological Studies.

The observational epidemiological method is used to investigate the contribution of several psychosocial factors to the development and course of CVD at the population level. Typically, a cohort free of CVD or other diseases under investigation (the outcomes) is selected and the sociodemographic, clinical and psychosocial variables of interest (the exposure) are measured from questionnaires, interviews or medical screening at baseline. The cohort is followed up over time so that the outcome of interest (e.g CV events or mortality) can be measured and evaluated at different points in time. In this way, the association between exposure

and outcomes can be evaluated using statistical techniques such as multilevel modelling, or multivariate analysis. These techniques allow to estimate the size of the association between several psychosocial factors alone or in combination, as well allowing adjustment for factor that covary with the disease under investigation, the exposure or both. The main advantage of this method is that it provides robust data on the association between stress and future CV risk factors such as hypertension, lipids or inflammatory activity as well as hard outcomes such as death. The contribution of psychosocial factors to CV morbidity and mortality can therefore be evaluated. In addition, with this method investigators are able to construct theoretical models of underlying biological pathways that may mediate these associations. However, the main disadvantage of the epidemiological method is that the assessment of the exposure factor may not be particularly sophisticated and relies mainly on questionnaires. Furthermore, this method is subject to bias since causality cannot be established. Despite these limitations the epidemiological method can provide rich and robust data on the association between stress and CV outcomes.

1.3.2.2 Naturalistic Studies.

This method of investigation is also called ecological and involves the sampling of several affective, behavioural, biological or physiological variables over several days. The range of measures that can be sampled in this kind of studies include saliva for the assessment of hormones or enzyme such as cortisol, corticotrophin realising factor, alpha amylase or various catecholamine metabolites that are relevant in CV disease. Other measures include the assessment of autonomic

function such as blood pressure, heart rate and heart rate variability over the day and night, objective recording of daily levels of physical activity using accelerometer devices, and several affective measures such as momentary stress, fatigue or mood that can be aggregated over a day or more. Many of the instruments used in this kind of research are not or minimally intrusive therefore allowing to capture the dynamic of the physiological or behavioural response in everyday life. Accelerometers such as the ActiGraph for example are tiny devices that are worn around the waist or on the wrist and provide rich information on the dynamic and frequency of physical activity. The sampling of saliva throughout the day is a stress free and easy method that can be implemented by the participant as he or she goes about their normal life without having to visit a laboratory or undergo potentially stressful procedure such as blood sampling. In this way, more detailed measures of an individual CV risk can be obtained that would not otherwise be possible in an epidemiological study. Various methods can be applied to analyse the rich data provided by this naturalistic methods. For example, in this thesis I will use saliva samples to index the diurnal cortisol profile and examine whether this profile is different in individuals who are more or less physically active. In the second study I will use data derived from an accelerometer wore over four weeks to index the degree to which participants have reduced their exercise regime. In this way an objective check of compliance with the intervention protocol can be established.

A meta-analysis has shown that ambulatory blood pressure recording obtained over a 24 hour period was associated with mortality from cardiovascular causes (Conen & Bamberg, 2008). This suggests that information collected in everyday life can provide added value to the measure obtained in the clinic or in population studies. However, this method has also some limitations. The main problem is that

the range of measures that can be obtained without burdening the participants is still limited. In addition, some behavioural activities including smoking or eating and drinking may affect the biological samples under investigation and confound the results. A further limitation of this technique is that participants may change their behaviour during the monitoring period or limit their activities due to awareness of being monitored or because they feel uneasy with the equipment or the sampling protocol. If this is the case what would be supposed to represent a typical or ordinary day or week might not be such but may be biased by participants' behaviour. Despite these limitations, the naturalistic method adds important information that may not be obtained in the laboratory or in population survey.

1.3.2.3 Mental Stress Testing.

The last method I describe that is used to investigate the association of stress and other psychosocial factors on cardiovascular risk factors is psychophysiological mental stress testing. It involves monitoring and recording psychobiological responses to various behavioural challenges in a control setting using assessment tools such as continuous heart and blood pressure monitoring, blood and saliva samples and other physiological parameters. This design allows for the assessment of various inflammatory, cardiovascular and neuroendocrine responses following the administration of acute stress tasks under tightly controlled conditions. In this way the dynamic of this autonomic response can be measured and related to the various psychosocial factors under investigation. For example, the blood pressure or cortisol

reactivity to a public speaking scenario may be indexed as the change in systolic or diastolic pressure from baseline values and compared between a sedentary and a physically active group. If one is interested in the link between depression and inflammatory responses to acute stress, the inflammatory marker under investigation may be compared between different groups categorised by their depression status. Investigators employing the mental stress model may also be interested in investigating effects rather than associations that requires an experimental manipulation. For example, affective states such as anger, depression or positive affect may be induced in the laboratory before having participants perform mental stress tasks. Their responses can then be compared against a control group so that it may be possible to evaluate the effect of anger or other factors on autonomic responses.

This method has the advantage that greater control over confounding factors and standardization of protocols can be achieved that would not normally be possible in the other two methods outline above. In addition, sophisticated biological measures can be obtained during stress testing including repeated blood sampling, detailed hemodynamic and neuroendocrine responses, endothelial dysfunction and catecholamine activity. Importantly, blood samples obtained in the laboratory before and after mental challenge can also be used for further immunological challenges (i.e ex-vivo) in order to measures immune tissue sensitivity or inflammatory inhibition that may be relevant in the association of stress with CVD risk.

Typically, a mental stress protocol involves a period of rest in which the subject is sitting comfortably on an armchair in order to establish baseline or resting physiological. Instrumentation of monitoring equipment is normally carried out to ensure proper calibration and functioning of equipment such as cardiovascular

monitors and indwelling cannula for repeated blood sampling. After the baseline values have been obtained subjects are given stressors that may range from cognitive tasks such as the Stroop word interference, or hand-eye attention coordination tasks such as the mirror drawing, or socially evaluative tasks such as public speaking or the Trier Social Stress Test depending on the protocol and on the type or response under investigation. Immediately after the stressors further samples are usually obtained. Depending upon the protocol participants will be required to rest for several minutes after the stress tasks so that more biological sample can be obtained. This post-stress period is termed recovery time and is important for several reasons that will be discussed in more detailed later. The main reason is that some physiological responses to stress can be detected in blood or saliva several minutes after stimulation. For example, cortisol levels tend to rise between ten and twenty minutes after stress while pro-inflammatory markers continue to increase for up to two hours after mental stress.

Therefore, a typical stress session may take between two to four hours although some protocol can be longer, and require an experimenter and a research nurse or research assistant trained in blood processing techniques. The main limitation of using this methodology is that the task used to elicit the various responses an investigator is interested in may not be representative of stressors or challenges found in everyday life. For example, tasks such as the Stroop interference might be criticized for not representing a typical scenario for the average person. Furthermore, only a fair range of short-term biological responses can be recorded in the laboratory setting. This indicates that factors such as chronic stress exposure rather than acute stress may result in different autonomic responses that can be difficult to account for. However, despite some criticism, mental stress testing remains a valid and popular

method to investigate and evaluate the contribution of stress response to CVD risk factors. Two of the studies presented in this thesis involve mental stress testing.

1.4 Pathophysiology of CHD

Atherosclerosis is the process that underlies CHD. This is the progressive thickening of the walls of the coronary arteries due to the accumulation of lipids, macrophages, platelets, smooth muscle cells and various haemostatic factors into the lining of the vessel that eventually leads to narrowing of the vessel lumen and impaired blood flow to the heart muscle. Central to the process of atherosclerosis is the inflammation of the wall of blood vessels and the endothelium with subsequent accumulation of lipid rich debris derived from dead cells that form the necrotic core of the atherosclerotic plaque. Inflammatory signalling mechanisms in the endothelium are thought to translate the effect of several risk factors for CVD. The inflammatory process leads to build-up of toxic materials in the arteries that supply blood to the heart (Ross, 1999).

The atherosclerotic process is initiated in the artery wall by various enzymes including 15-lipoxygenase (15-LO) and inducible nitric oxide synthase (i-NOS) which promote the oxidation of low-density-lipoprotein (LDL). Oxidized LDL (Ox-LDL) in turn is responsible for the proliferation of pro-inflammatory cytokines and acute phase reactant including C-reactive protein (CRP), fibrinogen, Interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and Tumour necrosis factor alpha (TNF-α). These factors promote vascular and endothelial dysfunction by regulating the expression of adhesion molecules and formation of foam cells in the endothelium. This metabolic activity leads to the proliferation of smooth muscle cells and consequent release of

fibrous elements that covers the atherosclerotic plaque (Bhagwagar, Hafizi, & Cowen, 2005). When this fibrous cap eventually fractures, it releases pro-coagulant materials that may trigger thrombosis (Libby et al., 2011; Hansson, 2005).

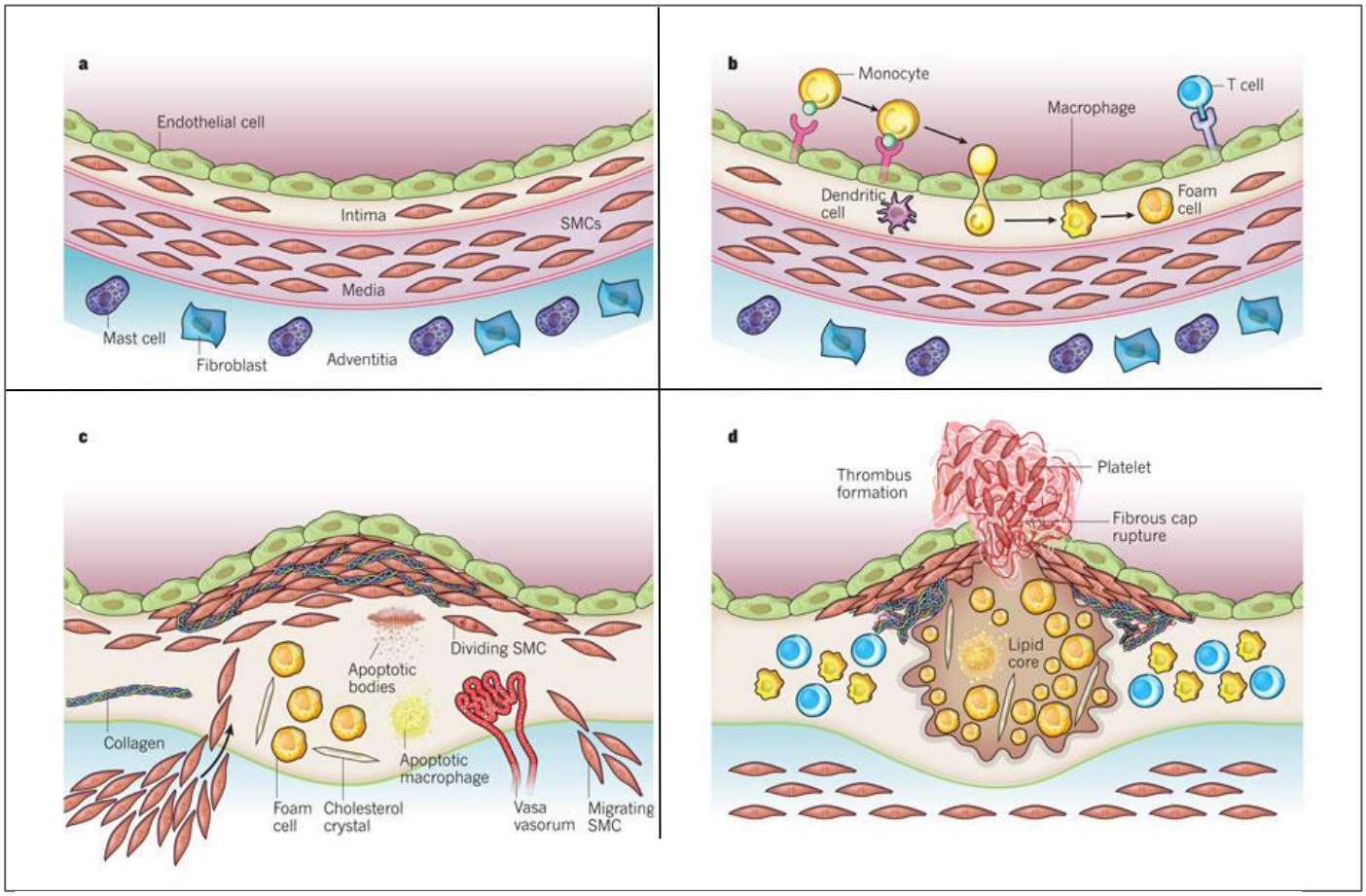


Fig.-1.1 Stages of atherosclerotic lesion development (Adapted from Libby et al, 2011)

Figure 1.1 illustrates the stages involved in the development of atherosclerotic lesions. It can be seen in part (b) of figure 1.1 that the early stage of endothelial dysfunction involves permeability of the endothelium and recruitment of monocytes and T cells which undergo maturation into macrophages and, through the uptake of lipids, eventually form foam cells. Part (c) of figure 1.1 illustrates how the initial stage of lesion formation initiates. The migration of smooth muscle cells (SMC) to the site

of the lesion is stimulated by cytokines and platelet derived growth factors released mainly by the now activated macrophages and T cells. Therefore, the migration of leucocytes, the formation of foam cells and the migration of SMC and lipids accumulation represents a central process in atherosclerosis development that develops through the years (Gratchev, Sobenin, Orekhov, & Kzhyshkowska, 2012).

The thickened wall of the artery hosting the atherosclerotic plaque undergoes arterial remodelling, that is a process of changing and adapting of the lumen of the vessel which allows the blood to flow. The final stage of plaque development and thrombus formation is shown in part (D) of figure 1.1. The accumulation of debris in the arterial wall lesion leads to a chronic inflammatory process that contributes to a narrowing of the lumen (stenosis). Eventually, the plaque is weakened by the accumulation of lipids and macrophages leading to its rupture. Plaque rupture involves the release of a fibrous cap with platelet aggregation and humoral coagulation to the site and the formation of a thrombus. The thrombus will impede the blood flow in the lumen of the vessel causing tissue ischemia and myocardial infarction. However, the thrombus may also detach from its original site becoming an embolus and block the flow of blood at a different site (Hansson & Hermansson, 2011).

Several risk factors for atherosclerosis are involved in this process, which generally take years to develop and may not present until in advanced stages. As an example, hypertension is thought to increase tension in the artery wall that results in impaired endothelial function and formation of an aneurysm.

1.5 Role of Inflammation in CVD

As it can be seen from the previous section, inflammation is a central part in the atherosclerotic process. For several years the accepted wisdom was that the passive accumulation of lipids and cholesterol into the arterial walls was responsible for atherosclerosis. However, it is now understood that atherosclerosis is a chronic inflammatory disease involving an interaction of lipid accumulation, vascular injury and specific inflammatory responses (Anogeianaki et al., 2011; Ross, 1999).

In recent years increasing effort has been devoted to investigating the role of systemic, low-level inflammation in the development and progression of atherosclerosis and CHD, and cardiovascular risk factors including the metabolic syndrome and diabetes (Sutherland, McKinley, & Eckel, 2004; Yudkin et al., 2004; Dandona, Aljada, & Bandyopadhyay, 2004; Libby et al., 2011). Systemic, low-level inflammation is defined as a two to four fold level increase in circulating pro and anti-inflammatory agents including cytokines and chemokines, and acute-phase reactants such as CRP and fibrinogen (Bruunsgaard & Pedersen, 2003). Cytokines are inflammatory polypeptides produced by the immune system that are involved in controlling local and neuroendocrine activities, and regulate immune and metabolic functions (see Figure-1.2).

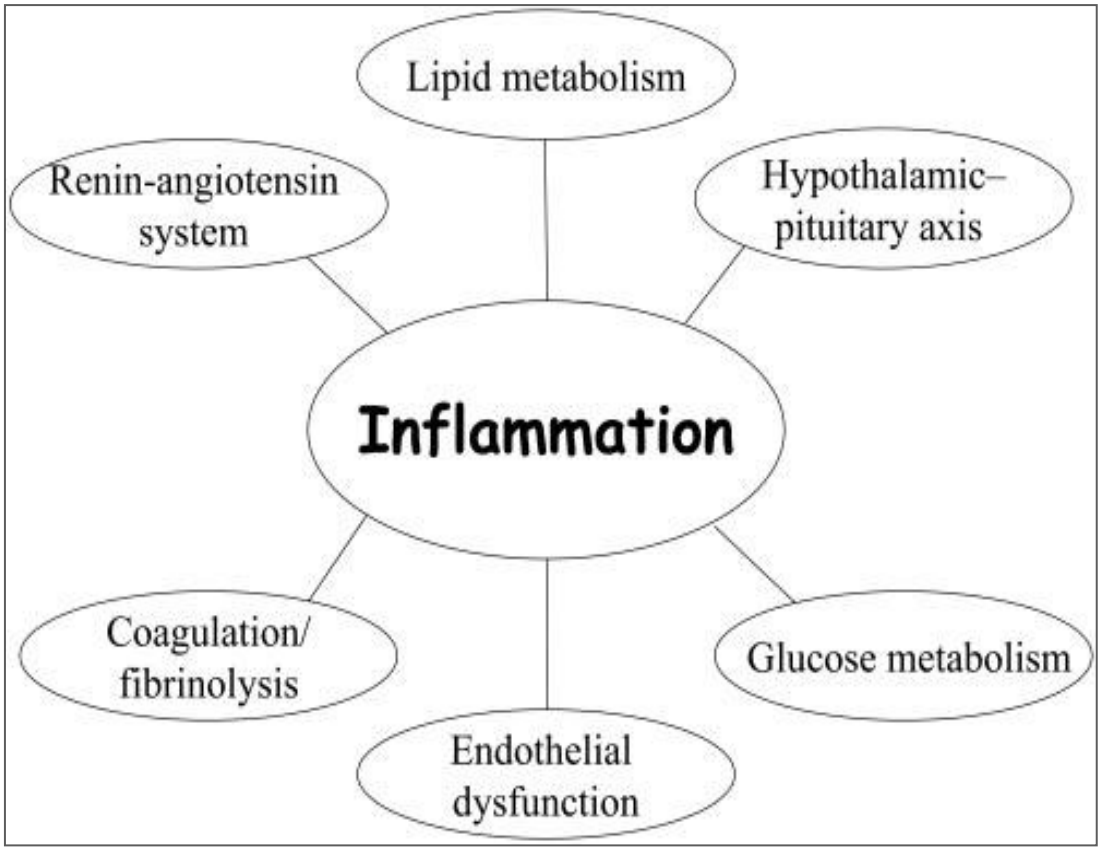


Fig-1.2 Pro-Inflammatory cytokines interact with the metabolic, vascular and endocrine system (Adapted from: Bruunsgaard, 2005).

It can be seen from Figure 1.2 that inflammatory cytokines are involved in several metabolic and vascular processes that are relevant in CVD. Inflammatory agents operate through bi-directional channels of communication – the immune system and the central nervous system (CNS) – and release inflammatory protein, neutrophils, natural killer cells and other factors involved in the pathophysiology of CVD and in cardiovascular mortality (Reuben et al., 2002; Hansson, 2005; Tedgui & Mallat, 2006).

CRP is an acute-phase protein produced by the liver which has been associated with the initial stages of atherosclerosis as well as the later stage. It induces expression of endothelial adhesion molecule cells, recruits inflammatory cells into

the artery wall and promotes plaque instability and eventually thrombosis (Li & Fang, 2004). CRP has emerged as a robust predictor of future CVD events due, among other factors, to its presence together with foam cells in atherosclerotic lesions. In a prospective study of over a thousand healthy men, it was demonstrated that the baseline level of plasma CRP was a strong predictor of risk of first myocardial infarction and ischemic stroke. Crucially, the reduction of risk of a first myocardial infarction associated with the use of an anti-inflammatory agent (aspirin) was related to CRP levels (Ridker, Cushman, Stampfer, Tracy, & Hennekens, 1997).

A recent meta-analysis offered evidence that CRP measured with a high sensitivity assay is a strong, independent predictor of mortality from cardiovascular causes (Kaptoge, Di Angelantonio, & Lowe, 2010). However, the causal association between CRP levels and CVD is still controversial (Kuper, Nicholson, Kivimaki et al, 2009), and genotype studies do not seem to support a causal link (Zacho et al., 2008). For example, it has been shown that although CRP levels independently predicts risk of CHD the causal association of CRP genotypes with CHD has not been established (Elliott et al., 2009). Therefore, CRP is considered to be an important marker of CV related outcomes rather than being causally related.

Inflammatory cytokines such as IL-6, tumour necrosis factor alpha (TNFα) and IL-1β are involved in the hepatic stimulation and production of CRP. IL-6 is produced peripherally by the skeletal muscle and the adipose tissue but crucially also in the vasculature by leukocytes and the endothelium (Rus, Vlaicu, & Niculescu, 1996; Mihara, Hashizume, Yoshida, Suzuki, & Shiina, 2012). It is implicated in the regulation of metabolic changes and the repair of damaged tissues (Papanicolaou, Wilder, Manolagas, & Chrousos, 1998). Some evidence also shows that IL-6 is a robust modulator of fat metabolism and has an augmenting effect on lipolysis and fat

oxidation (Van Hall et al., 2003). This suggests a central role of the inflammatory marker IL-6 in obesity and chronic disorders associated with obesity such as CHD, the metabolic syndrome and type 2 diabetes.

There is evidence for an association between plasma levels of IL-6 and CVD. For example, plasma IL-6 has been shown to be as strong a predictor as some of the major established risk factors in healthy older individuals (Danesh et al., 2008) conferring a 2.14 increased risk (95% CI 1.45 – 3.15). A nested case-control study in a population with stable CHD it was found that an increase of 1 pg/ml in plasma IL-6 was associated with a 1.70 increased relative odds of developing a myocardial infarction or sudden cardiovascular death (Fisman et al., 2006). Most importantly, a recent Mendelian randomisation analysis has offered robust evidence to support a causal role of IL-6 in CHD making this cytokine a likely target for intervention (Swerdlow et al, 2012).

TNF-α is a potent pro-inflammatory cytokine produced mainly by macrophages that mediates local inflammatory and acute-phase responses. It is mainly present in atherosclerotic lesions and is responsible for inducing the expression of adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1) in the vascular smooth muscle cells which contribute to plaque rupture and thrombosis (Couffinhal et al., 1993). TNF-α plays a central role in obesity, type 2 diabetes and the metabolic syndrome (Donath & Shoelson, 2011; Bruunsgaard & Pedersen, 2003), and elevated circulating levels of this inflammatory marker independently predicts risk of myocardial infarction and the severity of carotid artery atherosclerosis in healthy men (Wilund, 2007; Skoog et al., 2002). A large, prospective study of the incidence of cardiovascular events in healthy, older individuals has demonstrated an association between plasma TNF-α and CHD over a mean follow-up time of 3.6 years. The

relative risk (RR) per TNF-α standard deviation increase was 1.22 (95% CI 1.04 – 1.43) after adjustment for traditional risk factors (Cesari et al., 2003). There was also an association between TNFα and congestive heart failure (RR 1.59, 95% CI 1.30 – 1.95).

Therefore, inflammatory markers, cytokines and acute phase reactant are all involved in mediating cardiovascular risk. However, there is still some doubt regarding the causal association (Danesh & Pepys, 2009), and it has been suggested that some inflammatory agents such as CRP may be an intermediate marker that contributes to CVD via other mechanisms. Since psychosocial stress is associated with inflammation and with CVD, the next section will review evidence for a link between stress and inflammatory markers.

1.6 Stress and Inflammation

Although factors such as genetic influences are also known to influence inflammation and CVD risk, stress and psychosocial factors are important in inflammatory conditions and therefore CVD risk. In the previous sections I have outlined the methodology used to investigate the associations between stress and CV risk and in here the focus is on controlled laboratory studies since this method is used in the thesis. However, firstly I will briefly summarise some of the evidence from population studies showing association of psychosocial stress with inflammatory factors. Population studies are useful because of the large number of participants that allow statistical adjustment for several factors including ethnicity, gender and lifestyle characteristic that may confound the association of inflammation with stress.

Chronic psychological stress such as depressive symptoms, loneliness and caring for a relative with dementia seems to be associated with a low-grade inflammatory state (Hañnsel, Hong, Camara, & von Kanel, 2010). In a meta-analysis, depression was positively associated with CRP, IL-6 and IL-1 (Howren, Lamkin, & Suls, 2009) with body mass index (BMI) playing a moderating role. There is a wealth of research on depression and inflammation (Miller, Maletic, & Raison, 2009; Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008) suggesting that inflammation may be an important biological event in the development of stress-related disorders. However, other psychosocial stress factors are also important.

A consistent finding seems to indicate that low SES individuals have raised plasma level of CRP and fibrinogen which are independent of factors that are also associated with both inflammation and stress including blood pressure, smoking and socio-demographics characteristics (Nazmi & Victora, 2007; Brunner et al., 1996). The clotting inflammatory factor fibrinogen was also associated with psychological distress in a large representative sample independent of socio-demographic and lifestyle factors but no association was found for CRP (Goldman-Mellor, Brydon, & Steptoe, 2010).

Stress at work has not been found to be consistently associated with inflammatory markers. For example, one study reported a negative, independent association between fibrinogen and job control in men (Clays et al., 2005) but no association was found between CRP and work stress. Therefore, population studies seem to offer inconsistent evidence to support an association between stress and inflammation. More prospective studies testing whether inflammatory factors contribute to the association between stress and CVD will be able to address this issue.

1.6.1 Mental Stress Testing and Inflammatory Responses.

It has been suggested that chronic psychosocial stress is associated with chronic inflammatory responses that are relevant in CVD risk (Black, 2002). Mental stress testing allows for the evaluation of inflammatory responses under controlled conditions that can then be related to several psychosocial and stress characteristics.

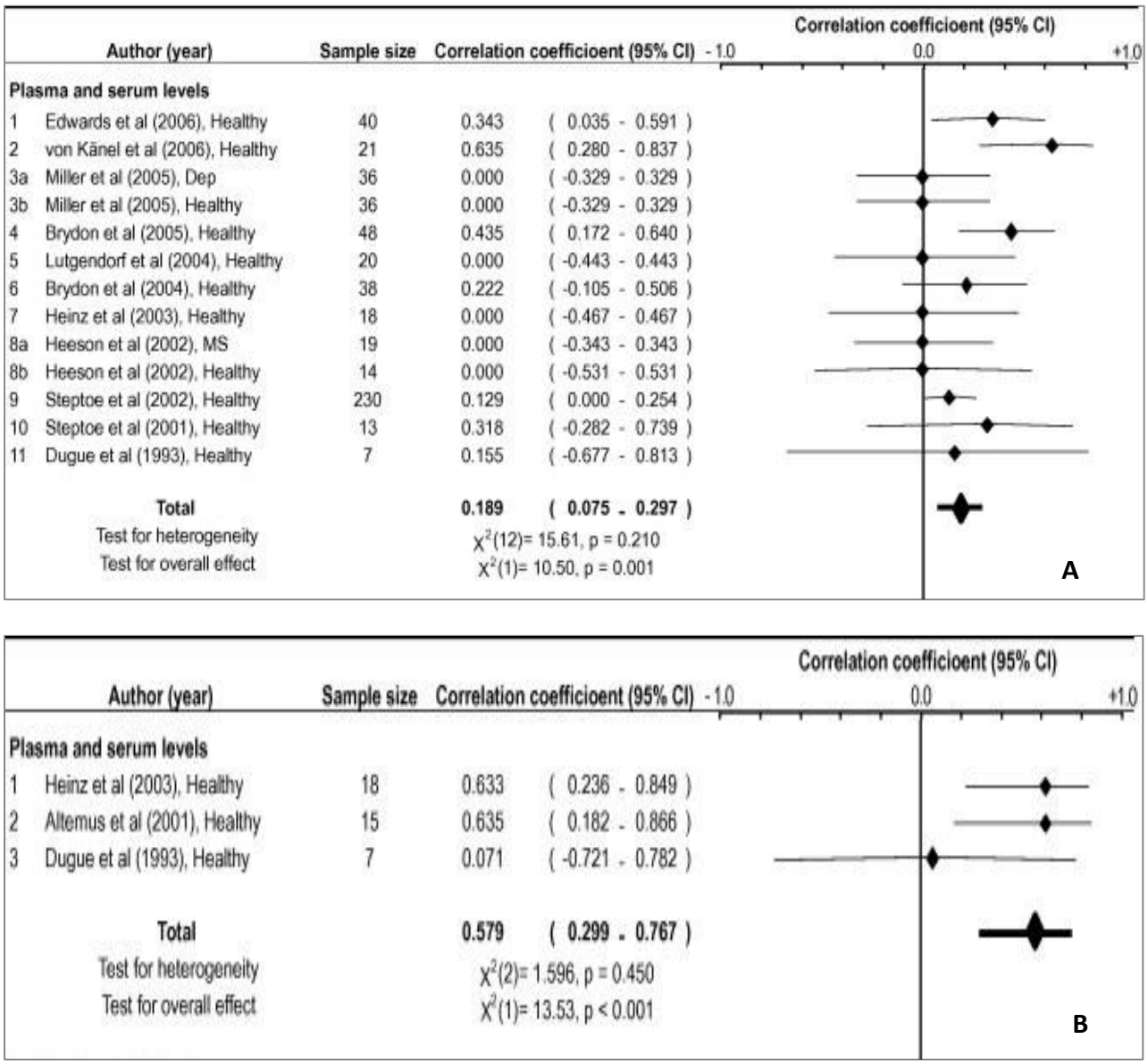


Fig-1.3 The effect of acute mental stress on IL-6 (A) and IL-1β (B) inflammatory responses. Includes healthy and clinical population. Adapted from Steptoe, Hamer & Chida (2007)

A meta-analysis of the effect of acute psychological stress on peripheral inflammatory markers has shown that acute mental stress in the laboratory induced transient elevation in IL-6 and IL-1β and only marginally in CRP (Steptoe, Hamer, & Chida, 2007). This effect of acute stress on IL-6 and IL-1β is illustrated in figure 1.3. The quantitative review noted that there was great overall variation between studies in the stressor tasks employed, blood sampling technique, and crucially the biochemical assay technique used to analyse inflammatory markers from plasma. The authors concluded that following acute psychological stress there is a modest increase in some inflammatory markers and highlighted methodological issues including the small sample size of most studies, the limited pool of markers examined and heterogeneity in the stress protocols.

There has been no prospective study examining whether inflammatory responses to mental stress predict the development of CVD or risk factors such as hypertension. However, one study has found that heightened IL-6 and fibrinogen responses to two stress tasks were positively associated with a three year increase in ambulatory blood pressure that was independent of confounding factors including smoking, BMI and ambulatory blood pressure at the time of stress testing (Brydon & Steptoe, 2005). Additionally, the fibrinogen and TNFα but not IL-6 stress responses were associated with reductions in the degree of arterial distensibility (a measure of carotid artery stiffness) measured at the three year follow-up independently of covariates including resting CRP, lipids and blood pressure (Ellins et al., 2008).

Therefore, although correlational, these studies suggest that inflammatory responses to acute psychosocial stress may predispose to an increase in CVD risk through alteration in hemodynamic and inflammatory functions and changes in the structure of the artery wall.

1.7 Neuroendocrine Factors in CHD.

Research on the contribution of neuroendocrine factors in CHD has mainly focussed on the HPA axis end product hormone cortisol. Briefly, the HPA axis links the hypothalamus in the brain to the adrenal glands situated near the kidneys. The axis initiates communication from the brain to the periphery via the release of the corticotrophin releasing factor (CRF) and adrenocorticotrophin (ACTH) hormones. These hormones stimulate the release of the glucocorticoid hormone cortisol from the adrenal glands whose role is to prepare the organism for mental or physical activities that require substantial energy expenditure. Cortisol has vital metabolic functions including stimulation of the cardiovascular system and suppression of the inflammatory response. A highly adaptive negative feedback loop system signals back to the brain to inhibit further release of CRF and ACTH hormones and ultimately inhibit further production and release of cortisol.

In physiologically normal conditions, cortisol has a marked and reliable circadian rhythm characterised by high levels upon awakening that peak between 30 to 40 minutes post-awakening (cortisol awakening response [CAR]) followed by a steady decline thereafter reaching a trough in the late evening (illustrated in Figure 1.4).

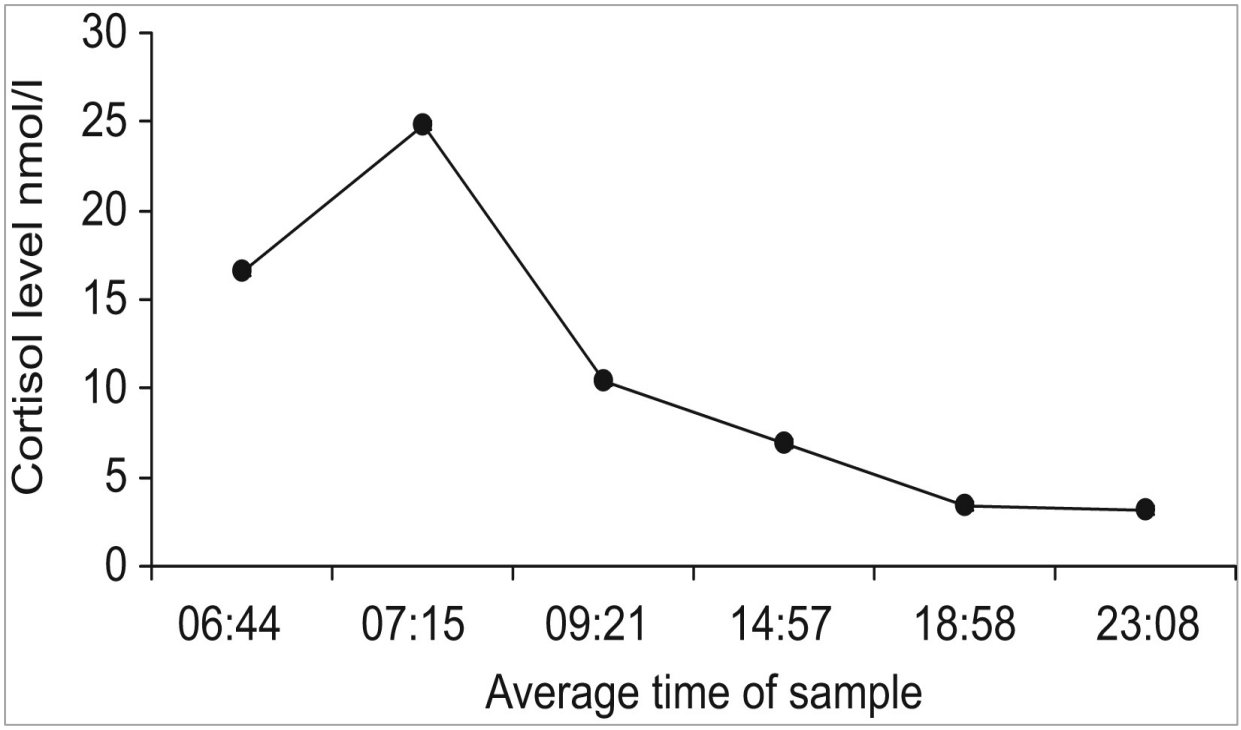


Fig-1.4 The cortisol diurnal cycle in 542 healthy men and women from the Whitehall II longitudinal cohort adapted from (O'Donnell, Badrick, Kumari, & Steptoe, 2008).

Acute and chronic psychosocial stress can impair or dysregulate this adaptive diurnal rhythm resulting in an impaired feedback signalling system, dysregulated HPA axis activity and heightened cortisol levels. The strongest evidence to date supporting a role for impaired diurnal cortisol rhythm in CV outcomes comes from a longitudinal study of over 4000 individuals (Kumari, Shipley, Stafford, & Kivimaki, 2011). The authors found that a cortisol pattern characterized by a flatter diurnal decline with higher evening levels was an independent predictor of cardiovascular mortality during a six years follow-up.

Indeed, several aspects of the cortisol diurnal profile are associated with psychosocial stress factors. A meta-analysis of studies that examined the association between psychosocial factors and the CAR (Chida & Steptoe, 2009)

concluded that the dimensions that were most reliably associated with a heightened CAR were work stress and life stress. However, other psychosocial factors such as fatigue and burnout were related to a reduced CAR, whereas there was evidence for an association of both heightened and reduced CAR for depression. Depression and depressive symptoms measured with the Center for Epidemiological Study Depression Scale (CES-D) have been recently related to a flatter decline in diurnal cortisol in a relatively large sample of women independently of several covariates including age, BMI, SES and health behaviours (Knight, Avery, Janssen, & Powell, 2010).

A recent meta-analysis of depression and HPA axis function reported that depression is associated with hyperactivity of the axis resulting in higher cortisol levels compared to non-depressed individuals (Stetler & Miller, 2011). The authors argued that the results of their meta-analysis support a model wherein dysregulation of the HPA axis links depression with an increased risk for chronic illness such as diabetes and CHD. Indeed, a large cross-sectional study of cortisol and atherosclerosis plaques in an elderly population reported an independent association between total cortisol exposure during the day and the number of plaques present in the carotid arteries (Dekker et al., 2008). Therefore, HPA axis dysfunction and impairment in the diurnal cortisol profile is an important pathway contributing to the association between stress and psychosocial factors with CV risk factors and mortality.

The HPA axis is also responsive to mental stress induced in the laboratory. It has been shown that social evaluative stressors such as speaking in front of others or the Trier Social Stress Test induce robust cortisol increases (Dickerson & Kemeny, 2004) that alters the normal downward cortisol slope (see Figure 1.4).

However, stressors characterized by cognitive or non-motivational challenges are generally modestly associated with cortisol responses. Evidence is beginning to emerge for an association between cortisol responses to psychological stress induced in the laboratory and the development of CHD risk factors. In a cross-sectional analysis, Hamer and colleagues demonstrated that healthy individuals who responded with a small cortisol increase to two mild mental stress tasks had a greater degree of coronary artery calcification measured by electron beam computed tomography (Hamer, O'Donnell, Lahiri, & Steptoe, 2010). Crucially, the cortisol responses were also associated with the progression of coronary artery calcification three years later independently of several covariates including BMI, SES, resting cortisol and blood pressure (Hamer, Endrighi, Venuraju, Lahiri, & Steptoe, 2012).

Therefore, acute stressors that disrupt the adaptive cortisol diurnal rhythm if sustained or repeated over time may increase CV risk. These studies provide some evidence that psychosocial stress induced under controlled conditions influence CVD risk through HPA axis dysregulation and cortisol release.

2 Physical Activity, CVD and Stress.

2.1 Physical Activity and CVD.

Physical activity has recognised, far-reaching beneficial effects on the cardiovascular system as well as on psychological wellbeing. Physical activity is associated with reduced risk of CVD risk factors and mortality (Ahmed, Blaha, Nasir, Rivera, & Blumenthal, 2012; Szostak & Laurant, 2011), and this relationship persists

after traditional, biological cardiovascular risk factors such as blood pressure and lipids are taken into account (Bijnen et al., 1998).

In a recent analysis of data from the Scottish Health Survey prospective cohort study, individuals who did not take part in at least 30 minutes of moderate physical activity a day had a 53 per cent increased risk of a cardiovascular event independently of traditional risk factors including smoking and hypertension (Hamer, Molloy, & Stamatakis, 2008). Results from prospective cohort studies have shown a strong association between levels of physical activity and cardiovascular risk. In one large epidemiological study Tanasescu and colleagues (Tanasescu et al., 2002) reported that various physical activities such as running, weight training and walking were each associated with a large reduction in the incidence of coronary heart disease independent of traditional risk factors. For example, that study showed that in men running at least a hour per week was associated with a risk reduction for CHD of 42 per cent (RR 0.58 95% CI 0.44 – 0.77) compared with men who did not run.

In the McArthur studies of successful aging, a longitudinal cohort of older men and women, the combined effect of a high allostatic load score (a cumulative cluster of CV related risk factors) and low physical fitness at baseline were strong predictors of incidence cardiovascular disease and cognitive decline several years later (Seeman, Singer, Rowe, Horwitz, & Mcewen, 1997). Recently, a prospective study of more than 10,000 adults with a mean follow-up of 13 years (Reddigan, Ardern, Riddell, & Kuk, 2011) has shown that taking part in physical activity that is of light or moderate intensity (assessed by self-report) was associated with lower risk of CVD mortality. Interestingly, this association was independent of traditional and metabolic risk factors including hypertension, lipids and inflammation.

A major weakness in this area is the reliance on self-report physical activity data. Self-report requires individuals to recall their usual level of activity so that people can generally be categorised into non-active or moderately and vigorously active. This is a limitation because physical activity and sedentary time is somewhat arbitrary and there is no consensus about the optimal cut-off point that define intensity levels (Pate, O'Neill, & Lobelo, 2008). Indeed, concordance between self-report and objective level of physical activity is low (Prince et al., 2008). Therefore, in order to examine the contribution of exercise and physical activity to CVD risk factors and mortality with greater precision, objective measures of physical activity participation are becoming increasingly important. Accelerometers devices such as the ActiGraph or the Gravity Estimator of Normal Everyday Activity (GENEA) watch that measure body movements in term of acceleration and use algorithms to estimate the intensity of physical activity over time are being increasingly used (Chen & Bassett, 2005; Butte, Ekelund, & Westerterp, 2012).

For example, in a cross-sectional analysis of a representative sample of adults there was an association between objectively recorded moderate to vigorous physical activity and better self-rated physical health which is a predictor of morbidity and mortality (Hamer & Stamatakis, 2010). In a prospective study of over 4,000 adolescent boys and girls objective physical activity level at age 12 was strongly associated with reduced fat mass measured by dual emission absorptiometry (Riddoch et al., 2009). The authors were able to show that an extra 15 minutes of moderate to vigorous physical activity each day was associated with 20 per cent lower fat mass in boys. This suggests that objective measures of physical activity behaviour have greater power to detect changes in important CV risk factors.

2.2 Physical Activity and Inflammation.

Midway through this chapter I discussed the role of inflammatory factors and psychosocial stress in CVD risk factors. Physical activity is a strong stimulus for the production and release of pro and anti-inflammatory cytokines and immune regulatory cells. Following an acute bout of exercise, cells in the skeletal muscle tissue and to a lesser degree the adipose tissues release the cytokine IL-6 into the circulation (Febbraio & Pedersen, 2002). This stimulates the production of the anti-inflammatory cytokines IL-1ra, sTNF-r, IL-10 and, to a lesser extent, the acute phase reactant CRP. The release of pro-inflammatory markers such as TNF-α and IL-1 is not stimulated by acute exercise (Pedersen, Ostrowski, Rohde, & Bruunsgaard, 1998; Pedersen, 2011). During this acute bout there is an actual increase in free radicals (oxidative stress) which is due to the sharp increase in oxygen consumption. The increase in free radicals was thought to promote LDL oxidation in the artery wall and contribute to atherosclerosis. However, ox-LDL produced following acute exercise is promptly processed by the liver in a different fashion compared to the ox-LDL present in the vascular artery and does not promote inflammation or contribute to atherosclerosis (Van Berkel, De Rijke, & Kruijt, 1991; Wilund, 2007).

The inflammatory factors released during exercise originate from working skeletal muscles exerting specific endocrine effects on several signalling pathways including the glycogen/p38 MAPK (Pedersen, 2011). In contrast, the inflammatory markers that are released during psychosocial stress are thought to be dependent upon the activation of the signalling transcription pathway Nuclear Factor kB (Bierhaus et al., 2003; Brydon et al., 2005). The differential activation of relevant pathways by stress and physical activity suggests that inflammatory markers such as IL-6 may be of central importance in explaining the association between physical

activity, stress and CVD risk factors. Indeed, several observational prospective studies have shown associations between physical activity and lower plasma level of inflammatory markers including CRP (Kasapis & Thompson, 2005), IL-6 and TNF-α (Panagiotakos, Pitsavos, Chrysohoou, Kavouras, & Stefanadis, 2005; Bruunsgaard, 2005; Hamer, 2007; Cesari et al., 2003). Hence, attenuated circulating levels of these inflammatory markers may be atheroprotective through various mechanisms. Crucially though, through regulation of lipid production and activation or enhancement of several antioxidant enzymes, in particular e-NOS in the plasma, muscles and in the vascular wall (Van Hall et al., 2003; Vollaard, Shearman, & Cooper, 2005), which all contribute to an anti-inflammatory environment.

Results from the few randomized exercise intervention trials that have been conducted however have been less consistent in finding reductions in inflammatory markers after physical activity interventions. Hence, there is only partial support for the hypothesis that regular physical activity is associated with lower levels of inflammatory markers (Ahmed, Blaha, Nasir, Rivera, & Blumenthal, 2012; Beavers, Brinkley, & Nicklas, 2010). It has been suggested that some of the inconsistencies may be explained, among other factors, by the effect of exercise on changes in fat mass as an inflammatory source, the lack of objective measurement of protocol adherence and the poor methodological qualities of some studies (Beavers et al., 2010; Hamer & O'Donovan, 2010).

2.3 Physical Activity and Stress-Related Processes.

2.3.1 Physical Activity and Mood.

Physical activity may also be relevant in several mental health outcomes including depression. This section reviews the evidence for an association of physical activity with mood, focussing particularly on studies in everyday life. A meta-analysis of prospective cohort studies has quantified the association (illustrated in Figure 1.5) between baseline physical activity and risk of depression during an average follow-up of 9 years (Hamer & Chida, 2008).

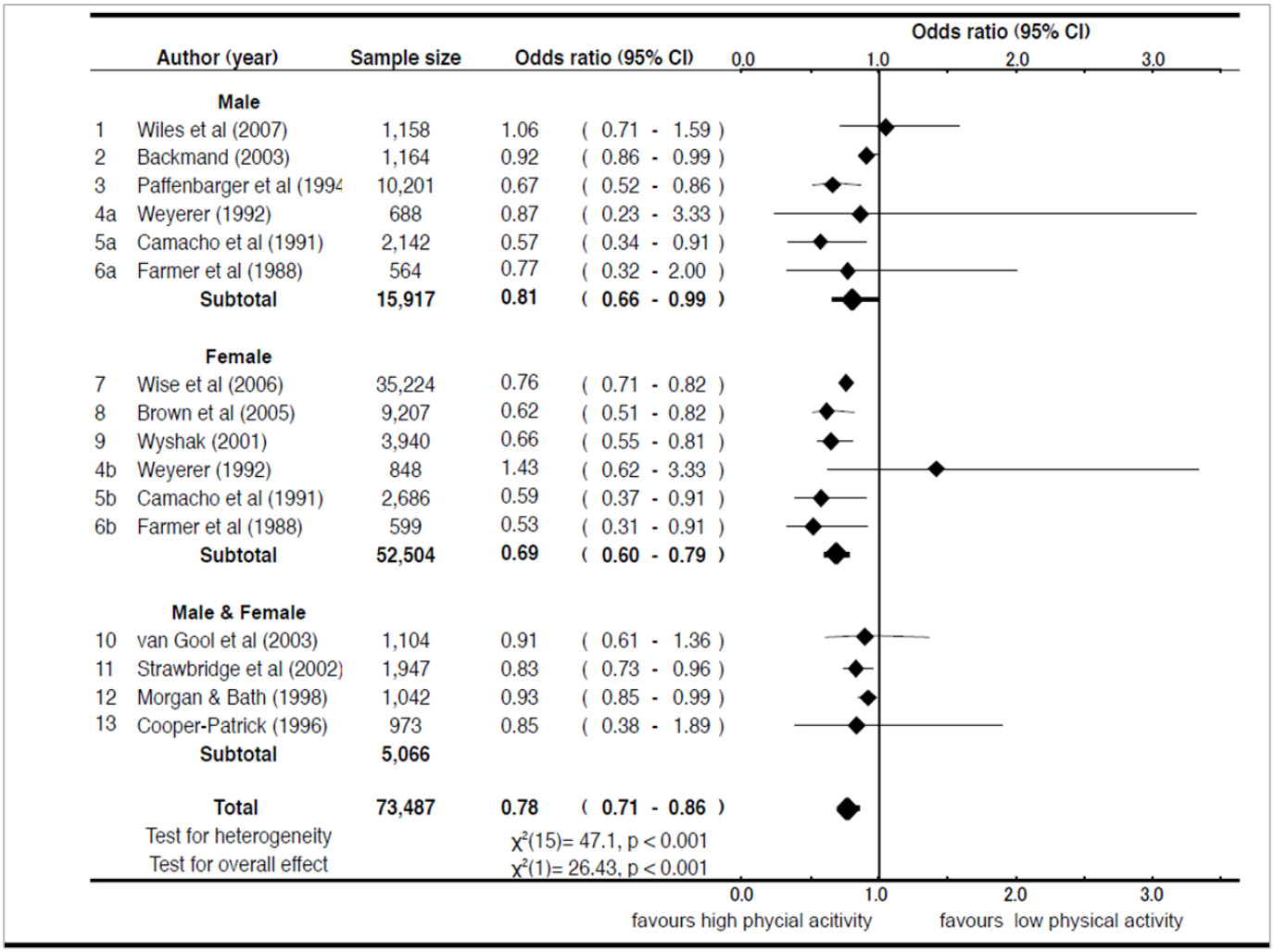


Fig-1.5 Effect sizes showing the prospective association between physical activity and risk of depression (adapted from Hamer & Chida, 2008).

Participants included over 73,000 healthy, non-depressed men and women. The pooled odds ratio for the risk of depression in physically active compared to sedentary individual was 0.78 (95% CI 0.71 – 0.86). Therefore, engaging in regular physical activity seems to be protective against future depression. A large population study reported an hazard ratio for the risk of depression in women with low level of physical activity of 1.80 (95% CI 1.29 – 2.51) compared to women with a high level of physical activity (Mikkelsen et al., 2010). Interestingly, there were no significant associations in men. Although the effect sizes included in the meta-analysis (Hamer

& Chida, 2008) were adjusted for confounding factors such as social position, health behaviours and smoking, several limitations remains in this area. The main methodological issue is the self-report nature of questionnaire measure used to gather data on depressive symptoms and on physical activity levels. In addition to the well-known problems shared by self-report data such as memory bias or the concurrent effect of a current mood state on the recalling of past mood, symptoms of depression may also cause reporting bias. For example, some individuals may be more likely to under- or over-report frequency of physical activity or sedentary time.

Few studies have examined the relationship of objectively assessed physical activity with depression either measured by questionnaire or by ecological momentary assessment or both. One large population study (Vallance et al., 2011) found that the level of moderate to vigorous physical activity (MVPA) measured with an accelerometer was associated with decreased odds of depression in a linear fashion (64 per cent reduced odds for depression in the most active compared to least active group). One study investigated the associations between objective time spent engaging in MVPA over a seven day period and daily ratings of mood in a small sample of healthy women (Poole et al., 2011). There was a moderate positive association between daily positive mood and objective MVPA. Importantly, MVPA was also negatively associated with mood measured by questionnaire (CES-D).

Overall, physical activity seems to be protective of future depression especially in women. There are however methodological limitations in interpreting self-report data. In study one I will investigate the relationship between self-report, habitual physical activity participation and mood measured by questionnaire as well as daily rating of general stress which may be an important precursor of mood disturbance. In study two I will use objective recording of physical activity over a four week period

to establish whether a causal association between mood symptoms and activity levels exists.

2.3.2 PA and psychophysiological responses

Tsatsoulis and colleagues (Tsatsoulis & Fountoulakis, 2006) have argued that there is evidence from animal models and human observational studies that poor levels of physical activity contributes to a dysregulated and inefficient adaptive stress response with sustained autonomic activity. This, in turn, results in an increase in cardiovascular risk and in the development of cardio metabolic risk factors. Indeed, it was demonstrated in a meta-analysis (Chida & Steptoe, 2010) that greater cardiovascular reactivity to, and impaired recovery from, psychological stress induced in the laboratory was associated with the development of important CHD risk factors including hypertension.

Physical activity may also act by inhibiting or modulate acute responses to stress challenges that if sustained over-time have negative effect on CV risk factors. This section reviews studies that have examined the association between physical activity and autonomic or inflammatory stress responses. Two fairly recent meta-analysis (Forcier et al., 2006; Jackson & Dishman, 2006) have also attempted a quantitative review of published studies examining the associations between physical fitness and cardiovascular responses. Jackson and colleagues review (Jackson & Dishman, 2006) concluded that fitness was related to improved recovery from mental stress only in the better quality studies reviewed. Forcier and colleagues instead (Forcier et al., 2006) concluded that higher fitness was related to attenuated systolic blood pressure and heart rate reactivity to mental stress. However, there were no

associations between cardiovascular recovery to stress and fitness. The limitation with these reviews is that they examined the effect of fitness rather than the levels of physical activity on stress reactivity. Most of the studies included were also based on non-objective measure of adherence to an exercise intervention. Furthermore, the outcomes under investigation were restricted to cardiovascular responses as there is at present a paucity of research examining the effect of physical activity or exercise on inflammatory and immune responses to stress.

The studies summarised in table 1.1 include cross-sectional as well as intervention studies that examined the effect of physical activity or fitness on stress-induced psychophysiological reactivity. About half of the studies were randomised exercise interventions, and only one cross-sectional study had inflammatory responses as an outcome.

Table-1.1 Summary of studies that examined the effect of physical activity or fitness on stress-induced psychophysiological responses.

Ref	Design	Sample	Physical activity measure	Stressor	Outcome	PA/fitness effect on reactivity/recovery
Albright et al, 1992	24w RCT	83 healthy	Aerobic ex vs control	MA	HR, BP	No
Blumenthal et al, 1990	12w RCT	37 type A	Aerobic ex vs strength training	15m MA	BP, HR, RPP, ADE, NAD	Yes BP, HR, RPP,
Blumenthal et al, 2005	16w RCT	134 IHD	Aerobic ex vs usual care	5m mirror + 5m speech	LVEF, WMA, FMD, HRV, BS	Yes (LVEF, FMD, BS) No (WMA, HRV)
Bond et al, 2008	8w Non-RCT	32 healthy	Aerobic vs control	5m Stroop, 4m CPT	BS, HR, AC, BP	Yes
Boutcher et al,1998	C-S	30 healthy	Trained vs untrained	5m MA + 5m Stroop	HRV	Yes (Stroop)
Degeus et al, 1993	16w QExp	62 healthy	Aerobic ex vs wait list control	2m CPT, + 2*10m RT	TPR, HR, BP, CO	No
Finkenzeller, et al, 2011	12w non-RCT	34 healthy	Skiing ex vs control	4*4m cognitive stress	HRV, SCL	No

Hamer & Steptoe, 2007	C-S	207 healthy	HR cycle ergometer	5m Stroop + 5m mirror	IL-6, TNFα, IL-1ra, HRV	Yes: IL-6, TNFα HRV
Palatini et al, 2010	C-S	119 hypertensive	Active vs sedentary (hypert) vs control	6m speech	BP, HR	Yes (BP)
Poole et al, 2011	C-S	40 healthy	Objective 7d PA	5m speech + 5m mirror	Cort, HR, BP	No
Ray & Carter, 2010	8w RCT	23 healthy	Aerobic ex. Vs sedentary control	5m MA	MSNA, RVR	No
Rimmele et al, 2007	C-S	44 healthy	Trained vs untrained	TSST	Cort, HR	Yes
Rimmele et al, 2009	C-S	92 healthy	Elite vs amateur vs untrained	TSST	Cort, HR	Yes
Sloan et al, 2011	12w RCT	149 healthy	Aerobic vs strength	5m MA+ 5m Stroop + 5m speech	BP, HR, RRV	No
Spalding et al, 2000	C-S	20 healthy	Trained vs untrained	2.5m Stroop, 2.5m MA, 2.5m problem solving	HP, RSA	No

PA physical activity, RCT randomized controlled trial, C-S cross-sectional, MSNA muscle sympathetic nerve activity, RVR renal vascular, RRV responses, TSST Trier Social Stress Test, HR heart rate, Cort cortisol, HRV heart rate variability, RRV RR interval variability, IHD stable ischemic heart disease, BS baroreflex sensitivity, LVEF left ventricular ejection fraction, WMA wall motion abnormalities, FMD flow-mediated dilation, CPT cold pressor test, TPT total peripheral resistance, CO cardiac output, QExp quasi experimental, RT reaction time, MA mental arithmetic, HP heart period, respiratory sinus arrhythmia, RPP rate pressure product, ADE adrenalin, NAD noradrenalin, PA physical activity, AC arterial compliance

Overall, no firm conclusions can be drawn from the studies listed in table 1.1, and there seems to be a lack of experimental studies that use objective physical activity measures and inflammatory outcomes. Due to these methodological shortcomings the study described in chapter four was designed to experimentally manipulate the level of physical activity and examine the effect of on inflammatory, neuroendocrine and cardiovascular stress responsivity.

3 Adiposity, Inflammation and CVD.

3.1 Adiposity in CVD

Obesity and excess adiposity are important risk factors for CVD and associated co-morbidities including atherosclerosis, type 2 diabetes and the metabolic syndrome (Weyer, Foley, Bogardus, Tataranni, & Pratley, 2000). In a pooled study of over one and a half million adults, overweight and obesity defined as a BMI greater than 25 was associated with all-cause mortality independently of age, physical activity, education and marital status (De Gonzalez et al., 2010). It has been estimated that the association between obesity and risk of mortality for cardiovascular causes is about 40 per cent (hazard ratio 1.41, 95% CI 1.37 – 1.45) higher for a five unit increase in BMI above the 22.5 – 25 range (Prospective, 2009).

The adipose tissue is an active endocrine organ secreting and activating several immune and adaptive inflammatory cells (Rocha & Libby, 2009). It has been shown that adiposity correlates with several pro-inflammatory markers in peripheral blood including IL-6, CRP, TNFα, IL-1β, RANTES and leptin which are also referred to as adipokines (Mohamed-Ali, Pinkney, & Coppack, 1998; Vozarova et al., 2001; Visser, Bouter, McQuillan, Wener, & Harris, 1999). Therefore, serum adipokine levels are raised in humans with excess adiposity especially central, visceral fat (Madani et al., 2009; Fried, Bunkin, & Greenberg, 1998). This systemic inflammatory state of metabolic tissues is associated with insulin resistance, metabolic dysfunctions such as the metabolic syndrome (Lorenzo, Williams, Hunt, & Haffner, 2007) and ultimately contributes to cardiovascular disease (Gregor & Hotamisligil, 2011).

Weight loss studies have generally shown reductions in basal levels of vascular inflammatory markers including CRP, IL-6, TNFα and increases in the anti-inflammatory marker adiponectin with a weight loss of at least six to ten per cent of initial body weight (Forsythe, Wallace, & Livingstone, 2008; Selvin, Paynter, & Erlinger, 2007). This suggests that weight loss is able to improve the low grade inflammatory state associated with obesity. It has been suggested that significant weight loss is not necessary in order to improve the metabolic derailment seen in obesity. Several changes in risk factors including insulin sensitivity, hemodynamic and inflammatory markers can be observed after initiation of moderate physical activity and healthy eating with minimal or not weight loss (Ross & Bradshaw, 2009).

3.2 Leptin.

Several inflammatory markers have already been reviewed in this chapter. However, the hormone adipokines leptin is relevant in study three of this thesis. Leptin is mainly secreted by cells of the adipose tissue in proportion to the amount of body fat present. It is implicated in appetite regulation through its direct effect on receptors in the hypothalamus, and energy expenditure. In obese individuals leptin levels are elevated suggesting that the cells targeted by this hormone become resistant to the effect of leptin. There is some evidence suggesting that leptin is associated with atherosclerosis and CHD risk (Oral et al., 2002; Wallace et al., 2001) possibly via its effect on central mechanisms such as blood pressure and on the vascular endothelium (Hou & Luo, 2011).

Leptin may also be involved in orchestrating the cytokine response to stress. In a cross-sectional study women with higher resting levels of leptin were characterized by greater stress-induced increases in pro-inflammatory IL-6 (Brydon et al., 2008a). This finding suggests that the adipose tissue may be an important source of stress responsive cytokines activation and provide a link between stress, inflammatory responses and cardiovascular risk factors.

3.3 Adiposity, Stress and Inflammation.

Stress has been linked to the development of adiposity and visceral obesity (Karalis et al., 2009). Low-grade inflammatory responses in obesity activate the HPA axis and the central and peripheral components of the central nervous system (Chrousos & Gold, 1992; Hotamisligil & Erbay, 2008). This results in the release of several hormones and immune cells that contribute to a chronic inflammatory state and therefore to increased cardiovascular risk (Purnell et al., 2009). Therefore, psychosocial stress may be a causal or predisposing factor in the development of adiposity and obesity. A recent meta-analysis of longitudinal studies examining the association between chronic stress exposure and adiposity (Wardle, Chida, Gibson, Whitaker, & Steptoe, 2011) reported a small effect size (r = 0.014, 95% C = 0.002 – 0.025). The authors concluded that although an association between stress such as job stress, caregiving and life stress was observed, there was considerable variability across studies suggesting that moderators of this association are likely to play an important role.

Cardiovascular and inflammatory activation in response to acute or chronic stress exposure might partly explain the link between adiposity and increased CVD

risk. Several cross-sectional and a few longitudinal studies have examined the association between obesity and psychophysiological responses to mental stress in the laboratory. These studies are summarised in table 1.2.

In cross-sectional studies, greater adiposity (waist circumference or waist to hip ratio) and BMI have been linked to greater inflammatory responses to mental stress. For example, the obesity related hormone leptin and IL-1ra responses to mental stress were found to be more pronounced in women with greater central obesity (Brydon et al., 2008b). Cross-sectional studies have generally found positive associations between obesity and cardiovascular and cortisol responses to mental stress but only a few cross-sectional studies have examined inflammatory responses in obesity. However, the largest prospective study to date showed that an attenuated heart rate response to mental stress at baseline was independently associated with an increased risk of becoming obese five year later (Carroll, Phillips, & Der, 2008).

Therefore, it is unclear whether obesity and adiposity are associated with a heightened or attenuated autonomic response to stress. Previous work in this area has been mainly observational highlighting the need for more experimental studies that systematically manipulate adiposity levels. Such studies may be able to elucidate whether reactivity to mental stress is one of the mechanisms linking adiposity to CVD risk factors, and to clarify the direction of the association. This issue will be addressed in chapter five by testing a group of overweight or obese women before and after weight loss. In this way it will be possible to examine the effect of adiposity on inflammatory and cardiovascular responses to stress.

Table-1.2 Summary of studies that examined the associations of adiposity or obesity on stress-induced psychophysiological responses.

Ref	Design	Sample	Obesity/adiposity measure	Stressor	Outcome	Obesity/adiposity effect on reactivity/recovery
Agapitov et al, 2002	C-S	46 healthy	BMI (Obese vs matched lean)	4m MA	BP, HR, FBF, SBF	Yes (SBF, FBF)
Benson et al, 2009	C-S	39 healthy	BMI (obese vs non-obese)	10m speech	ACTH, Cort, IL-6, HR, BP	Yes (cort, HR, BP)
Brydon et al, 2008a	C-S	94 healthy	Leptin	5m speech + 5m mirror	IL-6, HR, HRV, PEP	Yes (women only)
Brydon et al, 2008b	C-S	67 healthy	Fat mass, WC (continuous)	5m Stroop + 5m speech	BP, HR, IL6, IL1ra, leptin, Cort	Yes (leptin, IL1ra, BP)
Carroll et al, 2008	C-S & Long.	1647 (C-S) 1272 (Long.)	BMI, WHR,	3m MA	BP, HR	Yes (HR-neg C-S & Long.)
Davis et al, 1999	C-S	24 healthy	WHR (Central vs peripheral obesity)	5m Speech + 2m CPT	BP, HR, CO, TPR	Yes (BPreact, TPR higher, CO lower in central adiposity group)
Epel et al, 2000	C-S	59 healthy	WHR (low WHR vs high WHR)	15m (speech, MA, cognitive tasks)	Cort,	Yes

Georgiades et al, 2000	24w RCT Weight loss	99 high/normal HT	(exercise vs weight loss vs control)	5m speech + 3m anger recall + 3m mirror + 2m CP	BP	No
Kuniyoshi et al, 2003	C-S	35 healthy	BMI (obese vs matched lean)	4m Stroop + 2m CPT	MSNA, FVR, BP, HR	Yes (MSNA)
Moyer et al, 1994	C-S	41 healthy	WHR (low vs high)	15m MA	Cort	Yes
Ribeiro et al, 2005	RCT	39 healthy	(diet + exercise vs diet only)	5m Stroop	BP, HR, FBF	No
Seematter, et al, 2000	C-S	19 healthy	BMI, fat mass (obese vs lean)	5m MA + 5m Stroop	IS	Yes
Steptoe & Wardle, 2005	C-S & Long.	225 healthy	BMI, WHR (continuous)	5m Stroop + 5m mirror	BP, HR, CO, TPR	(C-S) yes (BP, CO) (Long.) yes (BP, CO)
Therrien et al, 2010	C-S	82 healthy	BMI, WC (lean vs obese vs reduce obese)	TSST	Cort	Yes (neg in men only)

FBF forearm blood flow, SBF skin blood flow, BMI body mass index, WHR waist-hip ratio, CPT cold pressure test, TPR total peripheral resistance, IS insulin sensitivity, HT hypertension, WC waist circumference, Long. Longitudinal, PEP cardiac pre-ejection period, TSST Trier Social Stress Test,

CHAPTER 2: AIMS, HYPOTHESES AND RESEARCH STRATEGY

2.1 Aims

The association between physical activity, adiposity, and inflammatory stress responses has gained little attention and therefore forms the focus of this PhD thesis. This project also examines the effect of physical activity and weight loss on mood symptoms. A further aim is to explore the associations between diurnal cortisol level, regular physical activity and daily level of stress using ambulatory methods.

At present, there is little experimental evidence regarding the effect of physical activity on pro-inflammatory reactivity and on the effect of adiposity on cardiovascular and inflammatory reactivity.

This PhD project experimentally investigates whether reduction in adiposity, achieved through caloric restriction, and reduction in physical activity levels achieved through exercise withdrawal, results in attenuated or improved responses to standardized mental stress. Heightened and sustained autonomic activation to behavioural stress under controlled conditions has been found to predict several cardiac risk factors. Therefore, by experimentally manipulating physical activity and adiposity levels one is able to examine the pathways through which these factors influence or protect against autonomic and inflammatory dysregulation.

2.2 Research Strategy and Methodology

In order to achieve the aims of this PhD project, I firstly carried out an analysis of data from a cross-sectional study and then set up and implemented two experimental controlled studies of acute psychophysiological stress testing. Study 1 is a cross-sectional investigation of self-reported physical activity participation, daily stress and mood, and the diurnal cortisol in a sample of working women. The data used in this analysis are from the Day-Tracker study which was conducted by the UCL Psychobiology Group between 2007 and 2008. I was involved extensively in the data collection process for this study.

Study two is a randomized cross-over study conducted with physically active participants using an exercise withdrawal paradigm (EWP) and objectively recorded physical activity levels. Study 3 is a non-randomized study of overweight or obese women tested in the psychophysiology stress lab at baseline and again after a 9 weeks weight loss intervention designed to modify adiposity. I planned, set up and implemented these two laboratory studies.

Psychophysiological stress testing involves the induction of psychological and biological responses in a controlled setting by means of validated, standard psychosocial stimuli such as public speaking, the Stroop colour-word interference paradigm, the mirror tracing task or similar protocols. These responses to laboratory stress are considered to be an index of the individual's stress reactivity, and are usually monitored for a period of time depending on the protocol used and the biomarkers under analysis. In addition to the reactivity index of any stress protocol, the rate at which psychobiological markers (i.e. blood pressure or heart rate) return to baseline levels after stimulation, termed recovery, is also of great interest. This is because evidence suggests that impaired stress recovery may be an indication of

sustained allostatic load and therefore detrimental to health in the long term (McEwen & Seeman, 1999; Chida & Steptoe, 2010; Mcewen, 1998).

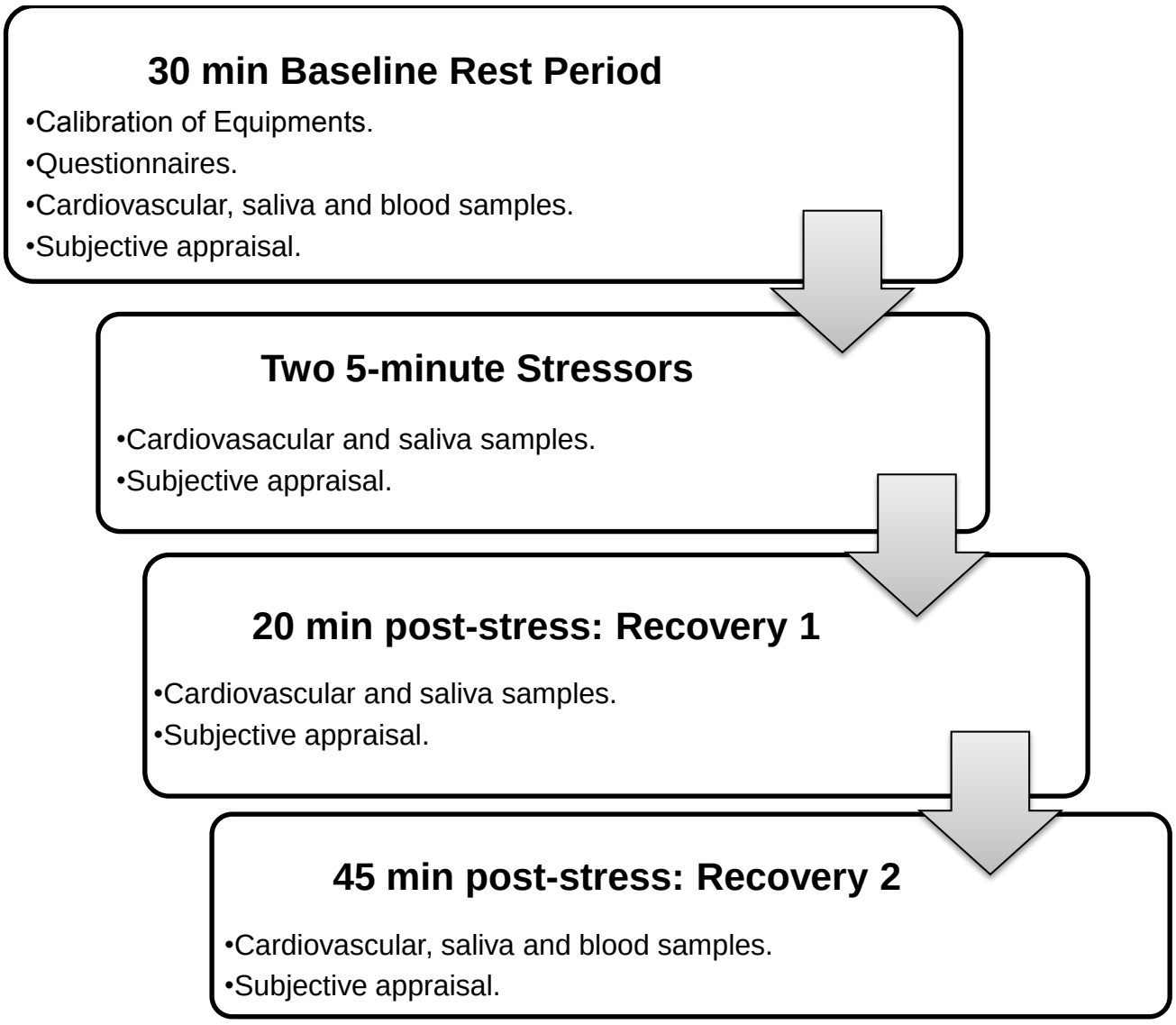
The two experimental psychophysiology studies involve the evaluation of cardiovascular, cortisol and pro-inflammatory cytokine IL-6 responses to mental stress under controlled conditions. The cross-sectional study involves the evaluation of the diurnal cortisol cycle in relation to habitual levels of physical activity.

2.3 Psychophysiology Stress Protocol.

A detailed description of the protocol will be provided under the methods section of each individual study, so in here only a general outline is provided. I planned to use two stress stimuli to induce psychobiological responses: a simulated public speaking task and a mirror drawing task. These stimuli have been used previously by our group and others and have been found to induce robust, reliable and reproducible stress responses. These tasks have been chosen because they do not require anyone other than the experimenter to be administered, unlike stress protocols such as the Trier Social Stress Test, and are of a relatively short duration.

The autonomic and immune parameters that are measured under stress-induced conditions include blood pressure, heart rate and heart rate variability, the noradrenalin metabolite 3-Methoxy-4-hydroxyphenylglycol (MHPG), the inflammatory marker C-reactive protein (CRP), the pro-inflammatory cytokine interleukin-6 (IL-6), the inflammatory hormone leptin, and the marker of hypothalamic pituitary adrenal axis (HPAa) cortisol. Figure 2.1 below gives an overview of the laboratory session protocol.

Fig-2.1 Overview of Psychophysiological Stress Testing Protocol.



2.4 Study 1: Physical Activity, Daily Stress and Mood, and Cortisol.

This study explored the cross-sectional association between self-reported levels of physical activity participation and daily rating of stress and general mood symptoms. In addition, the diurnal cortisol rhythm in relation to frequency of physical activity was also examined. It is possible that the beneficial health effects of physical

activity are in part mediated through improved HPA axis regulation but very little work has examined the activity of the hormone cortisol in relation to physical activity levels in a naturalistic sample.

The method of investigation for study one is ecological momentary assessment of general stress level during a working day and a leisure day as well as questionnaire ratings of mood and physical activity participation. Seven saliva samples obtained over a working day and again over a non-working day (leisure day) were used to estimate the diurnal profile of cortisol secretion which is an index of the HPA axis activity.

2.4.1 Hypotheses for Study 1:

I. Higher levels of physical activity will be associated with lower level of stress and mood symptoms.

   There will be group differences in rating of general daily stress and questionnaire measure of depressive symptoms between participants categorized into three levels of self-reported, habitual medium and vigorous physical activity (MVPA) participation: (1) No MVPA, (2) Little/some MVPA, and (3) Frequent MVPA.

II. Higher levels of physical activity will be associated with an attenuated cortisol diurnal profile.

   The following components of the cortisol diurnal profile: (1) mean evening concentrations, (2) cortisol awakening response (CAR), (3) total day cortisol

exposure (AUC), and (4) slope of cortisol decline through the day will differ between the MVPA participation groups.

2.5 Study 2: Physical Activity Withdrawal and Stress Study (PAWS).

If study one demonstrated a cross-sectional association between physical activity and mood symptoms in an ecological setting, the second study uses an experimental method to establish a causal association between physical activity and mood. In addition, the PAWS study examined the effect of physical activity on psychobiological responses to laboratory induced mental stress. A physical activity withdrawal paradigm was employed as an experimental model of physical inactivity in a cross-over, randomised, single-blind, controlled manner.

There are several advantages in using a physical activity withdrawal model rather than a formal active exercise induction trial, and therefore the strengths and weaknesses of this experimental model will be reviewed in greater details in the next chapters. Briefly, the manpower needed and the logistical and organisational costs required are reduced, because formal exercise supervision is not required. Moreover, a physical activity withdrawal approach overcomes the notorious problems of participant drop-out and poor adherence to protocol in exercise training studies.

Habitually active and physically fit individuals were assigned to two weeks of physical activity maintenance (control condition) or two weeks of physical activity withdrawal in a randomized, cross-over manner. Objectively measured physical activity levels over the four weeks duration of the study as well as self-report

assessment of mood symptoms were used to index the outcome of the intervention. This time period was chosen because previous work by our group and others has shown that two weeks of forced physical activity withdrawal is a robust and reliable method to induce mood disturbances in healthy, regular exercisers.

I examined psychobiological responses to the standard stress protocol relevant in cardiovascular disease risk before and after physical activity withdrawal. These psychobiological responses that were obtained in the psychophysiology laboratory include cardiovascular parameters (systolic and diastolic blood pressure, heart rate and heart rate variability, and total peripheral resistance); the neuroendocrine marker cortisol and MHPG; the inflammatory marker CRP and IL-6; and subjective stress appraisal ratings.

2.5.1 Hypotheses for Study 2:

I. Physical activity withdrawal will be associated with mood disturbances.
   a) A two week abstinence from regular physical activity compared to a two week maintenance of physical activity will cause significant changes in negative mood symptoms measured by questionnaire.
   b) The amount of objectively assessed change in physical activity will be positively and linearly associated with the mood disturbances following exercise withdrawal.
   c) The change in mood following physical activity withdrawal will be associated with changes in resting concentration levels of the inflammatory markers IL-6 and CRP.

II. Physical activity withdrawal will be associated with autonomic responses to stress.

a) Cardiovascular, inflammatory and neuroendocrine reactivity to mental stress will be higher, and recovery slower, following the two weeks of physical activity withdrawal compared to physical activity maintenance.

b) Subjective stress ratings will be higher, and stress task appraisal less favourable, following exercise withdrawal compared to exercise maintenance.

2.6 Study 3: Weight Loss and Stress Responses Study (WLSR).

This study examined the effect of adiposity on psychobiological responses to laboratory induced acute stress. Furthermore, mood changes following weight loss were also examined. I experimentally manipulated adiposity levels by inducing weight loss using a calorie restriction method with partial meal replacement and weekly support meetings. The weight loss intervention part of this study had been planned and implemented by the Diet Group in the UCL Health Behaviour Research Centre (HBRC). The participants were tested in the psychophysiology laboratory at baseline (before weight loss) and at follow-up (after weight loss) and the biological responses were compared. These psychobiological responses included the measures obtained in study two but with the exclusion of heart rate variability and cortisol. In addition, the adiposity-related biomarker leptin was assessed.

Overweight or obese, but otherwise healthy, women were recruited through advertisement in the local press for a study investigating the effect of adiposity and immune responses to stress. The total duration of the trial from study entry to the follow-up laboratory session was nine weeks. The weight loss intervention followed a

partial meal replacement method plus weekly nutritional advice and was designed by dieticians and Health Psychologists at the HBRC. This method aimed to achieve a 400 – 600 calorie deficit of calculated daily energy requirement. It was anticipated that this calorie deficit would induce an average weight loss of between 3 to 5 kg at three to six months follow-up. It was decided to use this method because standard behavioural counselling about nutrition does not produce substantial weight loss in the majority of participants.

The choice of this method was broadly based on a meta-analysis of weight loss clinical trials (Franz et al., 2007). As illustrated in figure 2.2, the meal replacement strategy was found to result in a mean weight loss of 6.7 Kg at 12 months follow-up. Importantly, the meal replacement method when compared to diet alone was found to result in significantly more weight loss at 12 months. The article also reported that the average attrition rate across the studies reviewed was 29 per cent at the one year follow-up.

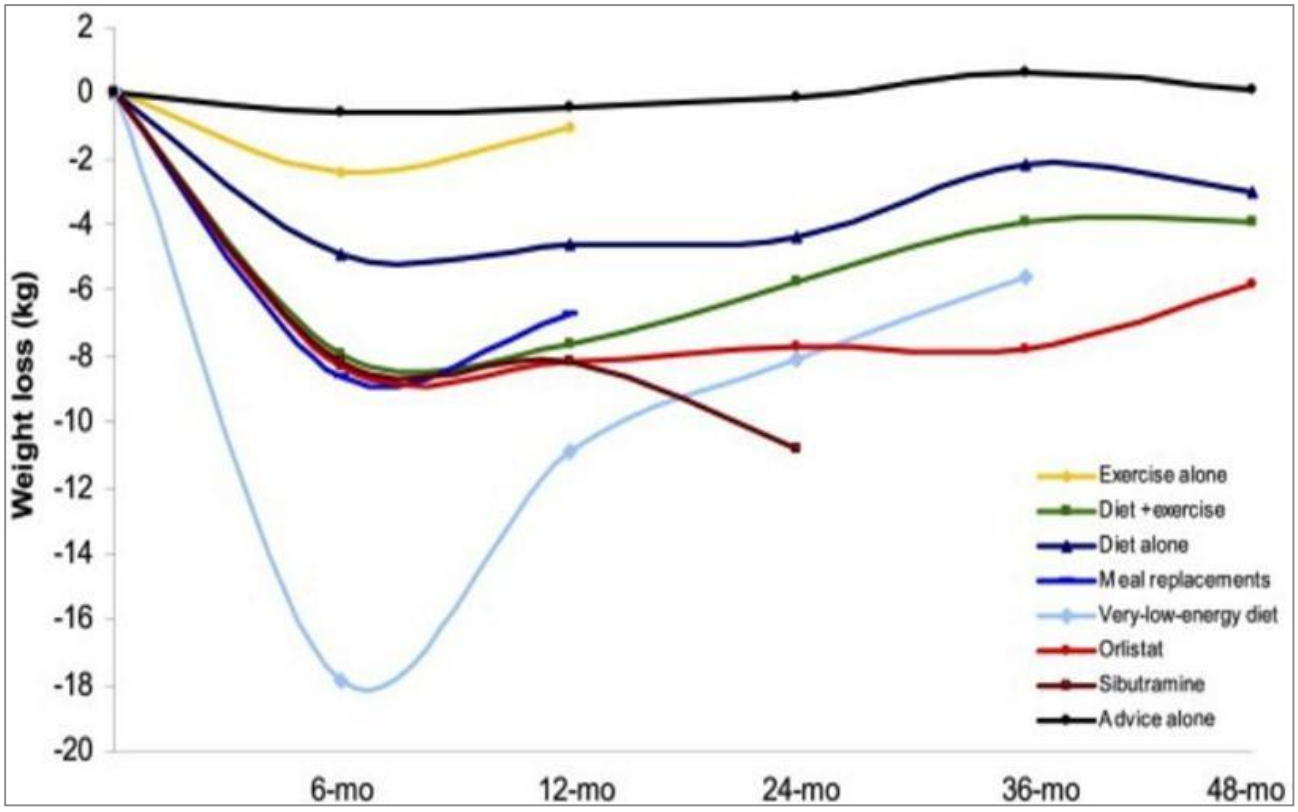


Fig-2.2. Systematic review of weight loss clinical trials (adapted from Franz et al, 2007)

2.6.1 Hypotheses for Study 3:

I. Weight loss will be associated with improvements in resting levels of inflammatory markers (CRP, IL-6 and leptin) and lipids.

II. Weight loss will be associated with attenuated autonomic responses to acute mental stress.

   a) Participants' cardiovascular and inflammatory responses to stress will be lower after weight loss compared to responses before weight loss, and the magnitude of this response will correlate with changes in adiposity measures.

b) Reductions in leptin following weight loss will be correlated with stress-induced inflammatory and cardiovascular responses.

III. Weight loss will be associated with improvements in depressive symptoms which will be mediated though changes in adiposity or inflammatory markers.

CHAPTER 3: PHYSICAL ACTIVITY, DIURNAL CORTISOL AND MOOD

3.1 Introduction

Regular physical activity has been consistently associated with morbidity and mortality and improved mental health and wellbeing (O'Donovan et al., 2010; Penedo & Dahn, 2005). The mechanism linking physical activity with health and wellbeing is important for several main reasons. Firstly, there is convincing evidence for an association between physical inactivity and depressive symptoms in healthy individuals (Strawbridge, Deleger, Roberts, & Kaplan, 2002) and in clinical samples (Krogh, Nordentoft, Sterne, & Lawlor, 2011). Depression is also associated with the development of cardiovascular disease risk factors (Nicholson, Kuper, & Hemingway, 2006; Hamer, Kivimaki, Lahiri, Marmot, & Steptoe, 2010; Knol et al., 2006), and mortality (Hamer, Bates, & Mishra, 2011; Moussavi et al., 2007).

Secondly, positive psychological wellbeing is an important outcome in itself not only for improving quality of life in general, but also because evidence point to wellbeing and positive affect as being independently associated with health outcomes (Chida & Steptoe, 2008). Therefore, elucidating the pathways through which regular physical activity exerts its beneficial effects is crucial, but at present these mechanisms remain poorly understood. Physical activity might exert its beneficial effects on mental health and cardiovascular function through shared mechanisms although little research has examined this.

Sub-clinical depressive symptoms are more prevalent in the general population than clinically diagnosed depression and have also been recognised as a strong risk factor for the development of major depression (Cuijpers & Smit, 2004). A recent large scale study of older women concluded that mild to moderate levels of depressive symptoms are strongly associated with poor psychological and physical functioning and accelerated cognitive decline (Vahia et al., 2010). Depressive symptoms measured longitudinally have also been shown to be predictive of coronary atherosclerosis in men independently of traditional risk factors (Hamer, Kivimaki, Lahiri, Marmot, & Steptoe, 2010). Therefore, convincing evidence suggests that depressive symptoms are associated with various health related outcomes.

Although some evidence suggests that cortisol hyper-secretion may be an important physiological component in depression, the association between depression and cortisol diurnal levels is inconsistent. Some studies have reported heightened cortisol in response to waking in patients with depression compared to healthy controls (Bhagwagar, Hafizi, & Cowen, 2005), and in healthy individuals reporting higher level of depressive symptoms (Pruessner, Hellhammer, Pruessner, & Lupien, 2003), but others have also reported a blunted response (Huber, Issa, Schik, & Wolf, 2006).

The discrepancy could be due to the different types of depression that have been investigated and the different instruments used to assess depressive symptoms. Depression is a heterogeneous diagnostic category which is often comorbid with other conditions. In addition, other factors such as chronic stress, use of anti-depressant and other medications, and behavioural and lifestyle factors are likely to confound the associations.

Other aspects of the cortisol diurnal cycle may also be relevant in depression. A recent meta-analytic review (Stetler & Miller, 2011) of the association of depression and HPA axis activity that included more than 18.000 individuals has found that the overall difference in cortisol concentration between depressed and non-depressed participants was more pronounced in the afternoon and smaller in the morning. This suggests that HPA axis dysregulation in depression may be observed in the entire diurnal cortisol cycle. The diurnal cycle of cortisol secretion is a well-documented phenomenon. In response to waking, and up to half an hour post-awakening, cortisol levels rise by an average of 50 to 160 per cent (Clow, Thorn, Evans, & Hucklebridge, 2004; Fries, Dettenborn, & Kirschbaum, 2009) in most healthy people. This phenomenon is called the cortisol awakening response (CAR). This is followed by a steady decline throughout the rest of the day that culminates in a trough at around midnight. Cortisol levels then rise again steadily through the early hours of the morning until the cycle is repeated. However, this diurnal rhythm is affected by several psychosocial and behavioural factors (Kirschbaum, 1994; Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005).

Chronic stress and stress-related factors have been more consistently associated with a heightened CAR (Chida & Steptoe, 2009). Since psychological stress is a strong risk factor for the development of depressive symptoms, an impaired or hyperactive HPA-axis might be an important mechanism. This is even more important in light of recent findings that HPA-axis dysregulation and high cortisol levels have been prospectively linked to cardiovascular disease mortality among depressed patients (Jokinen & Nordstrom, 2009) and healthy individuals (Kumari, Shipley, Stafford, & Kivimaki, 2011; Vogelzangs et al., 2010). Additionally, recent work has shown associations of the diurnal cortisol rhythm with the development of

cardiovascular risk factors (Reynolds et al., 2010). For example, in a large study of healthy, middle age adults (Matthews, Schwartz, Cohen, & Seeman, 2006), a flatter cortisol slope was associated with a greater presence of coronary artery calcification, an objective marker of atherosclerotic lesions which is a strong predictor of future cardiovascular events. In another study total daily cortisol exposure indexed as the area under the cortisol curve (Cort-AUC) was independently associated with a greater number of plaques of the carotid arteries (Dekker et al., 2008).

HPA-axis hyperactivity might also be relevant in response to challenge. Acute stress triggers a cascade of events that leads to the release of cortisol from the adrenal medulla into the blood stream. Cortisol has robust cardio-metabolic effects and it is thought that repeated stress-induced activation of the hypothalamic adrenal axis due to psychosocial stress or environmental demands leads to the development of cardiovascular risk factors. Our group has recently shown that heightened cortisol responses to mental stress predicted risk of having coronary calcium among healthy, older participants as well as being associated with higher calcium score among individuals with detectable coronary calcification (Hamer, O'Donnell, Lahiri, & Steptoe, 2010). Therefore, the associations between the HPA-axis and cortisol activity with risk factors can be observed in naturalistic settings as well as in controlled laboratory studies. This indicates that chronic activation of the axis in response to mental stress and the cortisol diurnal rhythm are important pathways in mediating these adverse effects.

The association between regular exercise and HPA axis function is at present unclear. Controlled studies in animals have shown that running exercise improved the glucocorticoid response during stress by facilitating hormonal habituation to repeated stress exposure when compared to a sedentary control group (Sasse et al.,

2008; Droste, Chandramohan, Hill, Linthorst, & Reul, 2007). In humans, it has been shown that the age related increase in stress-induced cortisol is attenuated in the more physically fit, older women (Traustadottir, Bosch, & Matt, 2005). Rimmele and colleagues (Rimmele et al., 2007) have also reported that professionally trained men had lower cortisol responses to stress compared to sedentary men, a finding that was replicated in a similar study comparing elite sportsmen to non-professionally trained individuals (Rimmele et al., 2009). However, one study showed that an acute bout of exercise is associated with a marked increase in plasma and salivary cortisol and greater monocytes sensitivity to glucocorticoids in endurance trained men (Duclos, Gouarne, & Bonnemaison, 2003) possibly suggesting that this increased glucocorticoid sensitivity acts to prevent a sustained, post-exercise inflammatory response.

Taken together these findings provide some preliminary evidence that the beneficial effects of physical activity may be mediated in part through improved HPA-axis control and regulation. Since the HPA axis is implicated in several physical and mental health outcomes, and that this pathway is sensitive to psychosocial and behavioural factors, it is important to investigate these associations further. Indeed the few studies that have attempted to address the issue were conducted in animals or mainly compared elite or amateur athletes with sedentary individuals. Although relevant, these studies are somewhat limited by the fact they miss out important aspects of the diurnal cycle of cortisol activity, the small sample sizes employed and the use of elite or well-trained participants, rather than the general population, that may reduce the generalizability of results. Furthermore, the association of regular physical activity with mood and the diurnal cortisol rhythm in women has received little attention. Women generally report more psychological distress and depression

than men (Kuehner, 2003; Silverstein, 2002) suggesting that this population may be more at risk of the adverse effects of poor mental health due to greater exposure to stress.

3.2 Hypotheses.

Therefore, the present study aims to investigate the associations of habitual physical activity, depressive symptoms and daily levels of stress, with the diurnal cortisol rhythm over a working and a weekend day in a large sample of office based female workers in full-time employment from two industrial European cities.

It was hypothesized that:

III. Higher levels of physical activity will be associated with lower level of stress and mood symptoms.

There will be group differences in rating of general daily stress and questionnaire measure of depressive symptoms between participants categorized into three levels of self-reported, habitual medium to vigorous physical activity (MVPA) participation: (1) No MVPA, (2) Little/some MVPA, and (3) Frequent MVPA.

IV. Higher levels of physical activity will be associated with an attenuated cortisol diurnal profile.

The following components of the cortisol diurnal profile: (1) mean evening concentrations, (2) cortisol awakening response (CAR), (3) total day cortisol exposure (AUC), and (4) slope of cortisol decline through the day will differ between the MVPA participation groups.

3.3 Methods

3.3.1 Study Design: Day-Tracker Study.

The Day-Tracker was a cross-sectional, ambulatory investigation of bio-behavioural factors and daily emotion regulation, involving the administration of a psychosocial questionnaire plus momentary ecological assessment of stress during a working day and a weekend day. In addition, saliva samples for the quantification of free cortisol were obtained over twenty-four hours during the two assessment days. It was designed to investigate the relationship between psychological well-being and biological function in everyday life using a cross-national design, so data were collected from comparable samples of working women in London, Budapest and Amsterdam. Results from the study have been published in various articles over the last two years (Dockray et al., 2010; Dockray & Steptoe, 2011; Jackowska, Dockray, Hendrickx, & Steptoe, 2011). This chapter reports analyses of data from the London and Budapest samples.

3.3.2 Study Sample.

A convenience sample of 388 healthy, full-time employed office-based women was recruited between April 2007 and April 2008 at University College London (UCL) and Semmelweis University in Budapest (Hungary). Exclusion criteria were: use of regular medication (except contraceptive pill), chronic illnesses such as coronary heart disease, cancer and diabetes, depression and related mental health impairments. Participants were screened over the telephone to determine eligibility

and scheduled to attend a research laboratory visit prior to commencing the working day monitoring period and again prior to the weekend period. The order in which participants commenced the study was counterbalanced so that roughly half of the participants started with the working day and half with the weekend day monitoring period.

3.3.3 Materials.

3.3.3.1 Self-Report Physical Activity:

Habitual physical activity (PA) was assessed by asking participants the frequency with which they engage in activities that are mildly, moderately, and vigorously energetic. Respondents rated the extent of their involvements in such activities on a scale ranging from *hardly ever/never* to *three or more times a week*. This scale was adapted from the Whitehall II longitudinal cohort study (Marmot & Brunner, 2005; Singh-Manoux, Hillsdon, Brunner, & Marmot, 2005), where it was shown that PA participation was strongly related to cognitive performance. Given the importance of moderate and vigorous physical activity (MVPA) for health (O'Donovan et al., 2010), participants were categorized into three physical activity groups: No MVPA (n = 48); Little/some MVPA (1d/ week, n = 144) and Frequent MVPA (≥ 2d /week, n = 196). These cut points were chosen according to the distribution of responses.

3.3.3.2 Mood and Daily Stress:

Mood and daily stress were measured in two ways. Firstly, participants were administered the Centre for Epidemiologic Studies Depression Scale (CES-D, (Radloff, 1977). The CES-D is a 20 item self-report scale that measures depressive symptoms in the general population by asking respondents how often they have felt or behaved in a certain way over the past week. The psychometric properties of the CES-D have been reviewed in a large sample of women in which the single depression dimension factor was found to fit the data well giving further support to the use of a single continuous score (Knight, Williams, McGee, & Olaman, 1997). Higher scores denote more depressed mood and cut-off values for categorical assessment have also been validated. In this study the continuous score system was used. Cronbach's alpha in this sample was 0.88.

Ecological Momentary Assessment (EMA) ratings of stress and the perception of being or feeling in control were recorded by the participant on 6 occasions during the working day and the leisure day. From these ratings a single measure called daily stress was calculated by converting the scores into standardized Z-score and averaging them. These ratings were obtained using a paper diary at the same time as the saliva sampling and consisted of a Likert scale ranging from 1 (not at all) to 5 (very much) in response to the following statement: In the last 30 minutes how much did you feel (-in control?, -stressed?). The control scale was reverse scored before further analysis. EMA measurement techniques aim to overcome the problems associated with questionnaires measures such as recall bias and the influence of current states on trait assessment (Shiffman, Stone, & Hufford, 2008).

3.3.3.3 Cortisol Sampling Technique:

Saliva was sampled on seven occasions during the working day and the weekend day using Salivettes® (Sarstedt, Leicester UK). At the research laboratory visit participants were provided with a sample collection kit made up of the saliva sampling diary and seven Salivettes. Participants were instructed to place the cotton swab in their mouth and chew gently for two minutes before returning the swab into the salivette container. They were asked to take a sample at the following times: [s.1] 17:00, [s.2] bedtime, [s.3] waking time, [s.4] 30 minutes after waking, [s.5] 10:00, [s.6] midday and [s.7] 15:00 in order to capture the cortisol diurnal cycle. Samples were to be kept in the participant's refrigerator until returned to the research team laboratory a few days later and stored in a clinical -80 degree Celsius freezer until analysis.

3.3.3.4 Sampling Diary:

The sampling diary served two main purposes: to monitor adherence to the saliva sampling protocol and as a memory jogger for the EMA ratings of stress and perception of control during the study days (described above). Each time a saliva sample was due to be collected, participants noted the clock time of actual sampling along with 'yes' or 'no' responses to the following statement: in the last 30 minutes before sample collection did you?: brush your teeth, drink caffeinated drinks, take medications, eat a meal, drink alcohol, exercise, smoke; and subsequently the EMA ratings were made. Participants were instructed not to exercise, eat or drink or brush their teeth for half an hour before saliva collection. In addition, they were asked not to take any anti-inflammatory medication or pain killers for the duration of the study.

3.3.4 Procedure.

The procedure is summarised in figure 3.1 below, and is the same for the working and weekend day. For the working day monitoring period, participants were required to attend the lab between Monday and Thursday at about 16:00 so that they could start the study at 17:00 which is when the first saliva sample was due. For the weekend day monitoring period instead, participants were required to attend on a Friday at 16:00. The study then terminated at 3 pm the following day. The starting day (weekend or workday) was counterbalanced among participants and the maximum lag between the two research days was 2 weeks and the minimum three days. Participants returned questionnaires and saliva samples using a pre-paid padded envelope.

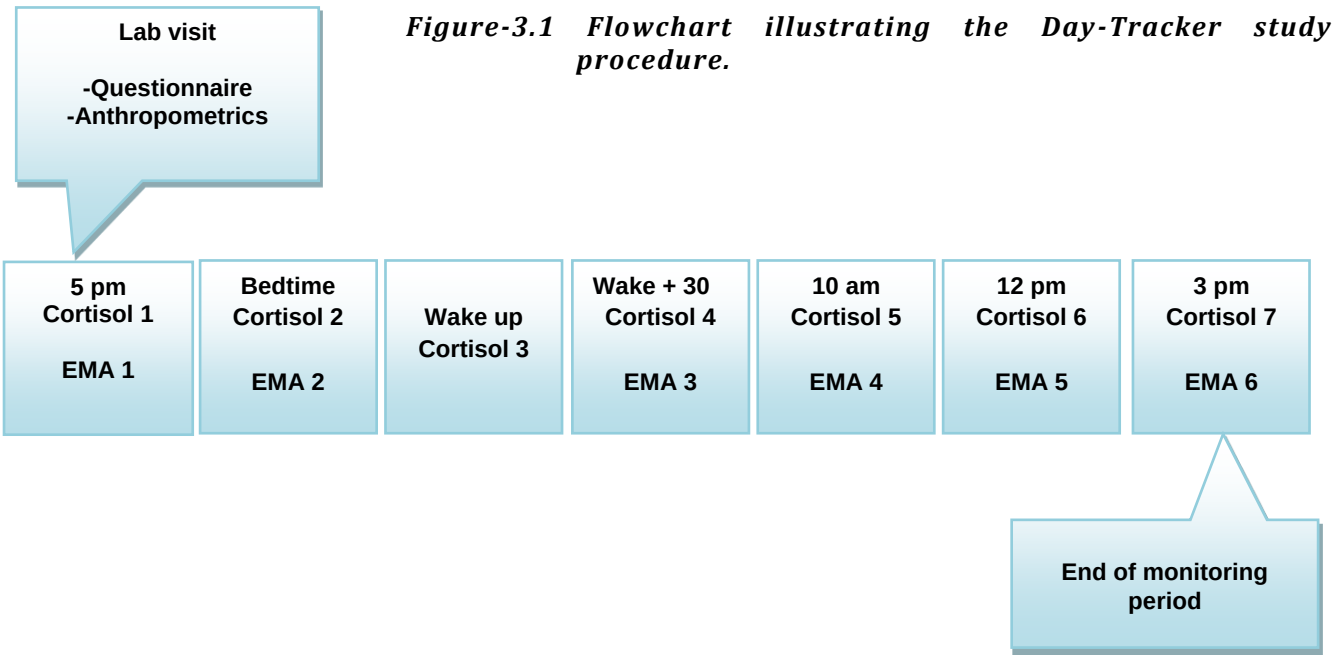


Figure-3.1 Flowchart illustrating the Day-Tracker study procedure.

3.3.5 Analytical Statistical Approach

Firstly, I compared the English and Hungarian sample on the between-subject factor habitual physical activity and the outcome variables diurnal cortisol, CES-D and daily stress using one-way ANOVA and Chi square. Then, I sought to establish whether differences in the diurnal cortisol cycle were present between the working and leisure days by using repeated measures ANOVA. The cortisol variables examined were: CAR (calculated as the mean difference between the waking and the waking + 30 minute samples), Cort-AUC (total cortisol output during the day calculated using the trapezoid formula as described in (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003), Cort-Slope (the slope of cortisol decline from waking through the last 3 pm sample calculated by statistically regressing the raw cortisol values on the time of day that the samples were collected).

The MVPA variable was used as the between-subject factor in the analyses involving the mood symptoms scores obtained by questionnaire (CES-D) and daily stress rating by aggregating EMA ratings. In this analysis the covariates were country (UK and Hungary), age and BMI. The association of habitual PA with cortisol was examined on the cortisol variables: CAR, Cort-AUC, Cort-Slope calculated as described above plus Cort-Ev (evening levels of cortisol calculated by averaging the last 2 samples). In this analysis the covariates where country, age, BMI, smoking and awakening time (for the CAR). Significance p values for linear trend and adjusted means ± standard error of the mean (SEM) are presented. In cases where the ANCOVA test was significant, LSD-adjusted test for multiple comparisons was used to examine post-hoc differences between MVPA groups.

3.4 Results

3.4.1 Sample Descriptive.

Table 3.1 shows the socio-demographic characteristics of the sample. Participants were full-time office based workers recruited in London and in Budapest. The mean BMI appears to be in the normal range and the mean depression score is lower than the cut-off value of 16 which is indicative of mild depressive symptomatology (Zich, Attkisson, & Greenfield, 1990). Table 3.1 suggests a relatively well-educated, healthy sample with few smokers with a great proportion of participants engaging in frequent physical activity.

Table-3.1: Descriptive Characteristic of the Study Sample (n = 388)

Variable	Mean ± SD
Age (years)	35.71 ± 10.17
BMI (kg/m^2)	23.82 ± 4.37
CES-D	11.88 ± 8.86
Variable	**N (%)**
Education	
Less than degree	146 (36.7)
Degree or higher	252 (63.3)
Income (£)	
< 35 K	123 (31.9)
≥ 35 – 70 K	159 (41.3)
> 70 K	103 (26.8)
Smoking	
Never	249 (63.4)
Former	81 (20.6)
Current	63 (16)
Alcohol	
Non-drinker	40 (10)
Drinker	358 (89.3)
Moderate & Vigorous Physical Activity (MVPA)	
No	48 (12.4)
Little – Some	144 (37.1)
Frequent	196 (50.5)

Table 3.2 reports the mean cortisol concentration and sampling time for the two study days. Overall, compliance with the scheduled sampling protocol was reasonable good, however, it has been shown in previous studies that in order to accurately determine the cortisol diurnal rhythm, and especially the CAR, participants who did not comply with the sampling protocol at the specified time should be excluded (DeSantis, Adam, Mendelsohn, & Doane, 2010; Dockray, Bhattacharyya, Molloy, & Steptoe, 2008). Therefore, I excluded from further analysis involving cortisol participants who delayed the waking sample for more than 10 minutes. In this way, the sample size for the working day was 326 and for the leisure day 314.

Table-3.2 Mean cortisol concentration and sampling time over the two study days

Sampling time	Work day n = 326		Leisure day n = 314	
	Sample time ± SD (hh:mm)	mean ± SD (nmol/l)	Sample time ± SD (hh:mm)	mean ± SD (nmol/l)
1 (5pm)	17.40 ± 1.23	4.51 ± 3.98	17.14 ± 1.39	4.45 ± 2.66
2 (Bedtime)	22.70 ± 1.05	3.34 ± 4.30	23.15 ± 1.48	3.43 ± 4.21
3 (Waking)	06.51 ± 1.02	15.70 ± 8.34	7.56 ± 1.36	13.99 ± 7.49
4 (Wake+30)	07.13 ± 1.08	22.60 ± 12.54	8.19 ± 1.37	18.25 ± 9.29
5 (10 am)	10.14 ± 0.31	9.39 ± 6.33	10.25 ± 0.61	10.48 ± 7.02
6 (12 pm)	12.15 ± 0.36	6.99 ± 4.60	12.22 ± 0.61	7.38 ± 4.62
7 (3 pm)	15.21 ± 0.61	6.50 ± 4.25	15.23 ± 0.53	6.33 ± 3.92

3.4.2 Cohort Differences.

Participants from London and Budapest did not differ in regard to CES-D depression score ($F_{1, 397} = 0.34$, $p = 0.56$) and BMI ($F_{1, 386} = 1.50$, $p = 0.22$). However, the Hungarian sample was slightly older (M age = 37.6 ± 10.69 years vs. 33.83 ± 9.28, p = <0.001) and reported less EMA assessed daily stress ratings in both the work-day (M = 3.81 ± 1.19 vs. 4.21 ± 1.20, p = 0.001) and the leisure-day (M = 3.48 ± 1.23 vs. 3.96 ± 1.10, p = <0.001).

Work-day Cort-Ev levels ($F_{1, 336} = 0.67$, $p = 0.41$), leisure-day Cort-Ev levels ($F_{1, 335} = 2.72$, $p = 0.10$), and leisure-day CAR ($F_{1, 335} = 0.01$, $p = 0.91$) did not differ between the 2 groups. Work-day Cort-AUC (M = 6892.14 ± 2992 nmol/l/min vs. 1354.98 ± 582, p = <0.001) and leisure-day Cort-AUC (M = 4918.96 ± 2150 nmol/l/min vs. 1098.16 ± 424, p = <0.001) were significantly higher in the Hungarian sample. Work-day CAR (M = 8.91 ± 12.21 nmol/l vs. 6.45 ± 9.22 nmol/l, p = 0.04) was also greater in the Hungarian sample.

A greater proportion of individuals in the English sample reported to engage in frequent MVPA (56.6% vs. 43.4%, p = 0.006). Therefore, in further analyses country sample will be added as a covariate.

3.4.3 Work Day vs. Weekend Day Cortisol Profile

Analysis of covariance was carried out in order to determine whether there were differences between the work-day and the leisure-day in the following diurnal cortisol variables: CAR, Cort-AUC and Cort-Slope. The CAR was adjusted for awakening time due to the fact that participants woke at different times in the two sampling days and therefore the absolute waking cortisol level might be different. This was

achieved by subtracting the waking leisure-day time from the waking working-day time. The obtained value, which represents the absolute waking difference expressed in minutes, was subsequently entered as a covariate into a repeated measure ANCOVA model. Figure 3.2 depicts the 24 hours cortisol diurnal profile in the two sampling days.

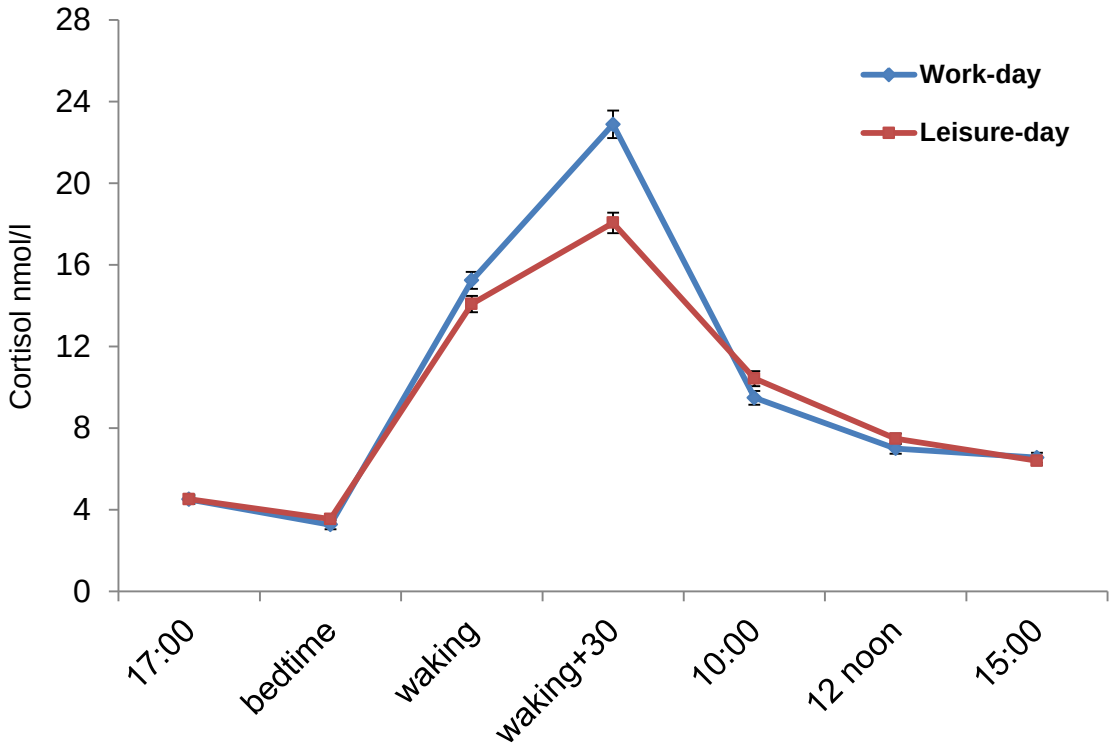


Figure-3.2 Un-adjusted 24h Salivary Cortisol Means in the two Study Days. Bars are standard error of the mean work-day n=326 – leisure-day n=314.

Results showed no main effect of CAR ($F_{1, 315} = 0.04$, $p = 0.83$) but a significant CAR by waking time interaction ($F_{1, 315} = 71.57$, $p = <0.001$) and a CAR by country

interaction ($F_{1, 315} = 5.26$, $p = 0.02$). Waking time and country sample adjusted CAR means (±SEM) were 7.62 ± 0.57 nmol/l and 4.46 ± 0.49 nmol/l for the work-day and leisure-day respectively. This indicates that when taking into account country sample differences in awaking time there was no significant difference in CAR levels between the work-day and the leisure-day.

The main effect of Cort-AUC was significant ($F_{1, 316} = 24.35$, $p = <0.001$) and there was a significant interaction effect of Cort-AUC with country sample ($F_{1, 316} = 78.48$, $p = <0.001$). Therefore, adjusting for country sample the Cort-AUC was greater in the work-day (M = 4150.42 ± 120.61 nmol/l/min) compared to the leisure-day (M = 2983.35 ± 81.36 nmol/l/min).

There was no main effect of Cort-Slope ($F_{1, 329} = 0.02$, $p = 0.87$) nor an interaction with country sample ($F_{1, 329} = 0.17$, $p = 0.67$) indicating no significant difference in the slope of cortisol decline between the work-day and the leisure-day.

Given that some differences in the cortisol diurnal rhythm in the two sampling days were observed, further analyses involving cortisol will be carried out separately for the work-day and the leisure-day.

3.4.4 Habitual Physical Activity and Mood Symptoms.

Analysis of covariance was carried out with MVPA groups as the between-subject factor and depression score (CES-D) as dependent variable while age, BMI and country sample were entered as covariates. A significant linear trend was apparent for the CES-D ($p = 0.02$), whereas age ($p = 0.94$), BMI ($p = 0.68$) and sample country ($p = 0.71$) were not significant covariates. The adjusted CES-D mean scores (±SEM)

were 14.36 (1.31), 11.96 (0.73) and 10.91 (0.64) for the no MVPA, little/some MVPA and frequent MVPA respectively. This association is shown in figure 3.3 below.

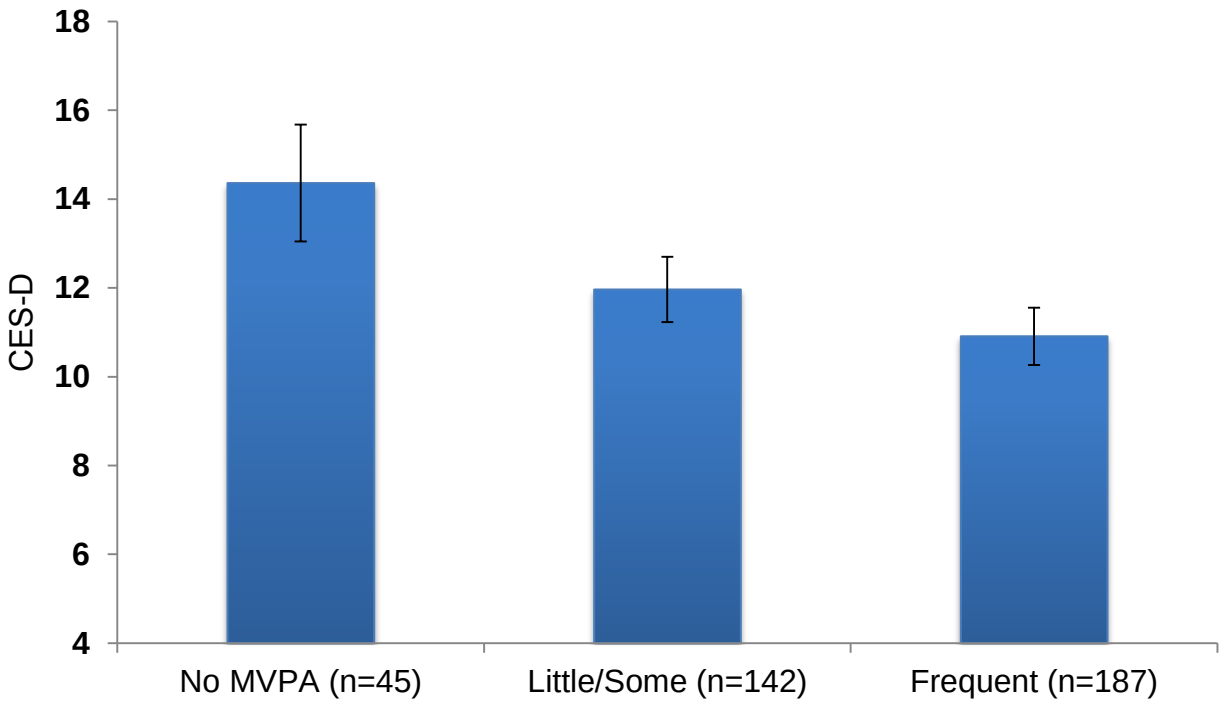


Figure-3.3 Associations of Habitual Physical Activity and CES-D Depression Score Adjusted for Age, BMI and Country Sample Error Bars are Standard Error of the Means

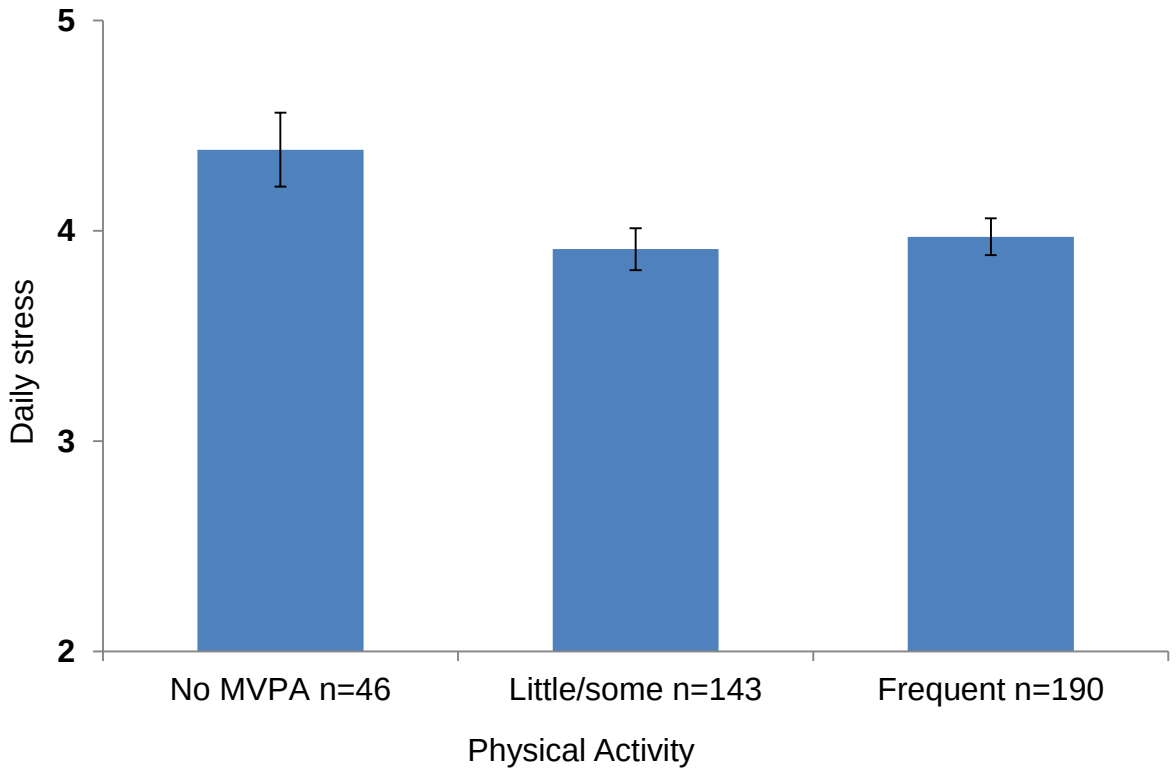
3.4.5 Habitual Physical Activity and Daily Stress (working day):

A total daily stress score was derived from the six aggregate EMA ratings of stress and perception of control for the whole day period during the work-day and the leisure-day. These measures reflect momentary feelings of stress rather than global mood as in the questionnaire measure (CES-D). The daily stress score was obtained by converting the score of each construct to a standardized z-score and then

averaging the two standardized scores (the feeling in control scale was reverse scored prior to standardization).

Daily stress correlated moderately with the CES-D (r = 0.36, p = <0.001) suggesting a certain degree of independence between the two measures. In order to test whether MVPA is associated with daily stress, ANOVA models for the work-day and leisure-day were run with the three MVPA groups as the between-subject factor and daily total stress score as the dependent measure adjusting for country sample.

Figure-3.4 Physical Activity and EMA Aggregate Ratings of Daily Stress During the Work-day



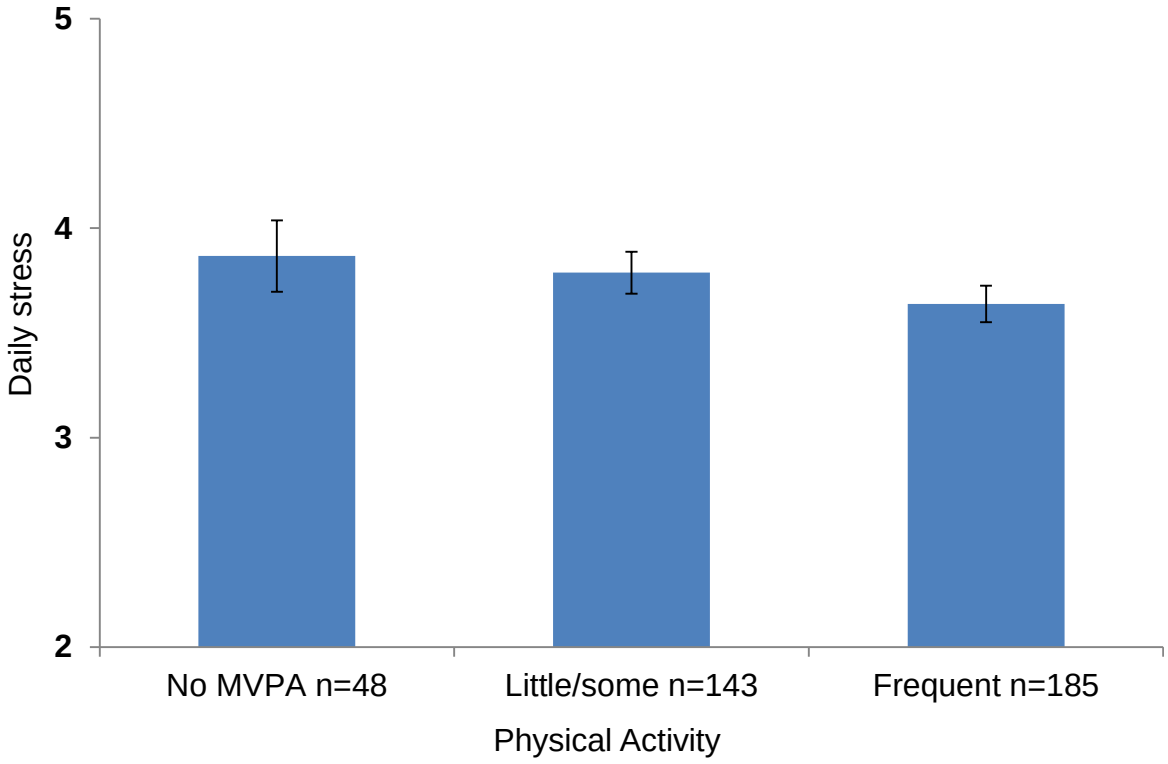
There was a significant linear association of daily stress with MVPA (p = 0.03) and there was also a near-significant quadratic trend (p = 0.06). The adjusted mean (± SEM) for the no MVPA group was 4.38 ± 0.17, for the little/some MVPA was 3.91 ± 0.1, and for the frequent MVPA was 3.97 ± 0.08 (shown if figure 3.4).

Post-hoc LSD adjusted analyses indicated that compared to the no MVPA, the little/some (p = 0.02) and frequent MVPA group (p = 0.03) rated significantly less daily stress. This indicates that after adjusting for differences in stress ratings between countries, daily stress measured during a work-day was lower in the more active group.

3.4.6 Habitual Physical Activity and Daily Stress (leisure day):

In the leisure day the correlation between total daily stress and CES-D depression was (r = 0.39, p = <0.001) suggesting again a good degree of independence. The adjusted daily stress mean scores for each MVPA group are shown in Figure 3.5.

Figure-3.5 Physical Activity and EMA Aggregate Ratings of Daily Stress During the Leisure-day

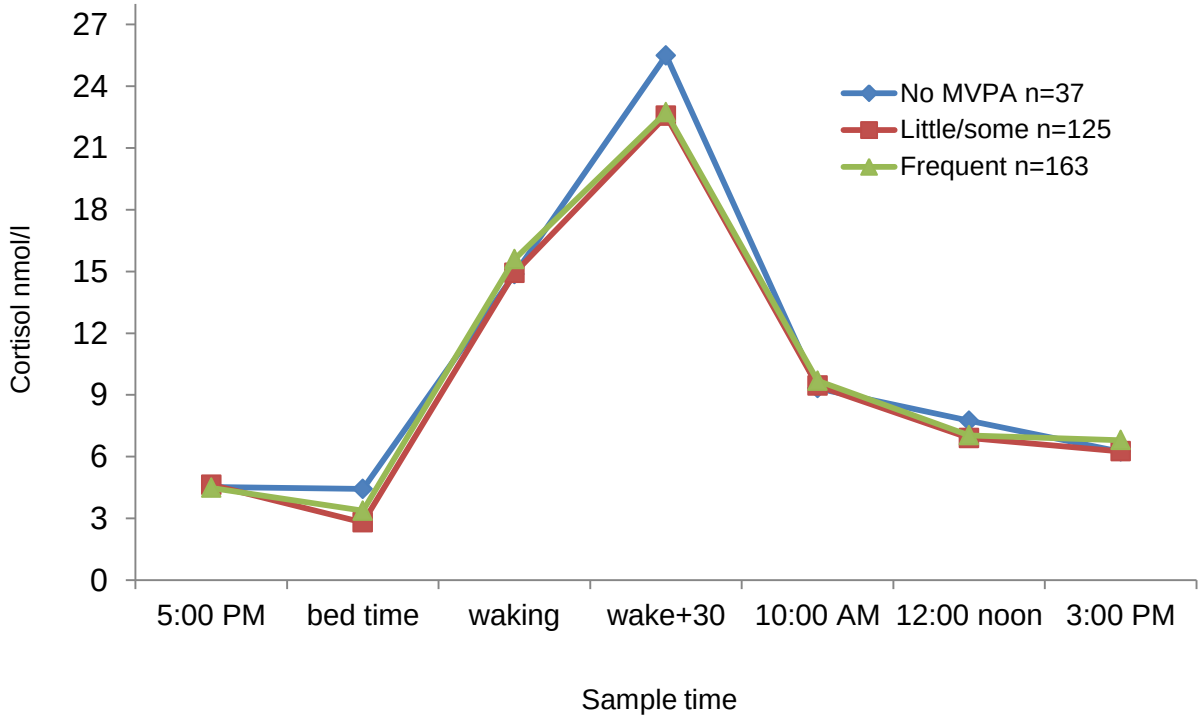


The linear trend was not statistically significant (p = 0.23). The mean scores (±SEM) were 3.86 (0.17), 3.78 (0.1) and 3.63 (0.08) in the no, little/some and frequent MVPA group respectively. This indicates that taking into account differences in country sample there were no significant differences in daily momentary measures of stress during the leisure-day between the physical activity groups.

3.4.7 Habitual Physical Activity and Diurnal Cortisol (working day)

Figure 3.6 shows the 24 hours salivary cortisol profile according to the frequency of habitual physical activity in participants who fully complied with the saliva sampling protocol (n = 326).

Figure-3.6 Unadjusted 24h Cortisol Means for the Work-day According to Self-report MVPA Participation.



Evening cortisol levels (Cort-Ev) were examined by averaging the concentration of the two evening samples (5 pm and bedtime) to provide a mean evening value that was used as the dependent variable with age, BMI, smoking status and country sample as covariates. The ANCOVA models ($F_{2, 303} = 2.94$, $p = 0.055$) showed that age ($p = 0.45$), BMI ($p = 0.20$), smoking ($p = 0.68$) and country sample ($p = 0.41$) did

not influence Cort-Ev levels. There was a significant linear trend (p = 0.02) suggesting higher Cort-Ev in the least active group. Adjusted Cort-Ev means (±SEM) were 5.41 (0.63) nmol/l, 3.76 (0.32) and 3.79 (0.28) in the no MVPA, little/some and frequent MVPA group respectively.

Post-hoc comparisons indicated that the group difference in Cort-Ev was significant between the no MVPA and the little/some and frequent MVPA (p = 0.02). Since figure 3.6 suggests that it may be the bedtime sample that is driving the effect of MVPA on Cort-Ev, the analysis was repeated using the bedtime cortisol sample instead of the average of the bedtime and 5pm samples. As in the previous analysis none of the covariates were significant. The linear trend was confirmed (p = 0.03) but there was also a quadratic trend (p = 0.04). Post-hoc comparisons indicated that compared to the no MVPA group, the difference in bedtime cortisol was greater in the little/some MVPA group (p = 0.01) than the frequent MVPA group.

The CAR is the net increase in cortisol concentration after waking and it was calculated as the mean difference between the waking and waking + 30 minutes sample (Chida & Steptoe, 2009). The covariates for the CAR included waking time. Age (p = 0.93), BMI (p = 0.23), smoking (p= 0.54) and waking time (p = 0.64) were not significantly associated with the CAR but sample country was marginally associated (p = 0.06) as noted in the previous section (3.4.2). There was no significant linear trend for MVPA groups and CAR (p = 0.12) and the adjusted means (±SEM) were 11.11 (2.09) nmol/l, 7.40 (1.02) and 7.59 (0.9) in the no, little/some and frequent MVPA group respectively.

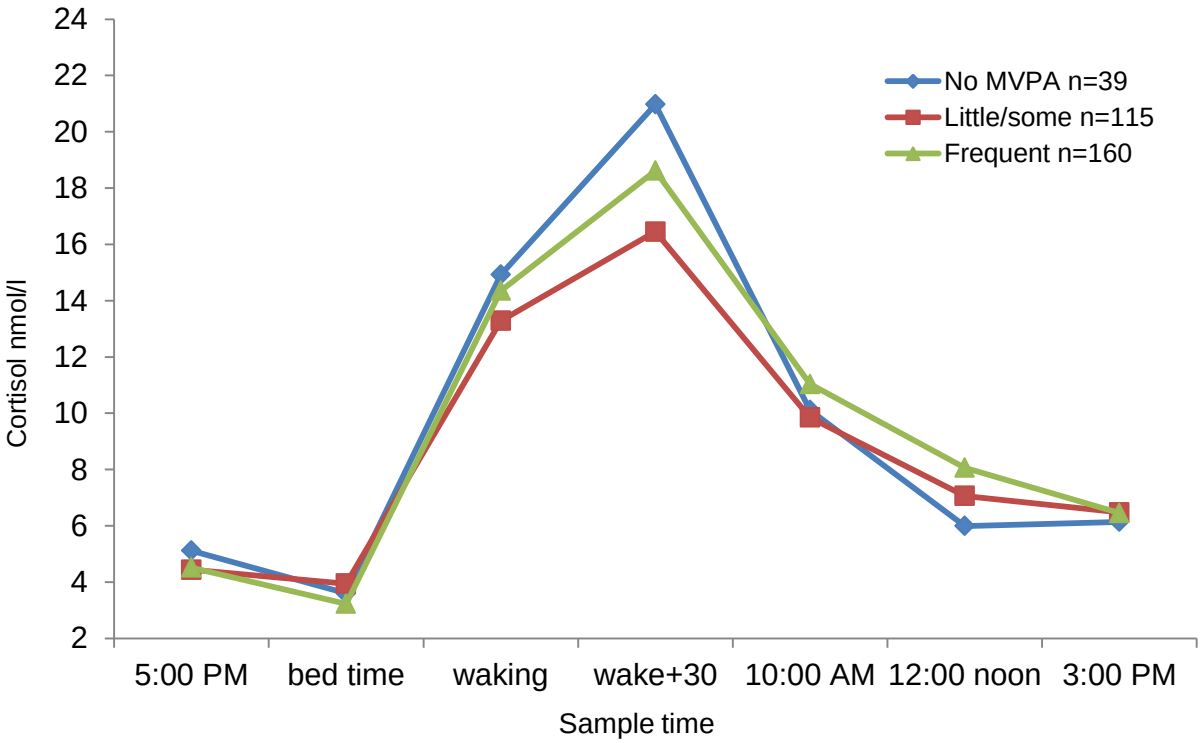
The Cort-AUC was calculated using the trapezoid formula (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Age (p = 0.52) and smoking (p = 0.38) were not significant covariates, but BMI (p = 0.01) and country sample were (p = 0.001). There was a significant linear (p = 0.05) and quadratic trend (p = 0.02). The adjusted Cort-AUC means (±SEM) were 4985.05 (370.48) nmol/l/min in the no MVPA group, 3966.38 (190.43) in the little/some MVPA and 4188.58 (165.91) in the frequent MVPA group. Post-hoc analyses showed a significant lower Cort-AUC in the frequent MVPA group (p = 0.05), and in the little/some MVPA group (p = 0.01) compared to the no MVPA group.

Finally, the Cort-Slope from waking through 15:00 was examined. A steeper slope is indicative of an improved cortisol decline. Age (p = 0.001) and country sample (p = 0.04) were significant covariates whereas BMI (p = 0.35) and smoking (p = 0.73) were not. There was no significant linear association between physical activity groups and Cort-Slope (p = 0.85). The adjusted Cort-Slope means (±SEM) were 0.016 (0.002) nmol/l/min, 0.017 (0.001) and 0.016 (0.001) in the no, little/some, and frequent MVPA groups respectively.

3.4.8 Habitual Physical Activity and Diurnal Cortisol (leisure day)

Figure 3.7 shows the 24 hours salivary cortisol profile according to the frequency of habitual physical activity in participants who fully complied with the saliva sampling protocol (n = 314).

Figure-3.7 Unadjusted 24h Cortisol means in the Leisure-day According to Self-report MVPA participation.



Analysis for Cort-Ev levels showed that none of the covariates were statistically significant: Age (p = 0.17), BMI (p = 0.36), smoking (p = 0.55) and country sample (p = 0.11). Cort-Ev was not linearly related to MVPA groups (p = 0.27). The adjusted means (±SEM) were 4.12 (0.41) nmol/l in the no MVPA group, 4.12 (0.23) in the little/some MVPA group, and 3.61 (0.2) in the frequent MVPA group.

For the leisure-day CAR age (p = 0.24), BMI (p = 0.93), smoking (p = 0.09) and sample country (p = 0.55) were not significant covariates but waking time was (p = 0.008). There was no association between MVPA group and CAR (p = 0.52), and the adjusted CAR means (±SEM) were 6.33 (1.51) nmol/l, 4.24 (0.87) and 5.24 (0.75) in the no MVPA, little/some and frequent MVPA group respectively.

For the leisure-day cort-AUC, age (p = 0.03) and sample country (p = 0.001) were significant covariates whereas BMI (p = 0.9) and smoking were not (p = 0.96). There was a non-significant trend towards a linear association of MVPA groups with Cort-AUC (p = 0.09). The adjusted means (±SEM) were 3481.24 (260.28) nmol/l/min in the no MVPA group, 3057.67 (148.77) in the little/some MVPA group, and 2995.36 (128.04) in the frequent MVPA group.

Finally, the Cort-Slope from waking through 15:00 was examined and none of the covariates were significant: Age (p = 0.21), BMI (p = 0.71), smoking (p = 0.13) and sample country (p = 0.63). There was no linear association between MVPA groups and Cort-Slope (p = 0.26). The adjusted means (±SEM) were 0.019 (0.003) nmol/l/min, 0.016 (0.001) and 0.016 (0.001) in the no MVPA, little/some and frequent MVPA groups respectively.

3.5 Discussion

The aim of this chapter was to explore the associations of habitual, self-reported levels of physical activity with mood symptoms and daily stress, and the cortisol diurnal cycle in a relatively large sample of office based workers. It was hypothesized that levels of physical activity would be linearly associated with mood measured by questionnaire, daily stress quantified with momentary techniques, and the diurnal cycle of cortisol secretion. Daily stress ratings and cortisol levels were assessed over a working day and a weekend day because there are likely to be differences in the cortisol dynamics and subjective level of stress between these two days (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004; Schlotz, Hellhammer, Schulz, & Stone, 2004). Therefore, in order to explore the associations between physical activity, daily stress and the cortisol diurnal cycle it is important to capture a typical working day as well as a non-working, leisure day.

The study sample consisted of working women recruited from London and Budapest who were asked to rate subjective stress levels and feeling of control several times through the study days, and collect saliva samples at the same time of the ratings. There were between country differences in regard to age, daily stress, and cortisol indexes. The Hungarian sample was older and reported slightly less EMA daily stress but greater cortisol output. In contrast, the English sample reported engaging in MVPA more frequently. Given the observed differences in several of the outcome variables the analyses were further adjusted for sampling country.

I sought to establish whether there were differences in the diurnal cycle of cortisol between the working and the leisure day. After adjusting for awakening time and country sample, there was no work – leisure day difference in the CAR. However, the whole day cortisol secretion indexed by the area under the curve was

greater during the working day independent of country sample whereas the slope of cortisol decline did not differ. Previous work has generally shown that the CAR is more pronounced during a working day compared to a leisure day (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004; Thorn, Hucklebridge, Evans, & Clow, 2006) independent of awakening time. In this study after controlling for country sample and difference in awakening time there was no working day – leisure day difference in CAR levels. A significant difference was nonetheless apparent in whole day cortisol production with greater levels during the working day.

Physical activity was assessed with an adapted scale used in the Whitehall II study that represents habitual engagement in activities and sports that require mild, moderate and vigorous energy expenditure. In line with the hypotheses, mood symptoms measured with the CES-D were inversely related to physical activity. Women who reported frequently participating in various forms of moderate and vigorous physical activity had a lower CES-D score than those reporting no MVPA, independently of age, BMI and country sample. These findings add to the physical activity and mental health literature (Stephens, 1988; Penedo & Dahn, 2005; Hamer, Stamatakis, & Steptoe, 2009) by showing that in a sample of women from two different European cities the frequency of weekly participation in physical activities and/or sport is associated with lower depressive symptoms scores in a linear fashion.

These differences were small and outside the cut-off points suggested for clinical or major depressive disorders. Nonetheless, it has been shown that even depressive symptoms at the sub-clinical level not only predict clinical depression but are also associated with worse health outcomes in older women (Vahia et al., 2010). Moreover, female office based workers such as those in this sample might be more

exposed to the risk of developing depression and low level physical activity than non-office based workers due to the high amount of time spent sitting (Teychenne, Ball, & Salmon, 2010).

I also examined daily levels of stress in relation to physical activity using aggregate scores of repeated ratings over 24 hours during the working day and leisure day. The subjective perception of stress and control may be important not only because chronic stress exposure is predictive of the development of depression (Alfonso, Frasch, & Flugge, 2005; Kasen, Chen, Sneed, & Cohen, 2010) but also because the appraisal of events or situations as stressful or demanding does, in turn, activate the coping strategies one will employ to deal with the situation. These coping strategies might be adaptive or maladaptive and have a long-term impact on health. The results showed that there was a moderate correlation of momentary stress with depression measured by questionnaire suggesting that although the two dimensions are related they have a large degree of independence. As predicted, during the work-day the group with the least physical activity participation rated higher daily stress compared to the other two groups. Importantly, this difference was independent of country sample since it was reported earlier that the London based sample rated greater daily stress than their Budapest counterpart.

This finding was not replicated in the leisure day. The reason for this is likely to be that perception of stress was lower in the leisure day compared to the working day and therefore the narrow variation reduced the power to find significant associations. Steptoe and colleagues found no difference in stress appraisals measured with EMA over 12 days between exercise and non-exercise days in a small sample of moderately active men and women (Steptoe, Kimbell, & Basford, 1998). However, participants reported more positive mood on the exercise compared

to non-exercise days. The reason for this discrepancy is likely to be attributable to the different methodology used.

Indeed, the differences in daily stress rating and questionnaire measure of depression between the physical activity groups in this study were small, and one may question the significance or the meaning of this finding. However, small differences in subjective appraisals such those found in this sample should not be dismissed or underestimated for several reasons. If a participant's rating represents a typical day, then, in the long-term, stress or lack of control may have negative health consequences. This notion may be in keeping with the allostatic load model of health and disease which suggest that sustained exposure to uncontrollable stress contributes to a disturbed or poor regulated homeostatic balance leading to vulnerability do disease.

An unexpected but interesting finding of this study was that the daily cortisol output indexed as the AUC was greater in the Hungarian participants in both sampling days compared to their English counterparts. This was in spite of slightly lower daily stress assessed by aggregating momentary assessment ratings only (but no differences in self-report mood symptoms). However, the physical activity effect observed in the total daily and evening levels of cortisol was independent of between sample differences in cortisol. Nevertheless, this finding might have important implications and needs to be interpreted. It may be that Hungarian participants experience slightly less day to day stress but perhaps more chronic stress or negative life events, which was not measured, and this may then result in sustained HPA axis activation with consequent greater daily cortisol exposure. An alternative explanation may be that since the Hungarian sample reported engaging in physical activity less frequently than the English sample, cortisol output may be elevated as a

result. Research on cross-cultural differences in HPA axis activity and cortisol levels is scarce so that at this stage it may only be possible to speculate what the reason for the difference in cortisol AUC might be. Nevertheless, this finding may suggest that diurnal cortisol exposure and momentary stress measure may not necessarily be positively related or that at least the association may be confounded by cross-cultural factors.

It was argued in the introduction that the beneficial effects of regular physical activity may act through HPA axis regulation. For example, cross-sectional studies have shown lower cortisol reactivity to mental stress in elite athletes and trained individuals compared to non-trained individuals and amateur sportsmen (Rimmele et al., 2007; Rimmele et al., 2009). In naturalistic settings no study has yet examined whether physical activity is associated with the diurnal cortisol profile. Results for the working day showed that evening cortisol level (cort-ev) was higher in the lowest MVPA group compared to the two other groups after adjusting for age, BMI and smoking. However, other parameters such as total cortisol output (cort-AUC) and the diurnal slope decline (cort-slope) were not significantly associated with physical activity. Only the linear trend of CAR and MVPA approached statistical significance.

In the leisure day none of the cortisol parameters examined was statistically associated with physical activity even though the associations were in the predicted direction. Only evening cortisol values approached significance level in this case ($p = 0.07$) so that the most active group again showed the lowest mean values.

These findings suggest that regular physical activity is associated with lower evening cortisol during the work-day and only marginally in the weekend. This is interesting because previous work has shown that perceived momentary stress is rated higher, and control and happiness lower, during the work-day compared to the

weekend day (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004; Schlotz, Hellhammer, Schulz, & Stone, 2004) possibly pointing to a link between regular physical activity, cortisol and work-day stress. Lower evening cortisol might therefore be a biological pathway that partly mediates the benefits of regular physical activity but there is currently only little evidence to support this notion. The largest prospective study of cortisol and cardiovascular mortality to date (Kumari et al., 2011) has shown that the slope of cortisol decline (flatter) but not the CAR or the mean diurnal output predicted an increased risk of cardiovascular mortality. Importantly, the flatter cortisol slope was driven by higher evening levels.

It is important to note that the non-significant associations observed for the other cortisol parameters, especially the CAR, warrant further investigation. It may be that the study was not sufficiently powered to find small associations between cortisol and physical activity, or that evening levels of cortisol are more important. Further investigation will benefit from the use of objective measures of physical activity over repeated days so that the association between the cortisol profile and daily level of physical activity can be quantified with greater precision.

Another significant aspect of cortisol homeostasis is the stress-induced cortisol increase in response to psychosocial demands that may occur in everyday life. This response is largely adaptive but if sustained can have negative consequences (Mcewen, 2008; Mcewen, 2004). Regular physical activity may also act by buffering the stress induced cortisol response, but no experimental study has so far examined this issue. Therefore, I decided to carry out a study in which physical activity levels will be experimentally manipulated so that its effect on cardiovascular, inflammatory and endocrine responses to standardized mental stress can be assessed and evaluated (see chapter four).

Strengths of this study include the use of a large sample with similar characteristics in terms of work environment, the assessment of cortisol over two days and the use of ecological momentary assessment of daily stress as well as depressive symptoms by questionnaire. Weaknesses include the use of self-report physical activity and the lack of objective checks for the saliva sampling protocol and time of waking. Indeed, the use of a self-reported physical activity measure might have introduced bias into the analysis because many people cannot accurately recall their activity levels, especially types of general every day activity that are not structured. It is also possible that the more physically active participants exercised during the sampling periods that might have impacted on the diurnal cortisol pattern. Therefore future studies in this area should incorporate objective assessments of physical activity.

In summary, this study has found that regular physical activity is associated with lower depressive symptoms and less daily level of self-rated stress. There was only partial support for an association between physical activity and HPA function. It is the first time that an association of physical activity with cortisol has been reported in an ambulatory study. Although the study has offered limited evidence linking regular physical activity with the HPA axis, it is important that these findings are replicated. Furthermore, it is necessary to investigate these associations in an experimental manner.

CHAPTER 4: PHYSICAL ACTIVITY WITHDRAWAL, MOOD AND AUTONOMIC RESPONSES

4.1 INTRODUCTION

Physical activity (PA) is associated with reduced mortality from coronary heart and vascular disease and also with better mental health (Heckman & McKelvie, 2008; Mora, Cook, Buring, Ridker, & Lee, 2007; Blomstrand, Bjorkelund, Ariai, Lissner, & Bengtsson, 2009; Deslandes et al., 2009). Lack of regular physical activity, by contrast, has been shown to predict the development of a myriad of modifiable cardiovascular risk factors and chronic disease. The autonomic nervous system (ANS) may be an important mediator of this association as it initiates a cascade of cardiovascular and inflammatory responses including acute phase proteins and immune functions which strive to maintain the organism's homeostasis. Acute and chronic psychosocial stress disturbs this homeostatic balance with deleterious long-term consequences (Mcewen, 1998). Regular PA may also act by buffering exaggerated and repeated stress-induced activation of inflammatory, cardiovascular and endocrine pathways that are implicated in several risk factors and health outcomes.

The mechanisms underlying this protective effect are not completely understood but it is thought that PA exerts positive effects on physical and mental health through shared biological pathways. Cross-sectional studies have generally shown that fitter individual have lower basal concentration of inflammatory cytokines (Petersen & Pedersen, 2006) and an improved hemodynamic profile (Hamer, 2006). Our

laboratory has shown that greater physical fitness is associated with lower stress-induced interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) responses, and greater heart rate variability (HRV) after adjusting for the effect of age, gender, body mass index (BMI), socio economic status (SES) and health behaviour (Hamer & Steptoe, 2007). Evidence from a meta-analysis of controlled laboratory studies has also demonstrated that an acute bout of exercise has a robust blunting effect on blood pressure reactivity to psychosocial stress (Hamer, Taylor, & Steptoe, 2006). These findings, although of a cross-sectional nature, suggest that PA modulates a wide range of autonomic responses including important inflammatory pathways.

Heightened responses to mental stress appear to have relevance for the development of a poor cardiovascular risk profile (Hamer, O'Donnell, Lahiri, & Steptoe, 2010). Larger inflammatory and haemostatic responses to stress have been related to heightened resting ambulatory blood pressure at three years follow-up after adjustment for age, sex, baseline blood pressure, BMI and smoking (Brydon & Steptoe, 2005). A meta-analysis of prospective studies (Chida & Steptoe, 2010) has shown small but significant positive associations of cardiovascular stress reactivity with incident hypertension and carotid intima-media thickness at follow up, suggesting that psychophysiological responses under controlled conditions have cardiac prognostic value in initially healthy subjects. Regular PA is also linked with improved psychological wellbeing (Hamer & Stamatakis, 2010; Rethorst, Wipfli, & Landers, 2009; Windle, Hughes, Linck, Russell, & Woods, 2010), and findings from exercise intervention trials suggest that structured exercise may be as effective a treatment as antidepressants in patients with major depression (Blumenthal et al., 2007).

This is noteworthy since depression has been associated with coronary heart disease (CHD), cardiac risk factors and poorer CHD prognosis (Knol et al., 2006; Nicholson, Kuper, & Hemingway, 2006). Indeed, a recent quantitative review of the effect of physical activity on depressive symptoms in individuals with a chronic condition (Herring, Puetz, O'Connor, & Dishman, 2012) showed that exercise training improves depression in this population by a mean effect size of Δ 0.3 (95% CI, 0.25-0.36).

At present, the relationship between physical activity, mood and stress-induced cardiovascular and inflammatory responses is poorly understood. In the previous chapter based on cross-sectional data, I reported an inverse association of habitual PA with depressive symptoms and daily stress, and partially with evening cortisol levels. The exercise intervention trial is the typical experimental design that has been used to examine the effect of physical activity on mood and autonomic responses to stress. However, exercise trials are costly and require a large amount of manpower in administering supervised exercise sessions. In addition, the effectiveness of the trial depends on several factors including type of exercise, intensity and bout duration, and individual responses to the intervention tend to vary greatly (Rethorst et al., 2009).

A novel method to experimentally investigate these associations is the Exercise Withdrawal Paradigm (EWP) in which fit and habitually active individuals abstain from structured exercise for a period of time. An early EWP randomized study of 40 habitual male found that two weeks withdrawal from running resulted in marked increases in fatigue, somatic symptoms and anxiety in the withdrawn compared to the running maintenance group (Morris, Steinberg, Sykes, & Salmon, 1990). A small study of habitual exercisers (Mondin et al., 1996) showed that mood disturbances

following exercise deprivation developed within 24 to 48 hours of stopping physical activity. However, that study was confounded by the effect of acute exercise since participants started exercise withdrawal shortly after their morning workout. Another small study reported increases in fatigue and pain and decreases in pain tolerance after one week exercise withdrawal in regular exercisers (Glass et al., 2004).

Interestingly, the participants that developed these symptoms were characterised by lower resting cortisol and slightly lower sympathetic activity suggesting a link between physiological functions and mood. The authors argued that a stressor like exercise withdrawal causes physiological changes resulting in the development of somatic symptoms. These early studies have shown that somatic symptoms, pain and fatigue reliably develop following one week or less of abstinence from habitual physical activity. However, these studies have limitations such as a small sample size or the use of a highly selected sample, the absence of objective recording of physical activity, limited assessment of psychological variable and the failure to control for acute exercise.

More recent work has sought to use the EWP as an experimental model of physical inactivity to investigate the link between exercise, mood and biological functions. For example, Berlin and colleagues (Berlin, Kop, & Deuster, 2006) have shown that negative mood and fatigue after exercise withdrawal may in part be explained by reductions in cardiovascular fitness pointing to a possible link between mood and autonomic activation. Further analysis found that negative mood induced by exercise withdrawal was related to a reduction in parasympathetic activity as measured by the ratio of high to low frequency heart rate variability (HRV) after controlling for age, gender, fitness level and weight (Weinstein, Deuster, & Kop,

2007). However, no significant changes in HRV were observed following exercise withdrawal in either group.

Two randomized studies have used objective physical activity measures to investigate whether mood changes and somatic symptoms that develop after two weeks of exercise withdrawal were associated with changes in immune markers and cardiovascular parameters. This idea developed from experimental work showing that an inflammatory stimulus resulted in transient but reliable mood disturbances in healthy individuals (Wright, Strike, Brydon, & Steptoe, 2005). It is therefore plausible to hypothesize that the marked mood changes and depressive symptoms that develop after exercise withdrawal may in part be mediated by changes in pro and anti-inflammatory activity.

The first study (Kop, Weinstein, Deuster, Whittaker, & Tracy, 2008) did not find an association of negative mood with inflammatory factors including IL-6, CRP and fibrinogen. In addition, resting levels of these markers did not change during exercise withdrawal compared to an exercise maintenance control group. The other study (Poole, Hamer, Wawrzyniak, & Steptoe, 2011) found lower resting plasma IL-6 levels after exercise withdrawal compared to exercise maintenance. This attenuation in IL-6 level was related to increases in negative mood induced by exercise withdrawal but no difference in the sympathetic/parasympathetic balance of the ANS was observed between experimental and control groups. The authors argued that the attenuation in muscle derived IL-6 levels may inhibit an anti-inflammatory activity and result in raised pro-inflammatory markers such as CRP and TNFα. However, resting levels of TNF-α did not change between groups which did not support this hypothesis. Therefore, at present there is no support for the hypotheses that pro-inflammatory processes following exercise abstinence are driving the mood disturbances.

The association between physical activity, health and inflammatory factors appears to be complex. During acute exercise, IL-6 released from the skeletal muscle prevents a sustained pro-inflammatory response mainly by inhibiting TNF-α IL-1β release and promoting IL-10 and IL-1ra anti-inflammatory proliferation (Pedersen & Febbraio, 2008; Pedersen, 2011). Since regular exercise is associated with lower systemic levels of inflammatory markers, this transient increase in cytokines and myokines is considered to be anti-inflammatory. Therefore, it is conceivable to speculate that following exercise withdrawal lower levels of IL-6 may indicate a switch towards a pro-inflammatory state. However, a small prospective study of middle aged, sedentary men found that IL-6 levels decreased significantly after a 12 week exercise intervention but returned to pre-exercise levels after two weeks of detraining (Thompson et al., 2010).

Another small study found that a week of exercise withdrawal in active, older men did not alter basal levels of CRP, IL-6 and TNF-α, but neither did a week of active exercise intervention in previously sedentary, older men (Lund, Hurst, Tyrrell, & Thompson, 2011). These inconsistencies are likely to be attributable to the different populations studied (i.e sedentary vs. active), the protocol adopted (withdrawal vs active exercise) and the time line duration of the intervention. In addition, the source of cytokine release may play an important part in accounting for the different results. Muscle derived IL-6 is likely to be anti-inflammatory and therefore decrease after exercise withdrawal whereas adipose tissue derived IL-6 is pro-inflammatory and may therefore increase following exercise withdrawal (Petersen & Pedersen, 2005). Unfortunately, it is difficult to investigate the source of cytokine release in humans without quite invasive techniques such as muscle biopsy. Nonetheless, the studies reviewed above have shown that the EWP is a robust model to experimentally

induce mood disturbances and investigate the links between PA, mood and physiological processes.

Another reason for the lack of significant differences in inflammatory markers or associations with mood using the EWP may be that in relatively young and healthy participants, and under un-stimulated conditions, these associations are difficult to detect. If the immune system is stimulated through acute mental stress for instance, an effect of physical activity withdrawal on autonomic or inflammatory responses may be detected. Indeed, evidence shows that acute mental stress activates a transcription factor (Bierhaus et al., 2003) resulting in pro-inflammatory and autonomic responses (Steptoe, Hamer, & Chida, 2007) that are reproducible over time (Hamer, Gibson, Vuononvirta, Williams, & Steptoe, 2006; von Kannel, Kudielka, Preckel, Hanebuth, & Fischer, 2006). Therefore, using an EWP it may be possible to experimentally examine whether changes in inflammatory markers or autonomic responses are present in response to acute mental stress and whether physical activity buffers exaggerated autonomic and inflammatory responses.

No study has so far investigated the effect of PA on stress-induced cardiovascular, inflammatory and endocrine responses using an EWP. This experimental design will help tease out the causal mechanisms involved in the previously reported cross-sectional associations of PA with autonomic responses to mental stress. In addition, since cross-sectional and self-report data on PA and mood are open to biases, this design will provide experimental evidence on the direction of the association between PA and mood observed in the previous chapter.

4.2 Hypotheses

The aim of this study is to experimentally reduce daily physical activity levels in order to examine the effect on mood and inflammatory, cardiovascular and neuroendocrine responses to standardized acute laboratory stress. The hypotheses of the study have been reported in chapter two but are repeated here:

1) Two weeks of forced PA withdrawal will result in significant negative mood changes and fatigue like symptoms compared to a control phase of two weeks PA maintenance.

a. Mood change will be associated with changes in objectively measured physical activity.

b. Negative mood following PA withdrawal will be associated with changes in basal peripheral concentration of the inflammatory cytokines IL-6 and CRP.

2) There will be differences in psychophysiological reactivity to, and recovery from, standardized mental stress within the PA withdrawal and PA maintenance phase.

a. Cardiovascular, inflammatory and endocrine reactivity to mental stress will be higher and recovery slower following the two weeks of PA withdrawal compared to the two weeks PA maintenance.

b. Subjective stress ratings will be higher, and stress task appraisal less favourable, following PA withdrawal compared to PA maintenance.

4.3 METHODS

4.3.1 Study Design: The Physical Activity Withdrawal & Stress Study

The Physical Activity Withdrawal and Stress Study (PAWS) is a randomized, cross-over, single blind, controlled intervention based on the EWP that has been described previously. The total duration of the intervention was four weeks. Study participants were required to attend the research laboratory at the beginning of the intervention to sign the informed consent form, undergo a fitness assessment (described later), complete a self-report questionnaire, receive an activity monitor, and finally be randomly allocated to the experimental condition (withdraw from all forms of PA and exercise) or control condition (maintain all forms of PA and exercise currently performed).

All participants switched (cross-over) group after having completed the first two weeks of the condition they were originally randomized to. Study participants attended the research laboratory a second time two weeks after randomisation, and a third time approximately four weeks after randomisation. In these two research visits, a validated psychophysiological stress test (outlined in the procedure section) was administered and stress-induced psychobiological responses measured. Figure 4.1 below gives an overview of the study design.

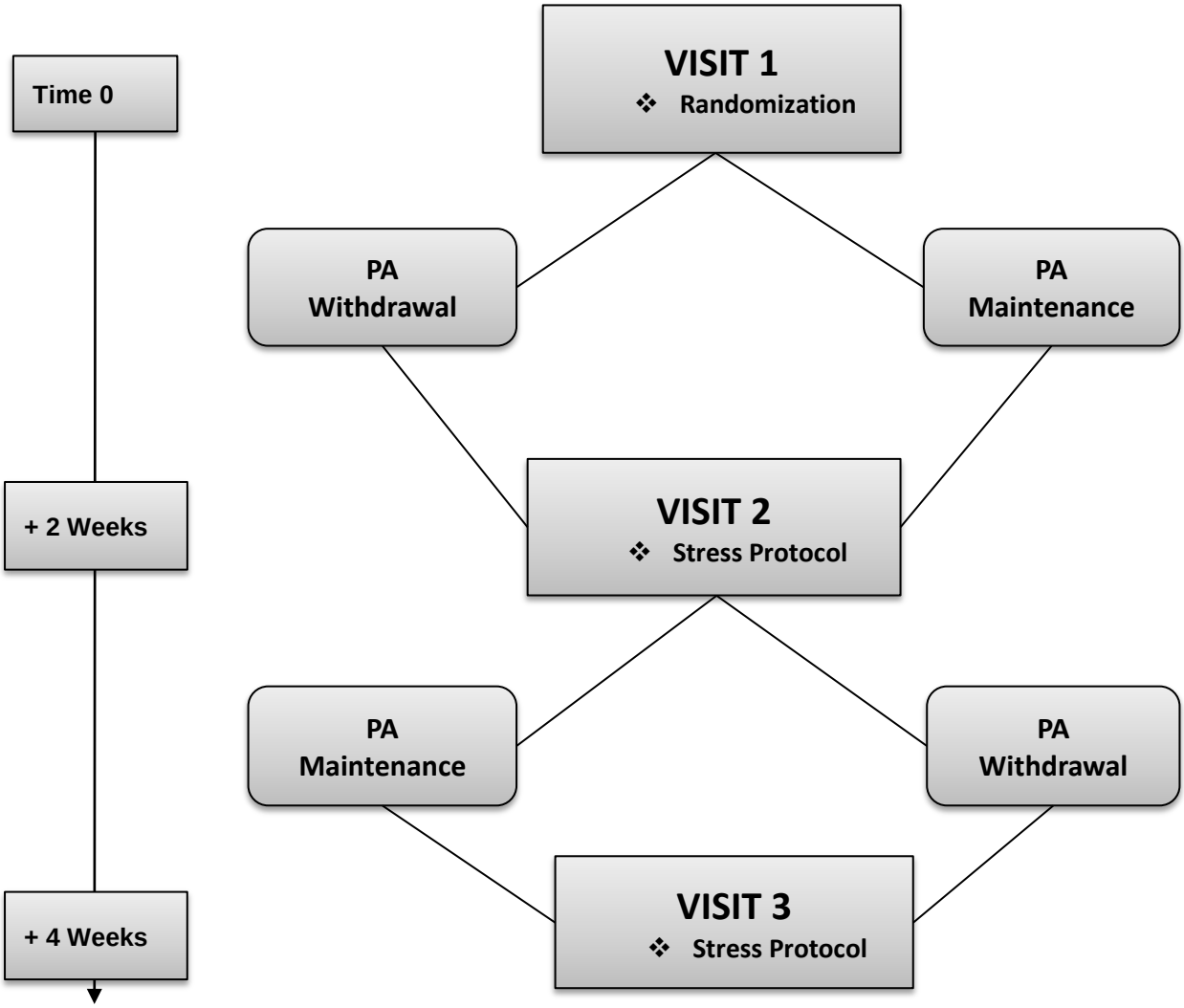


Fig-4.1The Physical Activity & Stress Study: Overview of Experimental Design.

4.3.2 Study Sample and Recruitment.

I recruited 51 fit, habitually active healthy men and women aged between 18 and 35 years from the student population at University College London (UCL), the University of London Union and various fitness centres in the UCL Bloomsbury catchment area. Volunteers were approached between February 2008 and

September 2009 through intercollegiate emails, posters in local fitness centres and the UCL campus and union, and the University of London Student Union.

Inclusion criteria were: Healthy individuals aged between 18 and 35 years, not on any regular medication (apart from the oral contraceptive pill), a BMI of between 19 and 25 kg/m^2, willing to wear an accelerometer for four weeks and to abstain from any form of PA that is of moderate, vigorous and very vigorous (MVPA) intensity for two weeks. Being physically active was defined as regularly engaging in vigorous physical activity at least three times a week for a minimum of one hour each session and having maintained such level of activity for at least six months.

Potential volunteers who expressed an interest in the trial received a standard email including the study information sheet (as shown in Appendix 1). Those individuals who met inclusion criteria and agreed to the study requirements were followed-up with a thorough telephone screening interview to ascertain suitability. Figure 4.2 below shows the recruitment selection process and response rate

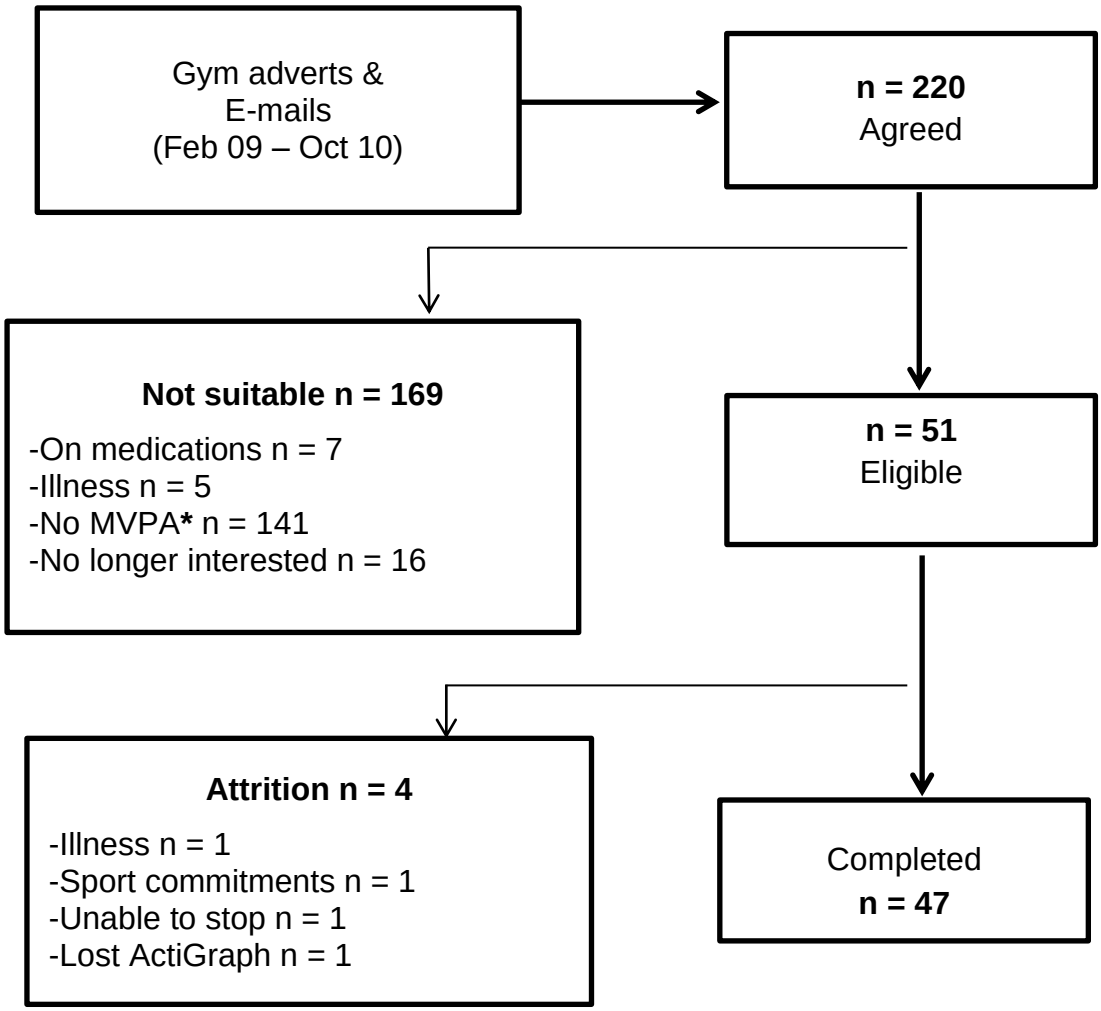


Fig-4.2 Consort diagram of participants' recruitment and response rate. * No MVPA = did not satisfy minimum physical activity level criteria.

4.3.3 Materials.

This section is divided into four parts:

I. Psychological, behavioural and fitness measures.

II. Anthropometrics measures and physical activity manipulation.

III. Laboratory stress protocol, cardiovascular and subjective measures.

IV. Blood and saliva protocol and outcomes.

I. Psychological, Behavioural and Fitness Measures.

At study entry (visit 1) between 09.00 AM and 12.00 PM all participants were required to review the information sheet, sign and date the informed consent form and retain a copy for their record. Participants then sat quietly on an armchair and completed a questionnaire made up of the following validated scales:

4.3.3.1 The International Physical Activity Questionnaire.

The International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003) is a validated measure of physical activity which measures three different levels of activity performed in the past week: walking, moderate, and vigorous intensity activity. Data are computed for metabolic equivalent minutes per week (MET-min) for each intensity level. A total physical activity (MET-min) score is also computed by adding up the three categories. Validation of the IPAQ and relative MET values were derived from work carried out by Ainsworth and colleagues (Ainsworth et al., 2000). Briefly, each type of activity is characterised by a correspondent MET value which is then multiplied by the frequency (days and minutes) with which such activity is performed. The MET values used in this study were published in the original IPAQ validation paper (Craig et al., 2003) and are as follow: Walking = 3.3 METs, Moderate activity = 4.0 METs, and Vigorous activity = 8.0 METs. It has been suggested that when the IPAQ is used in a very active population, the continuous score is to be preferred over the categorical score. In this study the continuous score system was therefore employed.

4.3.3.2 The General Health Questionnaire – 28 Item (GHQ-28).

The GHQ-28 (Goldberg & Hillier, 1979) is a widely used short form of the original 60 item scale (Goldberg D.& William P., 1988). It assesses the participant's current state of emotional distress as compared to his or her usual state and is therefore particularly sensitive to short-term changes in mood symptoms and psychological wellbeing. Four subscales derived by principal component analysis characterise the GHQ-28: somatic symptoms, anxiety/insomnia, social dysfunction, and depression.

Validation studies have shown that the GQH-28 has good sensitivity and specificity when compared to a clinical interview and performs as well as the longer form as a screening instrument (Goldberg D.& William P., 1988). The Likert scale continuous score has been suggested for the four subscales (Goldberg et al., 1997). Additionally, the subscales may be added up to produce a total GHQ psychological distress score which can then be used to identify caseness by means of validated cut-off values. Cronbach's-α coefficient in this sample were 0.78 at study entry, 0.9 at PA withdrawal, and 0.85 at PA maintenance.

4.3.3.3 The Perceived Stress Scale – 10 Item (PSS-10).

The PSS-10 (Cohen, Kamarck, & Mermelstein, 1983) is a brief instrument to assess stress or the generalised perception of stress. It measures the degree to which certain events or situations in one's life are perceived as stressful, that is, situations that are unpredictable and over which one has no or little control. The PSS builds on the person-environment model of stress appraisal in which potential stressful events are evaluated in relation to the individual's ability to cope (Lazarus &

Folkman S, 1984). The PSS provides a continuous score of perceived stress by adding up responses to each item but is not validated for categorical scores.

In this study the PSS-10 was used to monitor stress perception during the course of the study to ensure that any change in mood observed would not be driven by variation in naturally occurring stress. Data on validity are mainly from US where the PSS has been validated against other commonly used measures of psychological wellbeing, health behaviour, and life satisfaction (Cohen & Williamson, 1988). The PSS was found to have good internal reliability and was able to discriminate the appraisal of stress from other measure of psychological distress fairly well. In this sample Cronbach's-α was 0.82 at study entry, 0.85 at PA withdrawal and 0.83 at PA maintenance.

4.3.3.4 The Profile of Mood Scales – Short Form 36 Item (POMS-SF).

The POMS-SF (Shacham, 1983) is a measure of six dimensions of transient and distinct mood states. The subscales are: tension/anxiety, vigour/activity, depression/dejection, fatigue/inertia, confusion/bewilderment, and anger/hostility. A total negative mood score can also be computed by adding up the five negative mood subscales and subtracting the positive scale vigour/activity. The time reference used in the study is the past couple of days (prior to research sessions). The short form of the POMS improves from the original longer form in terms of better internal consistency and shorter completion time.

The validity of the scale has been established by showing high correlations between the POMS short form and the original POMS. More recently, factor analytic

work on the POMS-SF has confirmed the six factor structure of the scale and strong internal consistency as well as confirming convergent and discriminatory validity (Baker, Denniston, Zabora, Polland, & Dudley, 2002). In this sample Cronbach's-α was 0.79 at study entry, 0.84 at PA withdrawal, and 0.81 at PA maintenance.

4.3.3.5 Cardiorespiratory Fitness Assessment.

Participants completed a submaximal fitness test on a cycle ergometer bike (model 864, Monark, Varberg, Sweden) at study entry to estimate cardio respiratory fitness expressed as peak oxygen uptake (VO_{2peak} $ml*kg^{-1}*min^{-1}$). Prior to starting the test participants were fitted with a heart monitor and then rested for 20 minutes before two readings of blood pressure were taken using UA-779 digital blood pressure monitor (A&D Instrument LTD, Oxon, UK).

An initial four minutes warm-up on the bike was followed by a work load increment of one kg (55 watts) every four minutes to reach a maximum of 220 watts workload at the last four minute block (twelfth to sixteenth minute). Participants were required to keep a constant cycling speed of 60 revolutions per minute (RPM) throughout the duration of the test. Subjective appraisal ratings were also taken before every workload increment using the Borg perceived exertion rating scale (Borg, 1998) which is a reasonably good estimate of heart rate reactivity during strenuous exercise. All participants were expected to complete the test. However, when perceived exertion rating reached 18 or 19 (very hard to extremely hard exertion) the test ended and calculation of VO_{2peak} was adjusted accordingly. Out of 51 subjects

who underwent the test 47 completed the test at the sixteenth minute whereas four ended the test at the twelfth minute.

Baseline heart rate (beats-min, bpm) was calculated from the average of the last minute of the resting period before starting the cycling exercise. Maximum heart rate was defined as the average of the last sixty seconds of cycling. Maximum heart rate was then used was used to calculate VO_{2peak} value. A sex and work load adjusted nomogram for the calculation of maximal oxygen uptake from submaximal pulse rate has been used to estimate fitness (Astrand 1960). It is based on the linear association of heart rate with oxygen uptake (in Litres * min^{-1}) observed on the cycle ergometer (Astrand & Rodahl, 1986). Briefly, the participant's work rate expressed in watts is matched up with the average pulse rate during the last minute of cycling to give an oxygen uptake estimate (range 1.6 to 6.0 L*min^{-1}). This value is then multiplied by 100 and divided by the participant's body weight in kilograms to give an estimated VO_{2peak} value (ml /min/kg).

This measure varies greatly in the general population. Data from the Health Survey for England (HSE, 2008) has shown that, based on an in-house 8-min step test, the average cardiorespiratory fitness level of men is 36.3 ml/min/kg and 32 in women. A value of less than 33 ml/O_2/min/kg is considered unfit according to the HSE. In this study a more robust test was chosen due to the nature of the sample that consisted of young and highly active participants free from known chronic conditions.

II. Anthropometrics Measures and Physical Activity Manipulation.

4.3.3.6 Anthropometric Measures.

At each visit (study entry, 2 week, and 4 week) participants' height was measured by a research nurse or the principal investigator using a Stadiometer according to the standard protocol. Height was recorded to the nearest 0.1 centimetres. Body weight, fat mass, total body water and basic metabolic rate were assessed using a Tanita body composition analyser digital scale. Body mass index (BMI) was calculated as the ratio of weight in kilogram and the square of the height in meters (Kg/m^2).

4.3.3.7 Experimental Manipulation of Physical Activity (ActiGraph).

An ActiGraph® GT1M Monitor accelerometer device (Manufacturing Technology Inc. 2004) was employed in order to assess the level of compliance with the study protocol (i.e. PA withdrawal and maintenance). Participants wore the ActiGraph monitor around their waist during the PA maintenance and PA withdrawal phases of the study. The ActiGraph is a validated instrument widely used in physical activity research that records movement of the body using triaxial, solid state accelerometers to measure human motion against the force of gravity. It records the intensity and duration of physical activity in counts per minutes, as well as sedentary time.

The instrument has been validated to measure individual activity level, steps taken, energy expenditure and daily activity profile (Freedson, Melanson, & Sirard,

1998). The software used to analyse the raw data provided by the ActiGraph (MAHUffe) was developed by the MRC Epidemiology Unit at the University of Cambridge. The MAHUffe software processes data collected with ActiGraph instruments by processing raw data in predetermined intensity boundaries values to produce the following outcome variables: sedentary, light, moderate, vigorous and very vigorous activity. Average daily counts per minutes (CPM) and active wear time (RegTime) as well as a detailed hourly activity breakdown are also processed by the software.

The intensity of the cut-off values were chosen according to the population of interest and the activity of interest, but there is controversy about the optimal boundaries (Ward, Evenson, Vaughn, Rodgers, & Troiano, 2005). Generally, raw accelerometer counts may be translated into units such as MET minutes by employing suitable standardised equations. In this study the intensity boundaries for physical activity applied to the raw data were: (0-189 CPM) for sedentary behaviour; (190-572 CPM) for light activity; (573-2098 CPM) for moderate intensity activity; (2099-5898 CPM) for vigorous intensity activity and (5899 CPM) and above for very vigorous activity (Matthews, 2005). Participants were required to remove the monitor before showering or swimming and at bedtime. A minimum wear time of 10 hours/d was considered as valid. Any continuous 10 min periods of zero counts were considered as non-wear time.

The six outcome variables obtained provide a summary score expressed in average minutes per day and CPM/d per day of PA during the maintenance and withdrawal phases of the study. The number of days and the amount of time participants wore the ActiGraph was also analysed in the two exercise conditions

since some evidence suggest that there might be a behavioural effect of being monitored which may impact compliance (Costa, Cropley, Griffith, & Steptoe, 1999).

III. Laboratory Stress Protocol, Cardiovascular and Subjective Measures.

4.3.3.8 Mental Stress Tasks.

Two, 5-minute, standardised stress tasks which have been used previously by our group (Hamer & Steptoe, 2007; Brydon et al., 2008) and others were administered under time pressure in a random order in the two laboratory mental stress testing sessions. The tasks were a mirror tracing (MT) exercise and a simulated public speaking scenario (PS). In the MT task, participants were instructed to trace around the marked contour of a star with an electronic pen whilst looking at the star's own reflection in a mirror. The apparatus beeps and records an error every time the participant comes off the marked contour. Performance is judged by the number of times the drawing of the star is completed, as well as the number of mistakes made during the drawing. Participants were told that an average person usually completes the drawing of the star five times in five minutes with a minimum number of mistakes. Standard written instructions were given to participants before commencing the task, and up to one minute were normally allowed for practicing.

In the PS task participants were presented with a scenario in which they were either accused of stealing a purse in a large department store or threatened with redundancy at a company where they have been working for several years. Participants are allowed two minutes to prepare a speech and then three minutes to

talk without interruption in front of a video camera. General instructions for the stealing scenario required participants to pretend to be defending themselves in front of the store manager and the police. Instructions for the redundancy scenario instead required participants to argue their value and loyalty to the company's boss or face being made redundant. Participants were told that their performance on the speech task would be recorded and rated by three experts. The experimenter would be standing beside the camera during the speech and prompted the participant to continue in case they stopped talking before the end of the 5 minute period.

4.3.3.9 Cardiovascular Stress Responses.

During the stress sessions, heart rate (HR) and heart rate variability (HRV) were measured continuously using an ActiHeart monitoring device (Cambridge Neurotechnology, UK) attached to the participants' chest with ECG electrodes. The ActiHeart is a heart rate and movement monitor that is used in technical environments, and in resting or running conditions. Data on validity and reliability of the ActiHeart have been previously established and are published in (Brage, Brage, Franks, Ekelund, & Wareham, 2005). Autonomic nervous system activity can be investigated through changes in HRV which is regarded as an index of the relative influence of sympathetic and parasympathetic activity. The variable derived for HRV is the root mean square of the successive differences in interbeat intervals expressed in millisecond in the time domain index (rMSSD) (Task Force, 1996). The software used to analyse the raw data measured by the ActiHeart was the HRV

Analysis Software (Biomedical Signal Analysis Group, Dept. Of Applied Physics, University of Kuopio, Finland).

Before using the analysis software the ActiHeart data were cleaned by inspecting the distribution of IBI values and outliers were replaced by mean values. The time points analysed during the stress session were: Baseline (the average recording of the last five of thirty minutes resting time) stress task 1 (5 minutes mean), stress task 2 (5 minutes mean), post stress recovery 1 (mean of 15-20 minutes post-stress) and recovery 2 (mean of 40-45 minutes post-stress).

Blood pressure was monitored continuously during the two psychophysiology stress sessions. The device used was a Finometer Pro (Finapres Medical System, Amsterdam, The Netherlands) which records beat by beat pressure using a small finger cuff attached to the middle finger of the non-dominant arm. Although the Finometer does not collect direct measures of BP, the device provides measures of arterial pressure based on the volume clamp method and uses Modelflow modelling to derive hemodynamic parameters from pressure data, which have been previously validated using a range of stressors (Wesseling, 1996; Wesseling, Gizdulich, & Bos, 1996). The Finometer main unit is used to initiate calibration and to record participants' characteristics (age, sex, height and weight). A height sensor appropriately calibrated at each session accounts for hand movement and for differences in pressure between the finger and the arm.

The apparatus utilises the Beatscope® software to derive the appropriate variables. Systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MAP) and cardiac output (CO) were obtained from the Finometer. Total peripheral resistance (TPR) was derived with the formula (TPR = MAP/CO*80). Each time point was marked with an event button. For each laboratory session the time point

analysed were: Baseline (the average recording of the last five of thirty minutes resting time) stress task 1 (5 minutes mean), stress task 2 (5 minutes mean), post stress recovery 1 (mean of 15-20 minutes post-stress) and recovery 2 (mean of 40-45 minutes post-stress).

4.3.3.10 Subjective Stress Responses, Task Appraisal and Engagement.

Subjective ratings of stress and anxiety were taken at five time points during the stress testing sessions: baseline, task 1, task 2, recovery 1 (20 minutes post-stress) and recovery 2 (45 minutes post-stress). In addition, task appraisal ratings were taken at the end of each task. Task appraisal included the perceived level of difficulty, involvement and controllability. All subjective ratings were measured on a seven point Likert scale anchored at 0 (not at all) and 7 (very).

IV. Blood and Saliva Protocol and Outcomes.

4.3.3.11 Blood Sampling Protocol.

In each laboratory stress session blood was collected at the end of the baseline resting period and again at the end of the session (45 minutes post-stress). This sampling strategy was employed because previous work from our laboratory has suggested that inflammatory cytokines peak at 45 – 75 minutes following acute stress (Steptoe, Owen, Kunz-Ebrecht, & Mohamed-Ali, 2002). This time lag is

consistent with a hypothesis linking acute stress with de novo synthesis of cytokines. Blood was drawn from the antecubital vein of the forearm into EDTA coated vacutainers for plasma, and serum separator tubes containing a clotting gel for serum separation. The EDTA sample was immediately centrifuged at 1250rpm for ten minutes and the plasma obtained was transferred into appropriated microtubes in 0.5ml aliquottes and frozen at -80 C. The serum tube was left to clot for thirty minutes at room temperature before being spun. The supernatant was then removed and snap frozen in 0.5ml microtubes. All blood samples were taken by the PhD student after training at the University College Hospital phlebotomy ward.

4.3.3.12 Saliva Sampling Protocol.

Several saliva samples were obtained for the assessment of free cortisol as an index of the HPA-axis response to stress, and of 3-methoxy-4-hydroxyphenylglycol (MHPG) which is a metabolite of central catecholamine (noradrenaline) activity and therefore an index of sympathetic activation. Samples were collected during the two laboratory stress sessions at baseline, after the stress tasks, and at 20 and 45 minutes after stress. Samples were snap frozen at - 80°C and assayed in duplicate at the Kurume University in Japan using gas chromatography mass spectrometry (MHPG).

This method which is described in (Yajima, Tsuda, Yamada, & Tanaka, 2001) was found to have an intra-assay coefficient of variation of less than 4 per cent and to correlate highly with plasma levels of noradrenalin. This metabolite is therefore considered to be a valid marker of noradrenergic activity. Previous studies have also

shown robust increases in salivary MHPG in response to acute mental stressors (Hamer, Tanaka, Okamura, Tsuda, & Steptoe, 2007).

Salivary free cortisol was determined using a cortisol enzyme immunoassay kit by Salimetrics that has range of detection of 0.083 nmol/l to 83.01 nmol/l, and intra-and inter-assay CV of 3.6 and 5.4% respectively. Correlation of serum cortisol with salivary cortisol is r = 0.91 as reported by the manufacturer.

4.3.3.13 Inflammatory Markers.

The inflammatory cytokines Interlukin-6 (IL-6) and C - reactive protein (CRP) which are relevant in cardiovascular and metabolic disorders were examined. Peripheral concentrations of these markers were determined at baseline and in response to stress (IL-6 only). CRP was measured only at baseline since this marker indexes low-grade, systemic inflammation and may therefore be more sensitive to short term changes in physical activity. On the other hand IL-6 is released following acute stress as well as during PA, and therefore IL-6 is the primary stress-induced inflammatory marker in this study. Blood samples were analysed in duplicate using high-sensitivity Enzyme-Linked Immunosorbent Assay (ELISA) kits for quantitative determination of analytes in serum and plasma (R&D Systems, Oxford, UK). The limit of detection of the IL-6 assay is 0.016 pg/ml and the mean minimum detectable dose is 0.039 pg/ml. For CRP the limit of detection is 0.005 ng/ml and the mean minimum detectable dose is 0.010 ng/ml.

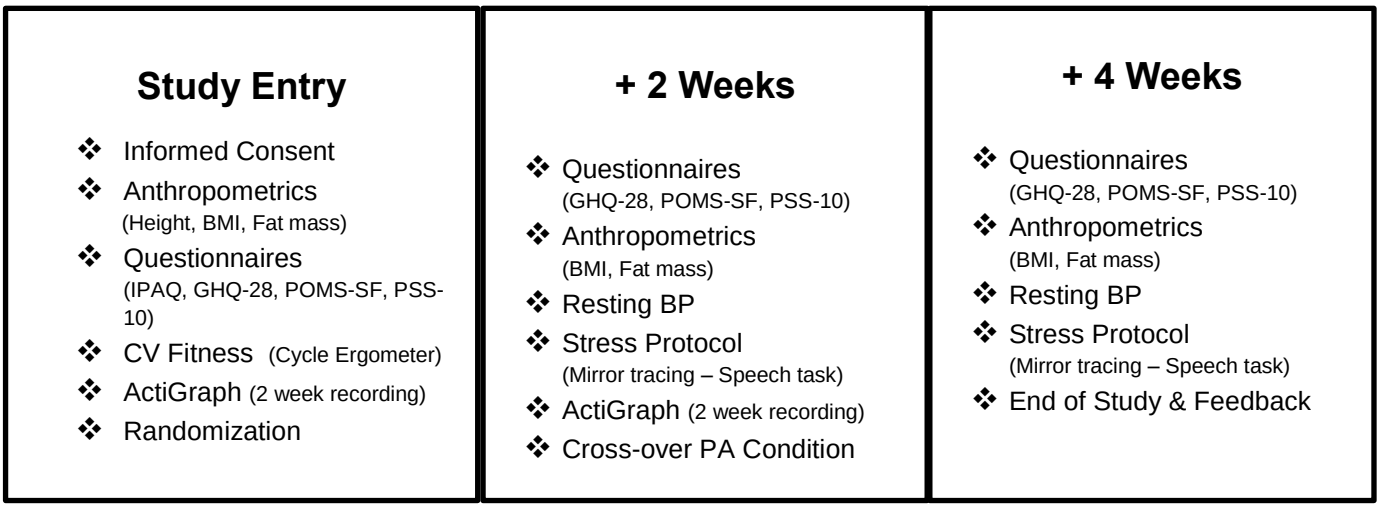
Participants with any sample that had a coefficient of variations (CV) of >10% were re-assayed in triplicate and the mean of the two readings with the lowest CV

was retained. By the end of the study each participant had 4 blood samples in total for IL-6 and 2 for CRP. The microplate reader used to determine the optical density of the ELISA plate was the Molecular Devices (UK) with software SoftMax® Pro 5 capable of reducing the data to a linear log/log parameter and therefore produce a standard curve and readings according to the ELISA kit protocol requirements. All samples were processed and assayed by the PhD student.

4.3.4 Procedure.

At study entry the researcher reviewed the study information sheet and the study protocol and answered participant's questions. The informed consent form was then signed and dated. All participants were seen between 09.00 AM and 12.00 PM. Figure 4.3 provides an overview of the study procedure adopted at each of the three research visits.

Fig-4.3 Summary of Study Procedure During each Research Visit.



The anthropometric measures were taken using standard protocols in the Psychobiology Group. The ActiHeart monitor was attached to the participant's chest, tested and initialised according to manufacturer's guidelines. Participants were then taken to the laboratory and rested for half a hour while completing the questionnaires. Resting blood pressure was measured twice using a manual electronic sphygmomanometer. Standard instructions were read out by the researcher and a few minutes were allowed for the participant to familiarize themselves with the cycle ergometer and testing protocol before formally starting the test. After the fitness test was completed, the researcher explained the main features of the activity monitor ActiGraph and emphasised the importance of wearing the device every day.

A third person blind to the study's hypotheses provided participants with a sealed envelope containing the random allocation to condition letter (see Appendix 2). The letter explained the study condition the participant was being allocated to (PA withdrawal or PA maintenance) with detailed instructions of the requirements of each condition. In order to maximise adherence, each participant received an email, on average a week into the study, containing a reminder of the study requirements. Participants also received standard instructions prior to coming to each of the two stress testing sessions reminding them not to exercise, drink caffeine and alcohol or take anti-inflammatory medications for a minimum of 24 hours before the scheduled visits.

At the two week follow-up research visit, the anthropometric measures were obtained according to standard protocol and participants returned the ActiGraph monitor containing the activity data of the previous two weeks. Psychophysiological testing was undertaken with the participant sitting comfortably on an armchair and

attached to the cardiovascular monitor devices. During the thirty minutes baseline resting time participants completed a short mood and stress questionnaire. At the end of the baseline rest period, blood and saliva samples, and subjective stress ratings were obtained according to protocol. The two five- minute stress tasks were administered in a random order with subjective stress and task appraisal rating obtained after each task. A saliva sample was also collected at the end of the tasks and participants were subsequently required to enter a 45 minutes recovery period.

The second blood sample was obtained at the forty-fifth minute which is when the stress protocol ended. A new initialised ActiGraph and letter of condition allocation containing instructions were then given to the participant by a research nurse blinded to the study design. The participant was required to do the opposite condition to which he or she had been originally randomized and return to the research laboratory for a final visit in two weeks time.

At the four week follow-up research visit the same stress testing protocol as in week two research visit was implemented. At the end of the session participants were given feedback on their fitness levels, resting blood pressure, BMI and the objective of the study. All participants were debriefed and given the opportunity to ask questions before being compensated with £60. Figure 4.4 below summarizes the stress protocol adopted and all the stress-induced outcome measures obtained.

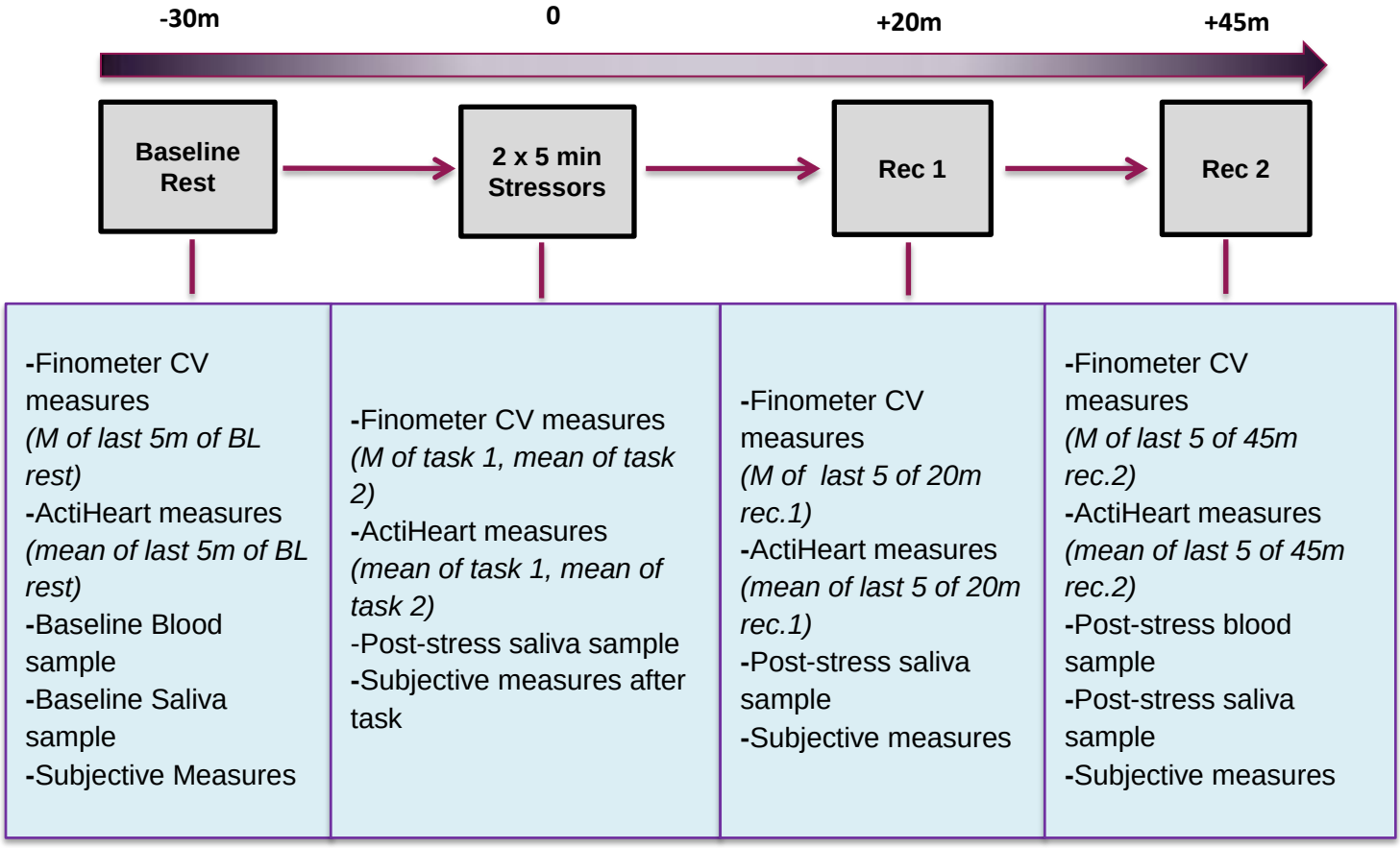


Fig-4.4 Summary of Stress Measure Recorded During the Psychophysiological Stress Protocol.

4.3.5 Statistical Approach.

The distribution of inflammatory markers, cortisol and MHPG data was inspected by examining the distribution and kurtosis/skewness values and where appropriate a log, or square root, transformation was applied to normalize the distribution. The data analyses strategy is as follow: Differences in objective physical activity data between PA conditions were used to check the experimental manipulation. This was achieved by computing a change score so that greater values indicate greater reduction in daily PA. Paired t-tests were used to test for the effect of PA withdrawal

on mood outcomes, and to explore any interaction term, while Pearsons correlation was used to explore the association of negative mood with objective PA data. Product-moment Pearson correlations were also used to investigate associations among baseline predictors.

The main analyses involving cardiovascular, cortisol and IL-6 responses to stress were carried out using mix analysis of variance (ANOVA) models with gender as the between-subject factors and time (baseline, stress and recovery) and PA condition (withdrawal and maintenance) as the within-subjects factor. The Greenhouse-Geisser correction for degree of freedom was applied when the assumption of sphericity was violated. Changes in the main outcome variables between the conditions were calculated by subtracting the PA withdrawal values from the PA maintenance values (absolute change) and by calculating a proportional change (relative change) making adjustment where appropriate.

Stress-induced reactivity scores were also computed as the mean difference between baseline and stress values while recovery scores were computed as the mean difference between baseline and recovery values. Secondary analyses were carried out using objective recording of physical activity data as an indicator of adherence to the study protocol in the first instance, and by using mood change following PA withdrawal as an index of the success of the intervention as a further manipulation check. Data are presented as mean (M) ± standard error of the mean (SEM) or standard deviation (SD) as appropriate.

Since the design of this study is randomized cross-over, half of the participants were assigned to each physical activity condition first. Therefore, there is a possibility, though unlikely, of an order effect (reactivity might be higher in the first stress session). In order to check for a possible order effect, a between-subject

factor was added to the analysis. This factor called “order” has two levels: PA withdrawal first then PA maintenance; PA maintenance first then PA withdrawal.

4.4 RESULTS

4.4.1 Sample Descriptive.

Table-4.1 Descriptive Characteristic of Study Sample.

Variable	Male M ± SD (range) n = 26	Female M ± SD (range) n = 21	p
Age y	23.78 ± 4.56 (18.75 – 34.75)	26.01 ± 4.79 (18.25 – 36.33)	0.11
BMI kg/m^2	22.88 ± 2.30 (19 – 27.3)	23.12 ± 2.42 (18.8 – 28.3)	0.74
Body mass kg	72.23 ± 6.75 (59.7 – 83.8)	64.07 ± 9.32 (46.6 – 78.5)	**0.001**
Sbp mmHg	115.07 ± 11.05 (98.0 – 131.0)	107.47 ± 9.15 (92.0 – 123.0)	**0.015**
Dbp mmMg	63.11 ± 7.16 (45.0 – 80.0)	67.24 ± 8.41 (50.0 – 83.0)	0.08
Hr Bpm	66.40 ± 10.40 (43.75 – 87.53)	67.27 ± 12.42 (48.88 – 93.84)	0.79
Hr (react) Bpm	162.74 ± 17.63 (132.23 – 194.65)	163.71 ± 12.25 (140.0 – 181.15)	0.83
Vo_{2peak} ml/kg/m	47.0 ± 9.42 (32.5 – 68.0)	52.0 ± 11.40 (39.0 – 82.0)	0.11
MET/min/w (*1000)	2.73 ± 1.97 (0.54 – 9.12)	3.21 ± 3.29 (0.96 – 15.84)	0.54
Perceived exertion	16.87 ± 1.95 (13.0 – 21.0)	16.9 ± 1.61 (13.0 – 20.0)	0.95

BMI =Body Mass Index, Sbp =Systolic Blood Pressure, Dbp =Diastolic Blood Pressure, Hr =Resting Heart Rate, Hr (react) = Maximum Heart Rate During Cycling Ergometer Test, MET =Metabolic Equivalent of Task in Minutes per Week.

The descriptive characteristics of the sample at study entry are summarised in table 4.1 above. Participants were 47 males and females who completed the study. As expected, males weighted more than females and had slightly higher resting systolic blood pressure. Female participants tended to have slightly higher resting

diastolic blood pressure but the difference only approached significance. The mean $VO_{2\ peak}$ values were above the general population mean (HSE, 2008) suggesting that the strict selection criteria had ensured the inclusion of mainly fit and active volunteers. As expected, VO_{2peak} correlated negatively with BMI ($r = -0.28$, $p = 0.06$), baseline heart rate ($r = -0.36$, $p = 0.01$), perceived exertion ($r = -0.35$, $p = 0.02$), and resting SBP though this did not reach significance ($r = -0.23$, $p = 0.11$).

Self-reported levels of physical activity in the week prior to study entry suggest that this sample exceeded the Governmental recommendation for the minimum amount of physical activity for health. Recommended amount of PA is that adults should be active at a moderate or vigorous intensity for at least 30 minutes five days a week (Chief Medical Officer, 2004) and therefore achieve between 450 – 750 MET/m/w. This is roughly equivalent to at least a two and a half hour of brisk waking per week. This sample achieved about 2900 MET/m/w in the week prior to study entry largely exceeding recommendations. This indicates that participant's CV fitness level and weekly amount of time spent doing PA of moderate and greater intensity is well above the population average values.

4.4.2 Experimental Manipulation.

Adherence to the experimental protocol is an important and crucial part of this study. Therefore, an accelerometer was used to monitor the degree of participants' compliance to the PA conditions. There was great variation in protocol adherence but overall results suggest marked differences in PA during the four week study protocol. One participant had missing data in the PA maintenance phase whilst one had missing data in the PA withdrawal phase. Firstly, I tested for a possible order

effect in recorded daily counts per minutes (CPM/d) in order to examine whether participants levels of PA was influenced by the order of condition allocation. As expected, there was no difference in mean CPM/d in the PA maintenance phase ($F_{1, 45} = 0.02$, $p = 0.88$) between order groups. Likewise, no differences were observed in CPM/d during PA withdrawal between order groups (F 1, 45 = 2.55, p = 0.12). Therefore, the order with which participants were allocated to the two PA conditions did not influence the level of recorded PA.

I then examined compliance with accelerometer wearing behaviour to check whether there were differences in the amount of time participants wore the ActiGraph during the study. In the PA withdrawal phase the mean ActiGraph wear time was 11.11 ± [SD] 3.17 days (range 2 – 15 days) with an average valid recording time of 774.67 ± [SD] 89.45 minutes/day. In the PA maintenance phase the mean ActiGraph wear time was 11.28 ± 2.95 days (range 4 – 16 days) with an average valid recording time of 797.30 ± 93.04 minutes/day. There was no statistically significant difference in the number of days ($p = 0.79$) the ActiGraph had been worn but there was a near significant difference in the amount of average daily wearing time indicating that participants wore the ActiGraph for 22 m/d (SEM = 11.97) longer during the PA maintenance condition compared to the PA withdrawal ($t_{44} = 1.85$; $p = 0.07$).

Next, I examined average recorded CPM/d as the primary indicator of protocol adherence and the data are illustrated in table 4.2. In the whole sample (n = 45) there was a significant difference (128.13 CPM/d [SEM] = 27.97; $t_{44} = 4.58$; $p = <0.001$) indicating that participants were more active during PA maintenance than PA withdrawal.

PA condition	n = 45	n = 41
PA Maintenance	516.22 ± 201.75	518.83 ± 205.12
PA Withdrawal	388.08 ± 162.50	385.01 ± 160.44

Table-4.2 Summary of ActiGraph Recorded Physical Activity. Values are counts per minute per day Means ± SD

When re-running the analyses excluding participants with less than six days of valid ActiGraph data in either condition (n = 4) this difference remained nearly unchanged (125.39 CPM/d [SEM] = 30.17; $t_{40} = 4.16$; p = <0.001). Overall, these data show that the manipulation of physical activity had been successful although there was clearly variation in the degree to which participants had decreased their daily activity (see Figure 4.5). Therefore, in light of this variation in compliance with study protocol, a secondary analysis will also be performed excluding participants whose daily CPM had not been reduced by at least 25 per cent compared to their PA maintenance period.

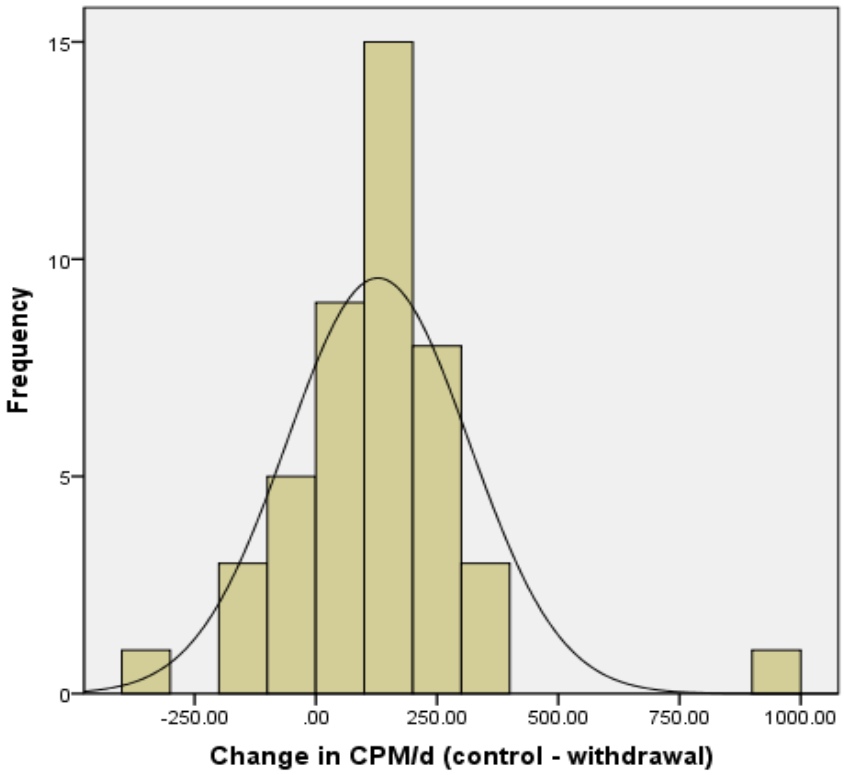


Fig.-4.5 Distribution of the accelerometer CPM/d reduction from PA maintenance to PA withdrawal in the physical activity and stress responses study n = 45. Positive values show a reduction in physical activity in the withdrawal phase, thus indicative of successful protocol compliance.

4.4.3 The Effect of PA Withdrawal on Mood Symptoms.

The effect of the intervention on mood symptoms is summarized in table 4.3. Mood in the two weeks before study entry was also measured in order to monitor pre-existing symptoms. One-way ANOVAs revealed that there was no gender difference in either the subscales or the total score in the GHQ (all $p \geq 0.8$), POMS (p range from 0.12 to 0.92) and PSS ($p = 0.82$) at study entry.

Table-4.3: The Effect of PA Withdrawal on Mood Symptoms n = 47.

Variable	Pre Intervention	PA Withdrawal	PA Maintenance	P*
GHQ Tot score	**15.83 ± 5.77**	**20.51 ± 9.38**	**14.19 ± 6.72**	<0.001
Anxiety/insomnia	4.42 ± 2.54	5.76 ± 4.07	3.70 ± 2.87	0.002
Depression	1.08 ± 2.10	1.00 ± 2.41	0.87 ± 2.25	0.77
Somatic symptoms	3.40 ± 2.18	5.85 ± 3.89	3.29 ± 2.23	<0.001
Social dysfunction	6.91 ± 1.99	7.89 ± 2.11	6.32 ± 1.72	<0.001
POMS Tot score	**5.70 ± 2.34**	**7.96 ± 3.08**	**5.23 ± 2.56**	<0.001
Tension/anxiety	6.51 ± 3.55	7.64 ± 4.33	5.55 ± 3.43	0.03
Vigour/activity	13.91 ± 3.58	8.02 ± 3.88	13.94 ± 4.08	<0.001
Depression/dejection	2.78 ± 3.68	3.68 ± 3.62	2.47 ± 3.07	0.03
Fatigue/inertia	5.43 ± 3.02	8.25 ± 5.14	5.02 ± 4.31	<0.001
Confusion/bewild.	5.94 ± 3.31	7.36 ± 4.15	4.95 ± 3.21	<0.001
Anger/hostility	3.47 ± 3.87	4.85 ± 4.36	3.29 ± 3.74	0.004
PSS Tot score	**12.76 ± 5.16**	**14.63 ± 6.08**	**12.25 ± 5.28**	0.01

**P values for difference between PA withdrawal and maintenance.*

Likewise, there was no gender effect in the PA maintenance phase for the GHQ (p range from 0.21 to 0.94) and PSS (p = 0.76) but female participants scored higher in the anger/hostility subscale of the POMS only (4.76 ± 4.60 vs. 2.11 ± 2.35, p = 0.01; all other p range from 0.21 to 0.60).

In the PA withdrawal phase the GHQ did not differ by gender (p range from 0.21 to 0.70) and neither did the PSS (p = 0.33) or the POMS (p range from 0.28 to 0.85).

Following the two weeks withdrawal from habitual PA participants experienced robust changes in mood and mood symptoms across all domains measured apart from the depression subscale of the GHQ. Results are as follows:

In the GHQ, the total psychological distress mean ± SEM within-subjects comparison score (PA withdrawal – PA maintenance) was 6.32 ± 1.46 ($t_{46} = 4.31$, $p = <0.001$). In the tension/anxiety subscale: 2.06 ± 0.61 ($t_{46} = 3.37$, $p = 0.002$); in the social dysfunction subscale: 1.57 ± 0.30 ($t_{46} = 5.18$, $p = <0.001$); in the depression subscale: 0.13 ± 0.43 ($t_{46} = 0.29$, $p = 0.77$); in the somatic symptoms 2.55 ± 0.60 ($t_{46} = 4.25$, $p = <0.001$).

In the POMS, the total negative mood mean ± SEM within-subjects comparison score (PA withdrawal – PA maintenance) was 2.73 ± 0.52 ($t_{46} = 5.23$, $p = <0.001$). In the tension/anxiety subscale: 2.08 ± 0.67 ($t_{46} = 3.11$, $p = 0.003$); in the vigour/activity: - 5.91 ± 0.79 ($t_{46} = - 7.40$, $p = <0.001$); in the depression/dejection subscale: 1.21 ± 0.54 ($t_{46} = 2.21$, $p = 0.03$); in the fatigue/inertia subscale: 3.23 ± 0.85 ($t_{46} = 3.82$, $p = <0.001$); in the confusion/bewilderment subscale: 2.40 ± 0.60 ($t_{46} = 3.99$, $p = <0.001$); in the anger/hostility subscale: 1.55 ± 0.51 ($t_{46} = 3.03$, $p = 0.004$).

In the PSS, the total perceived stress mean ± SEM within-subjects comparison score (PA withdrawal – PA maintenance) was 2.38 ± 0.93 ($t_{46} = 2.56$, $p = 0.01$).

There was no evidence of a group condition order effect in either the GHQ effect ($F_{1, 46} = 0.57$, $p = 0.45$), the POMS effect ($F_{1, 46} = 0.96$, $p = 0.33$), or the PSS ($F1, 46 = 0.19$, $p = 0.66$). This suggests that the PA condition with which participants started the study had no impact on the effect of PA withdrawal on mood symptoms.

The mood effect analyses were re-run excluding the 4 participants who did not have at least six days of valid ActiGraph data (n = 4) plus the 2 participants who had missing data on either PA condition due to equipment malfunction. However, the results remained virtually unchanged suggesting that participants for whom objective ActiGraph data were not available (equipment failure n = 2) or not of a sufficient length of time (less than seven days, n = 4) were unlikely to bias the study.

4.4.4 Mood Change and PA Withdrawal: Dose-Response.

In order to investigate whether these mood disturbances were related to objective or self-report PA data in a dose response manner, Pearson zero-order correlation analysis was applied. The negative mood change score was computed as the mean difference between GHQ total score during PA withdrawal minus PA maintenance (∆GHQ) so that a greater value indicated greater mood disturbance. The same method was used for the POMS total mood score (∆POMS). Changes in objectively recorded PA were computed as the mean difference in CPM/d between the two PA conditions (∆CPM/d) so that higher values indicate greater reduction in daily recorded PA.

Results indicated that the ∆GHQ did not correlate with self-reported MET-m/w at study entry ($r = -.03$, $p = 0.83$). Likewise, the ∆POMS did not correlate with MET-m/w at study entry ($r = -.15$, $p = .32$) suggesting that the degree of negative mood symptoms caused by PA withdrawal cannot be explained by background level of physical activity reported before the study had commenced.

There was a positive correlation between ΔPOMS and ΔCPM/d (r = .36, p = .02) indicating greater PA withdrawal induced negative mood in participants with greater reduction in daily CPM. No correlation was observed between ΔGHQ and ΔCPM/d (r = 0.16, p = 0.29). However, the positive association between the POMS and CPM/d became stronger (r = 0.42, p = 0.007) when the analysis was re-run with the exclusion of those participants without valid ActiGraph data due to either equipment failure (n = 2) or poor compliance (n = 4). The correlation is illustrated in figure 4.6 where it can be seen that higher mood disturbance is linearly associated with greater reduction in daily CPM. This suggests that changes in mood can in large part be accounted for by changes in objectively recorded PA, especially when participants with poor compliance with ActiGraph wearing behaviour are excluded.

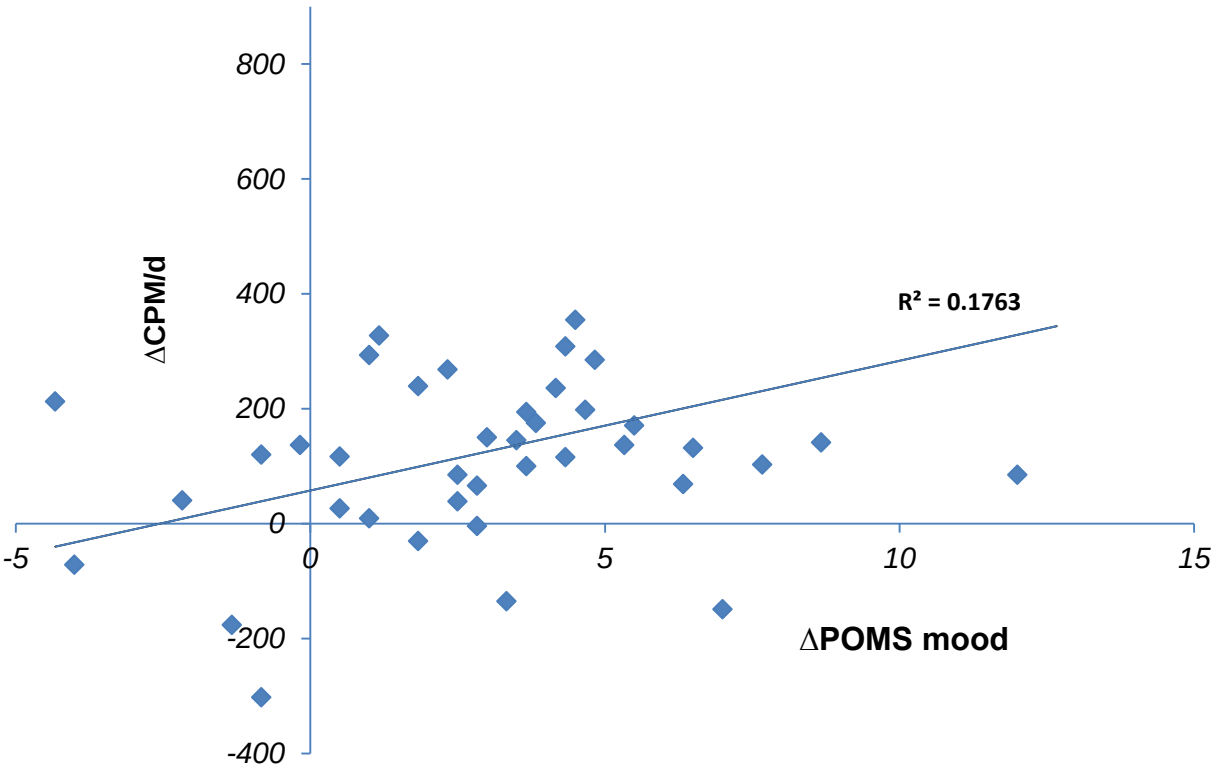


Fig-4.6 Scatterplot of the Association Between Negative Mood and Reduction in Objectively Recorded Physical Activity (n=41). Positive Values Represent Greater Reduction in Physical Activity (CPM/d) & Greater Negative Mood (POMS)

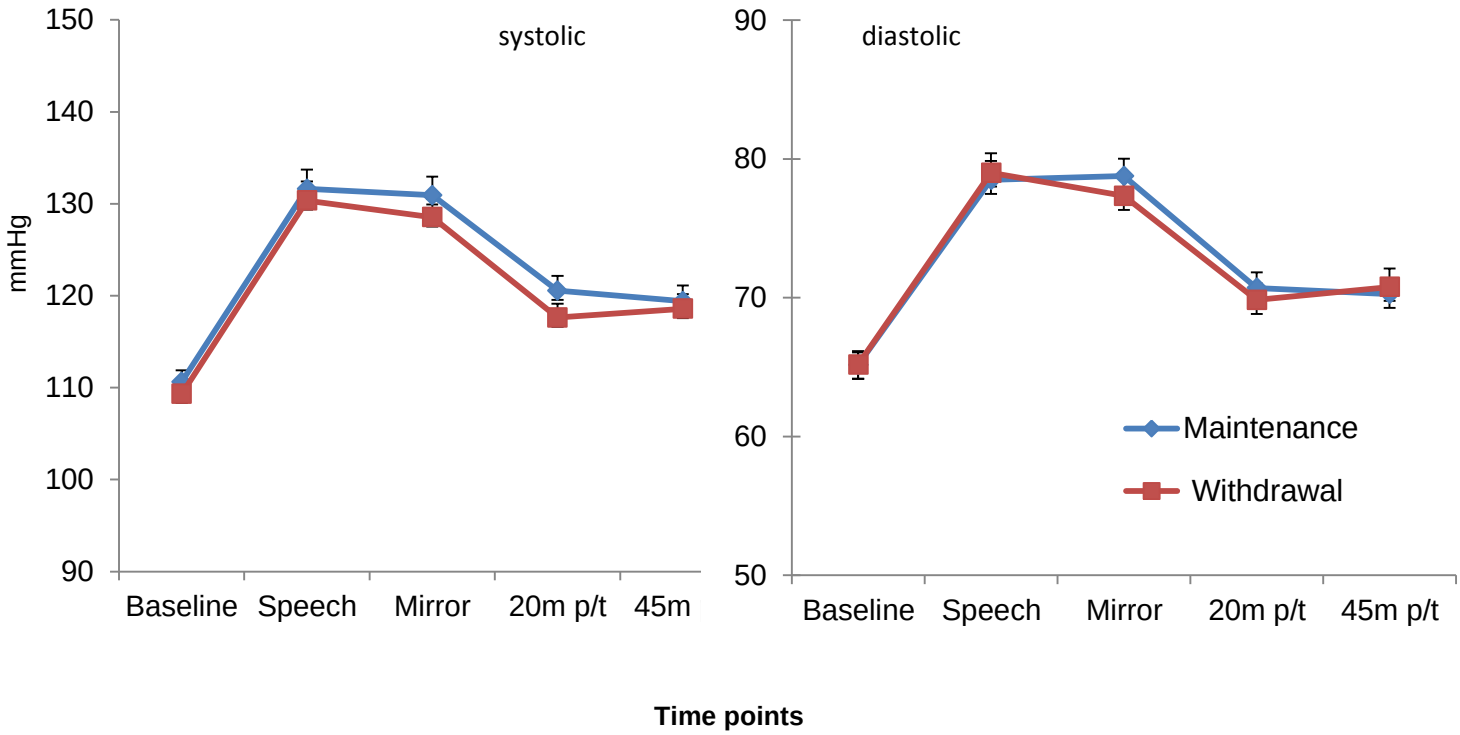
Participants' anthropometric measures were recorded at study entry, two and four week follow-up and in order to monitor changes in BMI as well as resting level of heart rate and blood pressure. As revealed by within-subject ANOVAs, BMI did not significantly change in the 4 weeks study period ($F_{2, 44} = 1.48$, $p = 0.24$) and neither did resting systolic blood pressure (SBP) ($F_{2, 43} = 1.11$, $p = 0.34$). However, there was a marginally significant increase in resting heart rate (HR) in the PA withdrawal condition compared to the PA maintenance (66.75 ± [SEM] 1.47 bpm vs. 64.32 ± 1.61 bpm, $p = 0.057$) even though the main effect of resting heart rate across the three repeated measures was not significant ($F_{2, 44} = 1.98$, $p = 0.15$).

The two mental stress tasks elicited significant increases in the cardiovascular (CV) parameters examined in both laboratory sessions. As an example, SBP increased during the task and returned towards baseline levels at 45 minutes post-stress (quadratic time effect: PA maintenance $F_{1, 42} = 122.64$, $p = <0.001$; PA withdrawal $F_{1, 42} = 124.87$, $p = <0.001$). As expected, SBP responses to the mirror task (MT) and the public speech task (PS) were highly correlated in both conditions (PA maintenance $r = 0.87$, $p = <0.001$; PA withdrawal $r = 0.72$, $p = <0.001$) indicating that the tasks elicited comparable blood pressure responses. I therefore averaged the CV responses to the MT and the PS in further analyses to create a single mean task response value.

The effect of PA withdrawal on stress-induced CV responses was analysed using mixed model ANCOVAs. In this model, the within-subject factors were PA conditions (withdrawal and maintenance) and time (baseline, mean stress, recovery 1 and recovery 2), and the between-subject factor was gender. Group order effect was entered as a covariate in order to check whether the PA condition to which participants were first randomized to had an effect on the CV responses. Two

participants had missing data due to equipment failure whereas two participants had data missing on at least one time point. Therefore, the sample size for blood pressure reactivity was 43. Figure 4.7 summarises SBP and DBP responses in the two stress testing sessions.

Fig-4.7 SBP and DBP responses to mental stress in the 2 PA conditions n = 43.



The assumption of sphericity was not met for SBP and Greenhouse-Geisser adjusted degrees of freedom are therefore presented. The main effect of time was significant ($F_{2.02, 78.71} = 51.37$, $p = <0.001$) indicating a change in SBP over time but there was no PA condition by time interaction ($F_{2.36, 92.36}, = 1.91$, $p = 0.15$). The gender effect was not significant ($F_{1, 39} = 2.05$, $p = 0.16$) and there was no evidence

of an order effect ($F_{1, 39} = 0.07$, $p = 0.78$). These results suggest that gender and condition allocation order effect did not influence the SBP responses but there were no differences in responses between the PA withdrawal and maintenance phases.

A similar pattern appeared for DBP. The main effect of time was significant ($F_{2.04, 77.65} = 59.87$, $p = <0.001$) indicating that SBP increased during stress returning closer to baseline values at 20 and 45 minutes post-stress in both stress sessions. However, as shown above for SBP, the PA condition by time interaction was not significant ($F_{2.53, 97.38} = 0.75$, $p = 0.50$) suggesting that PA withdrawal did not affect blood pressure responses to stress. Similarly, there was no main effect of gender ($F_{1. 38} = 2.33$, $p = 0.13$) nor order effect ($F_{1, 38} = 0.001$, $p = 0.97$).

Total peripheral resistance (TPR), or systemic vascular resistance, was calculated with the formula (mean arterial pressure/cardiac output)*(79.9) and is expressed in dyn.s/cm5. TPR responses during the MT and PS were highly correlated in both conditions ($r = 0.85$, $p = <0.001$) therefore a mean TPR task response value was calculated.

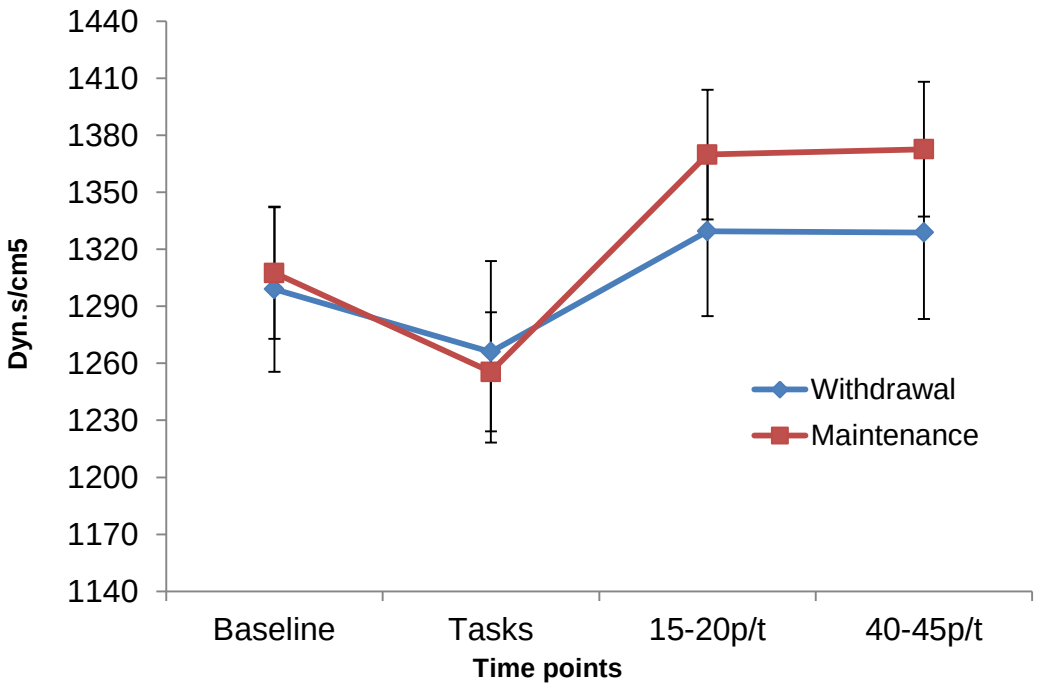


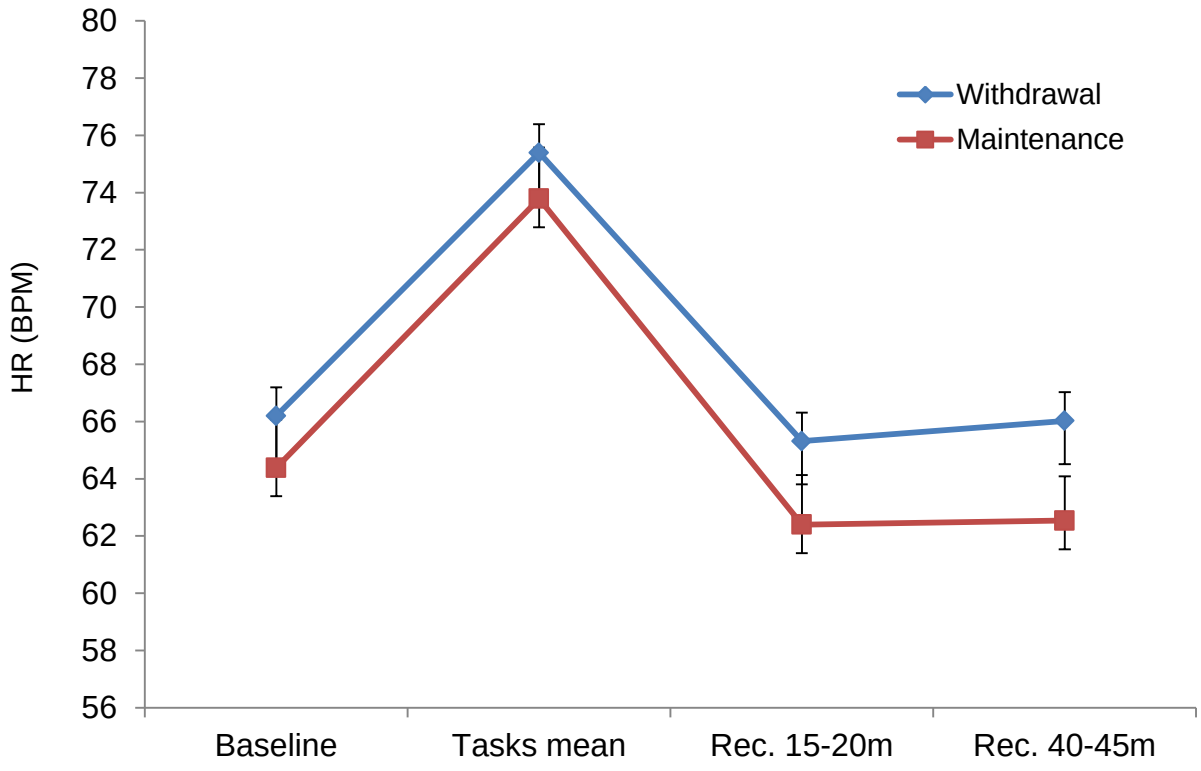
Fig-4.8 TPR mental stress reactivity and recovery in the two PA conditions.

The TPR response to stress is shown in figure 4.7. The mixed ANOVA showed a main effect of time ($F_{2.25,\ 87.97} = 12.38$, $p = <0.001$) indicating that TPR changed throughout both mental stress sessions but there was no main effect of PA condition ($F_{1,\ 39} = 0.40$, $p = 0.53$). The main effect of gender was also significant ($F_{1,\ 39} = 10.84$, $p = 0.002$) suggesting sex differences in TPR during the lab stress session but neither the time by gender ($p = 0.25$) nor the PA condition by time by gender ($p = 0.80$) interactions were significant. There was no significant PA condition by time interaction ($F_{3,\ 117} = 1.85$, $p = 0.14$) suggesting that stress-induced TPR responses were not affected by the PA withdrawal intervention.

HR and heart rate variability (HRV) were assessed using an Acitiheart as described in the Method section. HR responses to the MT and PS were highly

correlated in the two repeated stress sessions (r = 0.74, p = <0.001). Consequently, a mean task response value was computed by averaging the HR responses. As for the blood pressure analyses two participants were excluded due to the use of medications before the lab stress session and one participant had data missing due to equipment malfunction. Greenhouse-Geisser degree of freedoms correction is applied for the main effect of time only. The HR response to stress in the two stress testing sessions is depicted in figure 4.9.

Fig-4.9 Heart rate responses to mental stress in the 2 PA conditions testing n = 44.



The main effects of time ($F_{2.07, 89.32} = 51.47$, $p = <0.001$) was significant but the main effect of PA condition was not ($F_{1, 43} = 0.29$, $p = 0.59$). There was a significant PA condition by time interaction ($F_{3, 129} = 3.42$, $p = 0.01$). The gender effect was not significant ($F_{1, 43} = 0.74$, $p = 0.39$) and there was no evidence of a condition allocation order effect ($F_{1, 43} = 1.8$, $p = 0.18$). The interaction term was examined by computing a stress reactivity score (mean stress – baseline) and two stress recovery scores (recovery values – baseline). These values were then compared across PA conditions using within-subject t-tests. The HR reactivity did not differ across PA conditions ($t_{45} = - 0.24$, $p = 0.80$). When comparing recovery scores there were no differences in the 15-20m values ($t_{45} = 1.18$, $p = 0.24$) or in the 40 - 45m values ($t_{45} = 1.3$, $p = 0.19$). These results suggest that the lower HR values during stress observed in the PA maintenance phase can be largely accounted for by lower overall baseline values.

HRV stress reactivity and recovery data are expressed in milliseconds (mss). The distribution of HRV data was skewed and was therefore normalized with the square root method. HRV responses to the speech and the mirror task were only moderately correlated ($r = 0.42$) and were therefore analysed separately. Figure 4.10 illustrates the HRV responses in the PA withdrawal and PA maintenance stress testing session.

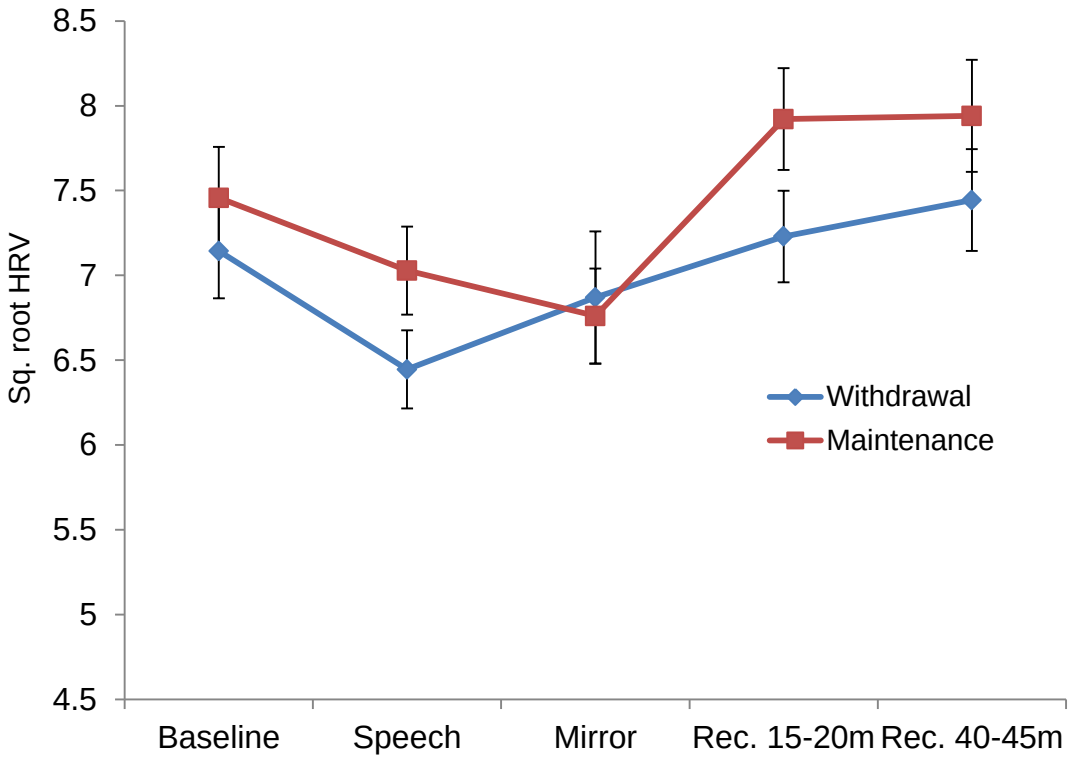


Fig-4.10 Heart rate variability responses to mental stress in the 2 testing sessions n = 42.

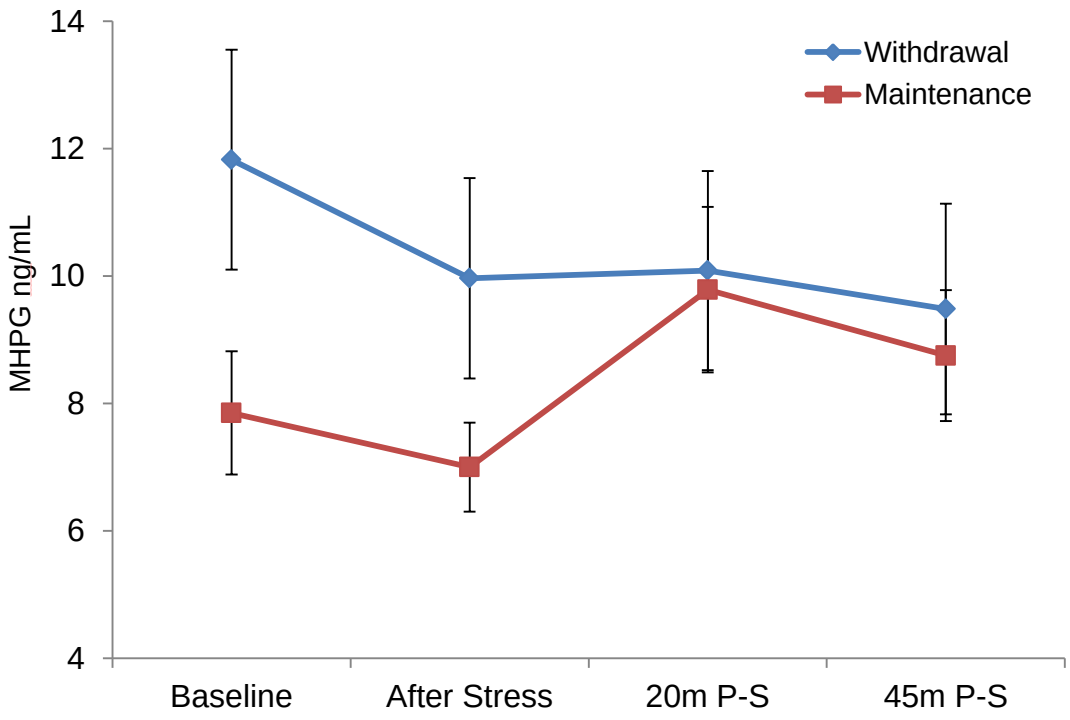
Mirror task: There was a main effect of time ($F_{1.7, 70.5} = 9.85$, $p = 0.001$) but not of PA condition ($F_{1, 40} = 2.81$, $p = 0.10$). The PA condition by time interaction was also not significant ($F_{2.03, 81.4} = 2.22$, $p = 0.11$). As expected, HRV was reduced during mental stress but increased in the recovery phases.

Speech task: The main effect of time was significant ($F_{3, 117} = 19.23$, $p = <0.001$) as was the main effect of PA condition ($F_{1, 39} = 6.59$, $p = 0.01$). However, the PA condition by time interaction was not statistically significant ($F_{3, 117} = 0.90$, $p = 0.44$). There was no main effect of gender ($F_{1, 37} = 0.002$, $p = 0.96$) or condition allocation order effect ($F_{1, 37} = 1.25$, $p = 0.96$) in the combined stress tasks. These results suggest that HRV responses to mental stress were not affected by PA withdrawal.

4.4.6 The Effect of PA Withdrawal on Stress-Induced MHPG Responses.

MHPG values were not normally distributed and were therefore logarithmically transformed but in the figure the raw data are presented. The MHPG response to stress pattern is illustrated in figure 4.11.

Fig-4.11 MHPG responses to mental stress in the 2 PA conditions (n = 43).



Two participants were excluded due to medication use and two participants had missing data because of insufficient sample for assaying. The result of the mixed ANOVA revealed that neither the main effect of time ($F_{3, 117} = 1.28$, $p = 0.28$) nor PA condition ($F_{1, 39} = 1.90$, $p = 0.18$) or the PA condition by time interaction ($F_{3, 117} = 0.84$, $p = 0.47$) were statistically significant suggesting that MHPG did not increase

significantly after stress in the two stress testing sessions. The main effect of gender was also not significant ($F_{1, 39} = 0.39$, $p = 0.53$).

MHPG AUC values were normalized with a square root transformation and compared between PA conditions using a paired t-test. PA withdrawal MHPG AUC was 24.182 ± [SEM] 1.40 whereas PA maintenance MHPG AUC was 22.68 ± [SEM] 1.07 but this difference did not reach significant levels ($t_{42} = 1.34$, $p = 0.18$).

4.4.7 The Effect of PA Withdrawal on Stress-Induced Cortisol Responses.

The cortisol response to stress in the two PA conditions was analysed in three ways. First, by using a two (exercise condition) by four (cortisol samples) mixed model ANOVA with gender as between-subjects factor and Greenhouse-Geisser degree of freedom correction where appropriate. Second, by calculating the cortisol area under the curve (AUC), which is an index of total cortisol release during the stress sessions, and comparing it using a within-subjects test. Finally, the absolute cortisol values immediately after stress, at 20 and 45 minutes post-stress were compared within the PA withdrawal and maintenance conditions using paired t-tests.

Two participants were excluded from these analyses because they reported to have taken medication known to interfere with cortisol activity leaving a final sample of 45 participants. The stress-induced cortisol reactivity in the two stress sessions is depicted in figure 4.12.

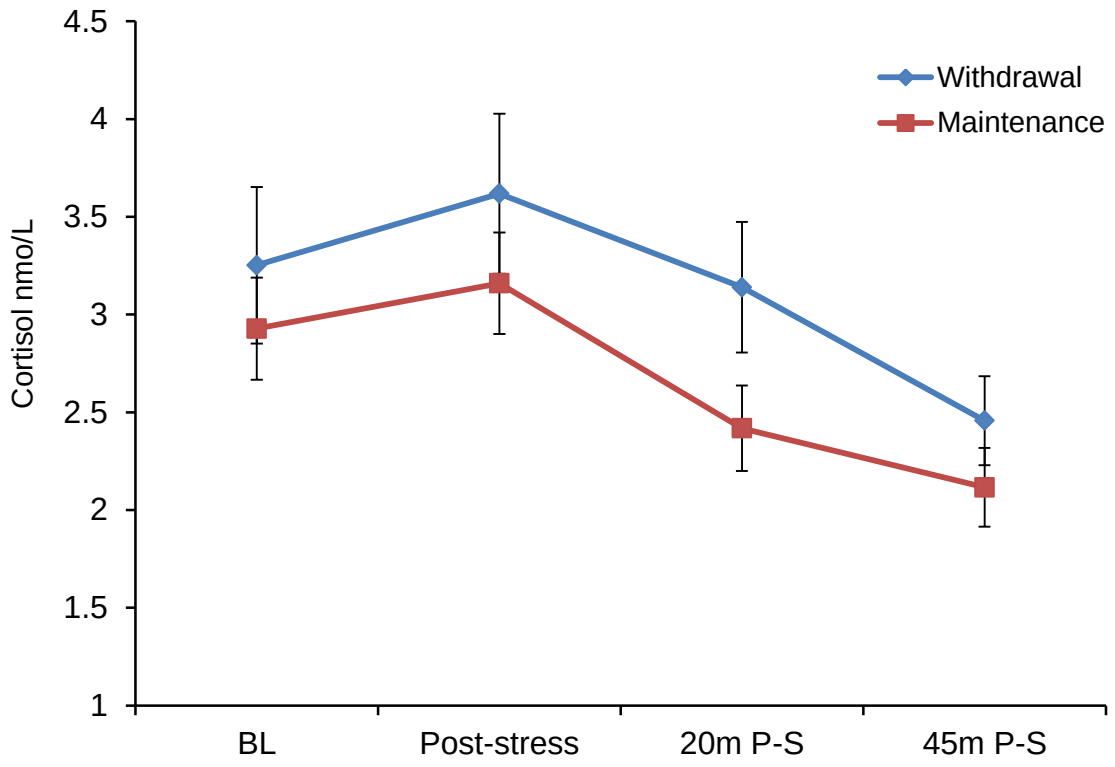


Fig-4.12 Salivary cortisol responses to mental stress in the 2 PA conditions (n = 45).

Overall, cortisol responses to stress in the two psychophysiology stress sessions were of small magnitude. The main effect of time was significant ($F_{2.45, 105.62} = 10.35$, $p = <0.001$) indicating that cortisol levels changed significantly in response to the tasks in the two stress sessions. There was no main effect of PA condition ($F_{1, 43} = 2.21$, $p = 0.14$) or PA condition by time interaction ($F_{3, 129} = 0.79$, $p = 0.49$) and no gender effect ($F_{1, 43} = 0.008$, $p = 0.93$).

Total cortisol released during the stress sessions (AUC) was examined using a paired t-test. Results indicated a higher AUC during PA withdrawal but this difference only approached statistical significance ($t_{44} = 1.58$, $p = 0.12$, M withdrawal 206.22 ± [SEM] 20.30 nmol/L/m vs. M maintenance 173.35 ± 12.86 nmol/L/m).

Stress-induced cortisol activity was analysed by performing paired t-tests at the immediately after tasks, the 20 and 45 minutes post-stress time points. No difference emerged immediately post-tasks ($t_{44} = 1.03$, $p = 0.30$) but cortisol level was higher at 20 minutes post-stress ($t_{44} = 2.0$, $p = 0.05$) during PA withdrawal (M withdrawal 3.14 ± [SEM] 0.33 vs. 2.42 nmol/L ± 0.22) compared to PA maintenance. This difference became not significant at 45 minutes into the post-stress recovery ($t_{44} = 1.58$, $p = 0.12$). These results show that a small difference in stress-induced cortisol can be detected 20 minutes post-tasks when cortisol released from the adrenal medulla in response to stress reaches a peak.

4.4.8 The Effect of PA Withdrawal on Basal and Stress-Induced Inflammatory Cytokines.

For the analyses of the cytokine IL-6 one participant was excluded due to anti-inflammatory medication use prior to the lab visit, one participant refused blood sampling and another participant was excluded for very high levels at both visits (<10 pg/ml), possibly indicative of an underlying illness such as an acute infection.

Baseline IL-6 at PA withdrawal averaged 0.84 ± [SD] 0.89 pg/mL (range = 0.23 – 5.37 pg/mL) and 0.87 ± 0.94 pg/mL post-stress (range = 0.16 – 5.60 pg/mL) but this increase was not significant ($t_{43} = 1.49$, $p = 0.14$). In the PA maintenance stress session, the baseline IL-6 was 0.81 ± [SD] 0.66 pg/mL (range = 0.17 – 3.05 pg/mL) and 0.83 ± 0.73 pg/mL post-stress (range = 0.17 – 3.37 pg/mL) but this increase was again not significant ($t_{43} = 0.63$, $p = 0.53$). Figure 4.13 shows the IL-6 stress reactivity data.

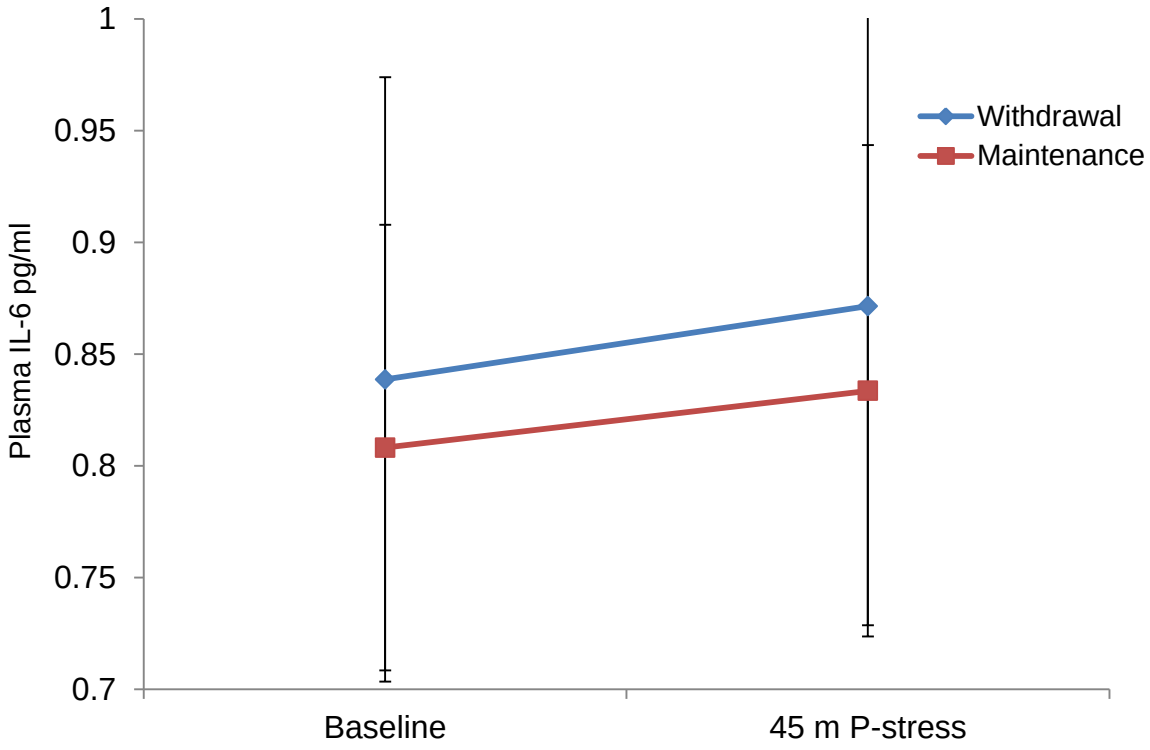


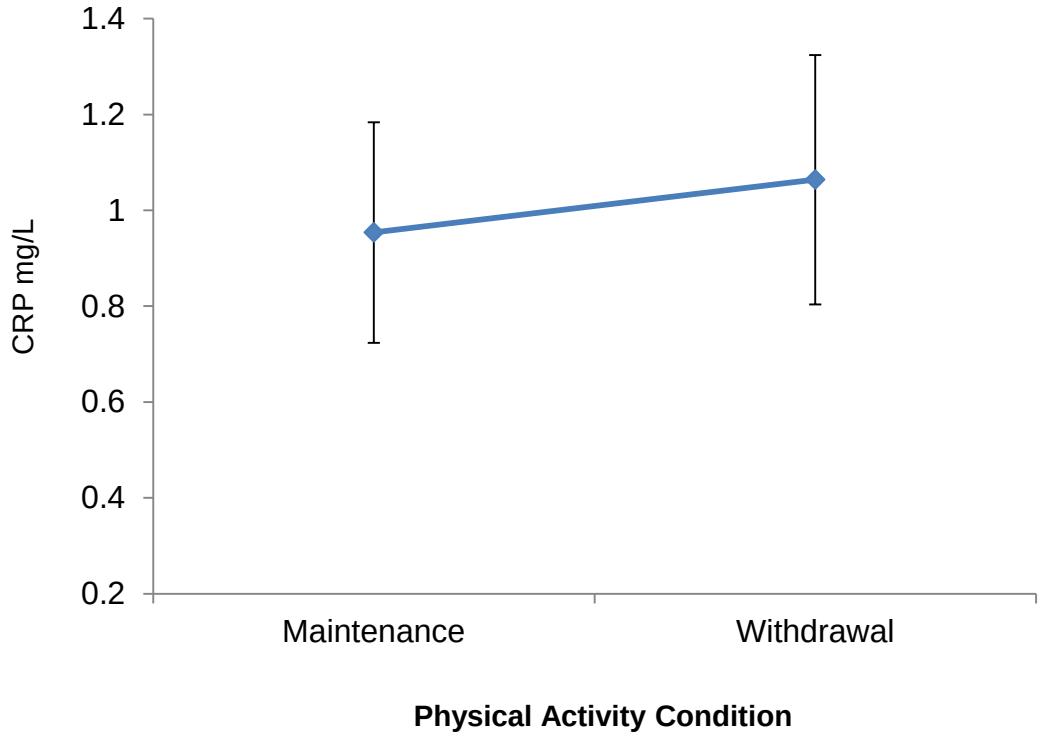
Fig-4.13 IL-6 responses to mental stress in the 2 PA conditions (n = 44).

Baseline IL-6 levels did not change significantly between conditions ($t_{44} = 0.20$, $p = 0.84$) suggesting that two weeks PA withdrawal did not alter basal IL-6 levels. Since there was great variability in plasma IL-6 in response to the stress task, a relative per cent change was calculated. During PA withdrawal participants had a 5.85% increase in IL-6 relative to baseline whereas during PA maintenance the increase was 1.94%. However, this difference in relative IL-6 stress reactivity was again not statistically significant ($t_{43} = 0.72$, $p = 0.47$).

Basal CRP levels were also assessed at the end of each of the two weeks PA condition in order to determine whether PA withdrawal had an effect on this stable

marker of systemic inflammation. CRP data were available for 43 participants and no value was above the 10 mg/L threshold that might indicate an underlying infection. Since the data were skewed a log transformation was applied but the data in Figure 4.14 are presented as untransformed mean ± SEM.

Fig-4.14 Basal CRP levels during the PA maintenance and withdrawal conditions (n = 43)



Mean CRP at PA maintenance was 0.95 ± [SD] 1.57 mg/L and 1.06 ± 1.71 mg/L at PA withdrawal. The mean baseline CRP per cent increase from PA maintenance to PA withdrawal was 11.5. A within-subjects t-test performed on the log transformed values showed that the increase in CRP following PA withdrawal

was not statistically significant ($t_{42} = 0.43$, $p = 0.67$). Therefore, neither systemic levels of CRP nor IL-6 were significantly affected by the PA withdrawal intervention.

4.4.9 The Effect of PA Withdrawal on Subjective Stress Measures.

Data on subjective stress measures were available for all participants. In order to assess whether an equal level of engagement was maintained in the two stress testing session, participants were asked to rate the degree of involvement in each task using a self-report scale ranging from 1 to 7. Results showed that participants had the same level of engagement when performing the speech scenario during PA withdrawal and PA maintenance testing (4.68 ± [SD] 0.21 vs. 4.62 ± [SD] 0.22; $t_{46} = 0.29$, $p = 0.77$), and when performing the mirror tracing task (5.81 ± 0.18 vs. 5.74 ± 0.19; $t_{46} = 0.38$, $p = 0.70$). Therefore, it can be concluded that participants maintained the same level of engagement in performing both stress tasks in the two stress testing sessions.

Subjective stress and relaxation during the testing sessions, perceived control over the tasks, and perceived difficulty of the tasks were assessed. These subjective responses to speech and mirror tracing were only moderately correlated and were therefore analysed separately. Figure 4.15 illustrates the stress-induced subjective responses in the two stress sessions.

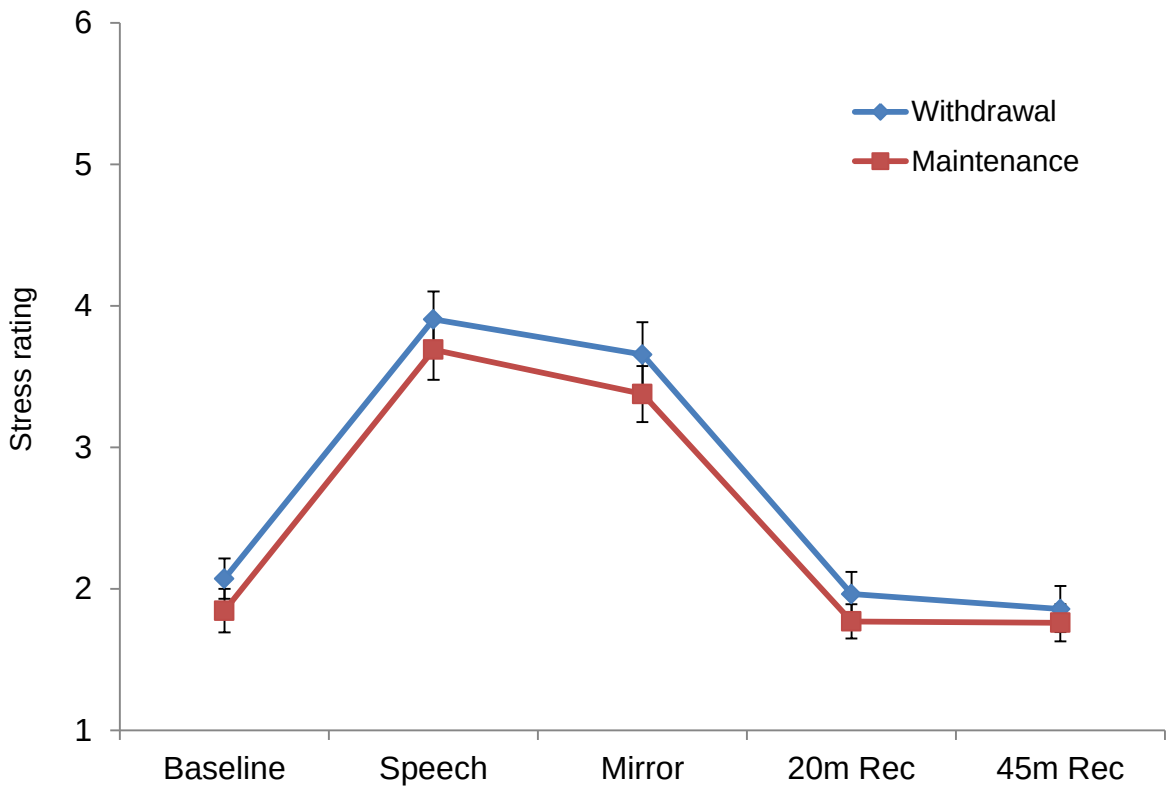


Fig-4.15 Subjective stress ratings in the 2 PA conditions n = 47.

There was a main effect of time ($F_{2.31,\ 103.89} = 51.5$, $p = <0.001$) indicating that subjective stress increased during the tasks and returned below baseline levels by the end of the session. The same was true for subjective relaxation ($F_{2.5,\ 112.47} = 94.48$, $p = <0.001$) in that participants' level of relaxation decreased during the tasks and returned above baseline levels by the end of the session. There was a main effect of PA condition for subjective stress ($F_{1,\ 45} = 4.43$, $p = 0.04$) but not for relaxation ($F_{1,\ 45} = 0.23$, $p = 0.63$), and neither stress by PA condition ($F_{4,\ 180} = 0.16$, $p = 0.96$) nor relaxation by PA condition interaction ($F_{4,\ 180} = 0.21$, $p = 0.93$).

Within-subjects comparisons revealed that participants rated the speech task and the mirror tracing task as slightly more difficult during PA withdrawal compared

to PA maintenance (4.36 ± [SEM] 0.22 vs. 3.89 ± [SEM] 0.22; $t_{46} = 1.76$, $p = 0.08$); (4.13 ± [SEM] 0.23 vs. 3.70 ± [SEM] 0.21; $t_{46} = 1.75$, $p = 0.08$). However, no difference emerged for perceived control over the speech task ($p = 0.74$) or the mirror task ($p = 0.41$) in the two PA conditions suggesting no effect of PA withdrawal on subjective feelings of control over the stress tasks. There was no gender effect in subjective stress ($F_{1, 45} = 1.81$, $p = 0.19$) or relaxation ($F_{1, 45} = 0.57$, $p = 0.45$).

4.4.10 Associations Between Changes in MHPG and Stress-Induced Parameters.

Correlation analysis was used to examine whether changes in basal levels of MHPG after PA withdrawal correlated with changes in mood and reduction in CPM/d as well as stress-induced markers. A baseline MHPG change score (∆bl-MHPG) was computed as the mean difference between baseline values. In this way a high score represents greater changes in basal MHPG following PA withdrawal which is indicative of a heightened sympathetic drive. ∆bl-MHPG values were not normally distributed so Spearman rho correlation analysis was used.

There was no association of ∆bl-MHPG with reduction in ActiGraph recorded CPM/d (rho = 0.08, $p = 0.57$) but negative mood following PA withdrawal measured with the GHQ-28 showed a marginally significant association with ∆bl-MHPG (rho = 0.25, $p = 0.09$) suggesting that individuals with greater mood disturbance tended to have a slightly higher level of sympathetic activation as indexed by the noradrenaline metabolite MHPH.

In order to examine associations between stress-induced CV and inflammatory markers and MHPG AUC during PA withdrawal stress testing, Pearson's zero-order correlations were used. MHPG AUC correlated with IL-6 reactivity ($r = 0.31$, $p = 0.04$), DBP reactivity ($r = 0.36$, $p = 0.02$) and marginally with TPR reactivity ($r = 0.26$, $p = 0.09$) but not with any of the stress-induced cortisol indexes. These correlations suggest a link between total sympathetic activation during stress and CV and inflammatory reactivity.

4.4.11 Secondary Analyses.

In order to investigate whether variation in the degree of adherence to the experimental protocol had an effect on the primary outcomes of interest, subsidiary analyses were carried out in two ways. First, I excluded participants whose daily levels of objectively measured PA was not reduced by at least 25 per cent based on data from the accelerometer and expressed in average daily counts per minutes (CPM/d). This was achieved by calculating a proportional CPM/d change within the PA conditions so that lower negative values represent greater reduction in daily activity levels. As evidenced by the distribution of these score there was great variation in adherence to the experimental protocol (see Figure 4.5). The median per cent reduction in CPM was - 37.35% (25^{th} and 75^{th} percentiles - 59.68% - 9.11% respectively). The choice of this cut-off threshold was based on the distribution of the CPM data alone since there is no consensus in the literature on what constitutes a significant reduction.

Second, the mood change following PA withdrawal was used as an index of the effectiveness of the intervention and is based on previous findings that withdrawal from habitual exercise or regular PA is associated with the development of mood disturbance in healthy participants after one or more weeks (reviewed in the Introduction). This mood change score was used as a continuous predictor variable in several regression models. Mood change and CPM/d reduction may be considered two independent methods of the effectiveness of the intervention as they correlate only moderately well with each other. For example, as reported above the correlation between mood change measured with the POMS and reduction in CPM/d was 0.36 and with the GHQ was 0.16 (n.s.). In addition, excluding participants only on the basis of ActiGraph use may result in the exclusion of genuine participants who might have followed the instructions but forgot to wear the device. Likewise, participants might be unfairly excluded because certain activities such as swimming or cycling are not easily recorded by the ActiGraph.

4.4.12 Secondary Analyses: CPM Reduction.

In this analysis 18 participants were excluded (16 with less than a 25% reduction in CPM and 2 who did not have valid accelerometer data in either condition). The main effect of time for SBP and DBP was still significant but the PA condition by time interaction was not significant, as was also found in the previous main analysis.

The main effect of time for TPR was still significant but gender became only marginally significant ($p = 0.08$) whereas the main effect of PA condition stayed non-significant and the PA condition by time interaction did not change either ($p = 0.13$).

The main effect of PA condition became significant ($F_{1, 27} = 6.82$, $p = 0.01$) for HR responses but the PA condition by time interaction only approached statistical significance ($p = 0.09$). Paired t-test on the reactivity and recovery scores adjusted for the baseline did not show any significant difference as in the main analysis.

The main effect of time for HRV stayed significant but the main effect of PA condition became not significant. The Within-subjects comparison showed the same patterns of reduced HRV in response to the speech task but again not statistically significant.

Secondary analyses for stress-induced IL-6 responses showed a near significant PA condition by time interaction ($F_{1, 27} = 3.73$, $p = 0.06$) but again no main effect of time suggesting a small difference in the IL-6 response. This difference was explored using a within-subject test comparing the IL-6 reactivity score in the two PA conditions. Results showed a near significant greater IL-6 reactivity during PA withdrawal compared to PA maintenance (0.062 ± [SEM] 0.03 pg/mL vs. - 0.028 ± 0.04 pg/mL, $t_{27} = 1.93$, $p = 0.064$). These data suggest that when excluding participants with poor adherence to the study protocol, greater IL-6 responses to stress begin to emerge in the predicted direction. The difference in resting CRP levels remained statistically not significant (1.32 ± [SEM] 0.41 mg/l vs. 1.11 ± 0.37 mg/L, $t_{26} = 0.55$, $p = 0.58$ in the PA withdrawal and PA maintenance respectively) suggesting that even though basal CRP levels were slightly elevated following PA withdrawal it cannot be attributable to exercise withdrawal.

Cortisol changes were compared within the two PA conditions. As in the previous analyses, only the main effect of time was significant. Within-subjects comparisons showed that the higher cortisol concentration following PA withdrawal previously observed at 20 minutes post stress became non-significant ($p = 0.13$)

although it became nearly significant at 45 minutes ($t_{28} = 1.76$, $p = 0.09$). The total cortisol output during the stress session (AUC) stayed non-significant. There were no significant changes in the MHPH stress responses.

Reduction in CPM/d used as a continuous variable did not correlate with any stress-induced cardiovascular or inflammatory reactivity measures.

4.4.13 Secondary Analyses: Mood Change.

In these analyses two participants were excluded for use of medications before the stress session. Since the main findings illustrated above seems to suggest a weak effect of PA withdrawal on IL-6, cortisol and HR responses, a stress reactivity score was again calculated for these variables in the two testing sessions. Stress reactivity was operationalized as the mean difference between average stress values and baseline values (greater scores = greater reactivity).

Two steps linear regression models were run with the stress reactivity values during PA withdrawal as the dependent variable outcome and the equivalent reactivity value during PA maintenance as predictor at step one. The change in mood was therefore the predictor added at step two of the model. In this way it is possible to quantify the association of negative mood with stress-induced reactivity during withdrawal testing which is independent of the maintenance (control) testing.

Negative mood change was operationalized as the mean difference score between the total GHQ psychological distress during PA withdrawal and PA maintenance. The mean GHQ score difference was 6.6 ± [SEM] 1.52 (95%CI 3.54 to 9.66).

Results are presented (Table 4.3) as coefficients (B) and 95% confidence intervals (CI), and the amount of variance explained by PA withdrawal induced negative mood over and above the variance explained by stress reactivity during PA maintenance testing.

Table 4.4 ΔGHQ following PA withdrawal as predictor of stress-induced indexes n = 45.

Outcome	B	95% CI	ΔR^2
IL-6 reactivity	0.006	0.001 – 0.011*	0.10
HR reactivity	0.19	0.02 – 0.36*	0.08
Cortisol AUC	-4.83	-10.25 – 0.58 **	0.06

Outcomes are adjusted for the corresponding reactivity at PA maintenance stress testing.
**p = 0.03; **p=0.07*

Table 4.3 shows that controlling for PA maintenance IL-6 reactivity, PA withdrawal IL-6 reactivity was positively associated with greater mood changes (B = 0.006, 95% CI = 0.001 – 0.011, p = 0.03), accounting for about 10 per cent of the variance. This association is illustrated in figure 4.16 where the ΔGHQ has been computed into tertiles of negative mood and plotted against the stress-induced IL-6 reactivity.

PA withdrawal HR reactivity to stress was also positively associated with negative mood after adjusting for PA maintenance HR reactivity, accounting for 8 per cent of the variance (B = 0.19, 95% CI = 0.02 – 0.36, p = 0.03). This association is illustrated in figure 4.17.

Participants with greater mood changes had a near significant attenuated cortisol output during the PA withdrawal stress session controlling for cortisol AUC during their PA maintenance session. There were no significant associations of mood change with systolic or diastolic blood pressure, systemic vascular resistance, HRV and MHPG, or with stress recovery values.

Fig-4.16 Association of Negative Mood with Stress-Induced IL-6 Responses.

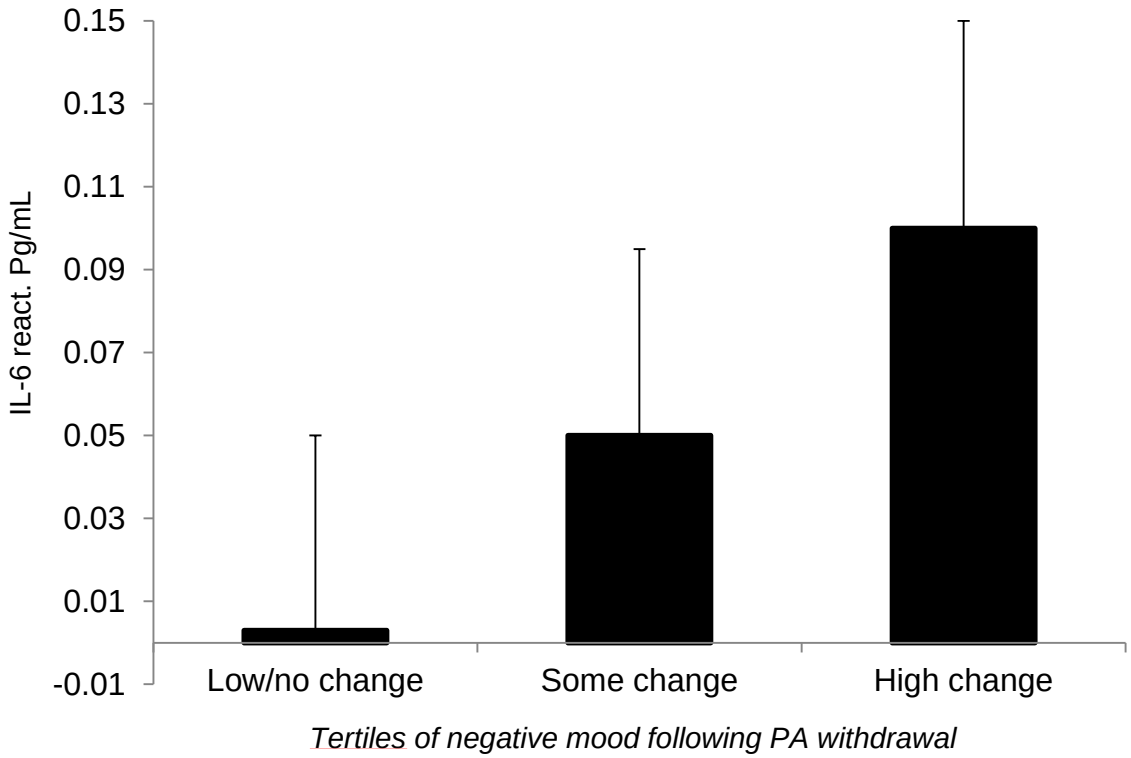
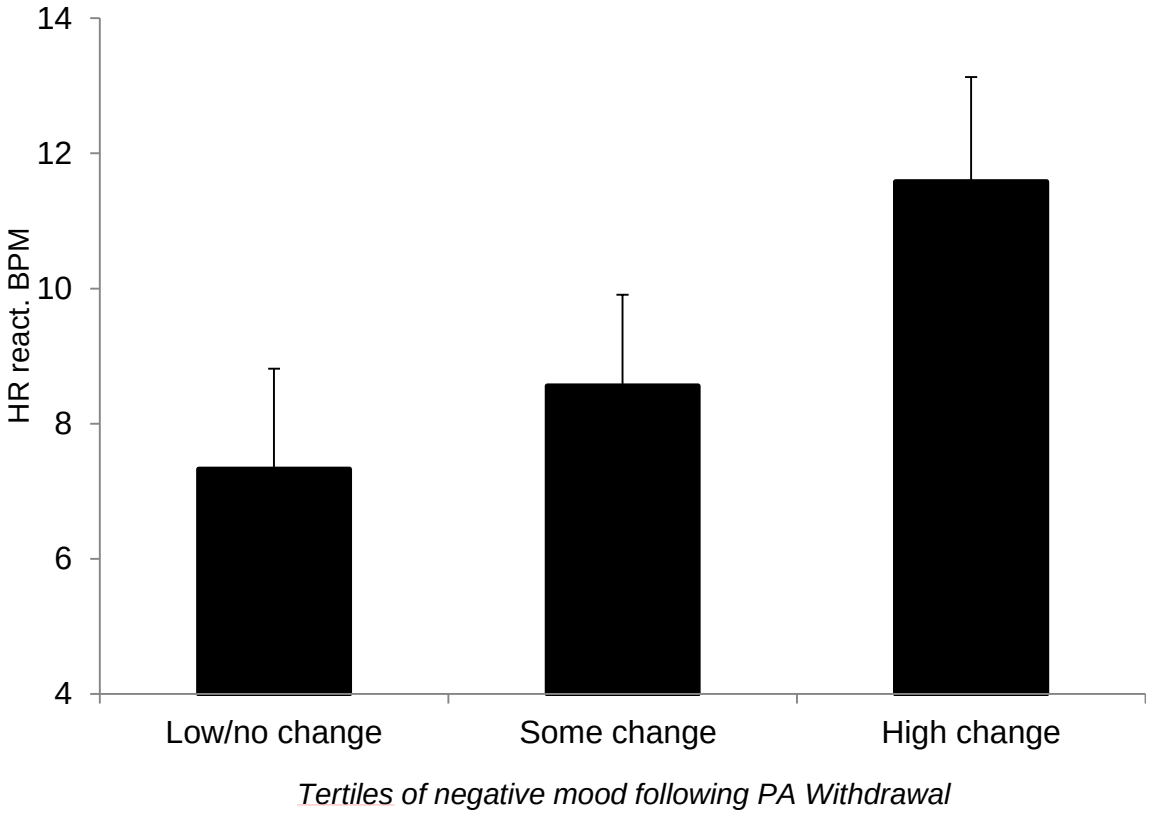


Fig-4.17 Association of Negative Mood with Stress-Induced HR Responses.



4.5 DISCUSSION

This study was designed to experimentally manipulate physical activity (PA) levels in order to investigate whether psychophysiological and immune responses to mental stress are altered by forced PA abstinence in healthy participants. This method, which has been termed exercise withdrawal paradigm (EWP), represents an experimental model of physical inactivity that can be implemented in a relatively short time course and presents several advantages compared to an active PA intervention trial. The EWP has been used previously to investigate the effect of PA on mood symptoms and other immunological parameters. This study has extended to incorporate a psychophysiological stress component in order to investigate the causal processes and mechanisms that may account for the association of PA, mood and physiological and immunological activity under stimulated conditions.

Cross-sectional work has shown associations of PA and fitness levels with attenuated stress-induced cardiovascular responses but not all randomized exercise trials have been able to show an effect of PA on CV reactivity to stress (Sloan et al., 2011; Forcier et al., 2006). No experimental study has so far addressed whether PA is associated with attenuated inflammatory responses. The main hypothesis was that physiological and immune responses to mental stress would be heightened following two weeks of PA withdrawal when compared to two weeks PA maintenance (the control condition). The secondary hypotheses stated that PA withdrawal would result in the development of negative mood symptoms and fatigue in a dose-response manner with the amount of objectively recorded change in physical activity.

The PA withdrawal intervention was successful in modifying the amount of daily PA. The relative within-subjects reduction in average daily counts per minute

(CPM/d) in the two PA phases was 34% and did not differ by gender. The participants recruited had higher than average cardiovascular fitness compared to the general population (HSE, 2008) and their self-reported amount of vigorous and very vigorous PA in the week before study entry was over two hours although there was large variation between-participants.

Two weeks of PA abstinence resulted in robust mood disturbances that were particularly marked in the somatic, fatigue and vigour subscales. Since these latter dimensions are reminiscent of, and phenotypically similar to, the symptoms of sickness behaviour, this finding is of particular significance given the hypothesized link between negative mood and inflammatory activity (Wright et al., 2005; Strike, Wardle, & Steptoe, 2004). The mood change was positively associated with changes in objectively recorded physical activity (CPM/d) suggesting a direct link between reduction in PA and the development of depressive symptoms. Interestingly, this dose-response between PA reduction and mood became stronger when participants with poor compliance with the ActiGraph were excluded. Overall, these results strongly indicate that a reduction in objectively measured PA is likely to drive the mood change rather than other non-specific factors such as social contact or interaction during exercise training.

Previous epidemiological work has shown that physically active individuals are less likely to develop depressive symptoms (Hamer & Chida, 2008). Data from exercise intervention trials were not consistent in showing positive effects of PA on depression (Lawlor & Hopker, 2001), which may in part be due to poor methodological quality (Rethorst et al., 2009). Although improvements in depression have been shown, and especially among depressed individual with a chronic illness (Herring et al., 2012), other non-specific factors related to exercise interventions

including increased social contact and general expectations may play an important role in mediating this effect (Steptoe, 2007). Therefore, I have shown that using an experimental approach, reduction in daily PA in regularly active and fit individuals induces negative mood symptoms.

This study provides further evidence in support of the PA and mood regulation relationship. However, the correlation between mood change and change in objectively measured CPM/d was not significant when mood symptoms were assessed with the GHQ-28. This could suggest that other factors besides an actual reduction in PA might play a role. However, there are two arguments that might not support this notion. First, the ActiGraph (accelerometers) device used to monitor compliance with the study protocol has some important limitations. For example, this device relies on the participant's compliance with the protocol (i.e. wearing the device as instructed) but ActiGraph wear time was mixed. In addition, not all physical activities are accurately recorded by the ActiGraph (Freedson, Bowles, Troiano, & Haskell, 2012) and there is little agreement in the literature on the cut-off boundaries that define moderate and vigorous/very vigorous PA intensity (Matthews, Hagstromer, Pober, & Bowles, 2012).

Second, this study used a cross-over, randomized design so between-subjects variability and individual difference is minimized. Participants were carefully interviewed and selected to reflect a sample of regularly active and healthy individuals free of any mental health issue. Therefore, the findings add to the cross-sectional and prospective literature by showing that experimental reduction in PA is associated with marked negative changes in mood and fatigue symptoms in a partial dose-response manner as hypothesized in the introduction.

A further objective of this study was to examine whether stress-induced autonomic and immune responses differed after PA withdrawal when compared to the usual PA maintenance regime. The main finding was that systolic, diastolic and total peripheral resistance responses to mental stress did not change following PA withdrawal and were remarkably stable over the two stress sessions. Heart rate (HR) recovery from stress was slightly enhanced in the PA maintenance condition but the difference could be explained by lower overall HR during the stress session. This suggests that stress-induced cardiac autonomic reactivity was not affected by PA withdrawal. However, higher overall HR activity in the PA withdrawal phase suggests that autonomic changes in cardiac modulation in response to exercise deprivation do not suddenly reflect in exaggerated reactivity or impaired recovery. It is possible that these differences may emerge after prolonged exercise abstinence.

According to the allostatic load theory of the effect of physiological stress activation, delayed post-stress autonomic return to baseline levels (pre-stress), in addition to heightened reactivity, is an important mechanism linking stress with the development of cardiac risk factors. However, this study offered no support for a causative model wherein experimental manipulation of PA results in heightened or disturbed cardiovascular responses to mental stress. This finding is not in line with a study showing that moderate intensity exercise was associated with reductions in sympathetic tone and greater peripheral vasodilation in response to mental stress mediated through enhanced beta 1 and beta 2 receptor sensitivity (Brownley et al., 2003). However, that study was an acute exercise model whereas we used an exercise withdrawal model that reduces acute exercise effects. Previous work has shown that although resting blood pressure is reduced after exercise training

(Cornelissen, Verheyden, Aubert, & Fagard, 2010) the effect on CV reactivity to and recovery from mental stress is mixed.

This is an important issue given that heightened and sustained CV responses to laboratory stress predict the development of cardiac risk factors (Chida & Steptoe, 2010). For example, a small, randomized eight week aerobic exercise training found no evidence of an effect of exercise on muscle sympathetic nervous activity and HR reactivity despite improvements in CV fitness (Ray & Carter, 2010). Jackson and Dishman (Jackson & Dishman, 2006) reported that fitness was related to greater heart rate reactivity but better recovery from mental challenge in 73 studies, although these effects were diminished when only randomized controlled exercise training studies were included and when fitness was measured as peak oxygen uptake. In contrast, Forcier and colleagues (2006) demonstrated an inverse association between fitness and heart rate reactivity in an analysis containing only trials with evidence of an exercise training effect. One of the difficulties in interpreting data from short term (often 8 – 12 weeks) exercise trials is that individual changes in fitness are usually modest, which suggests a short period of exercise training may not be sufficient to induce the type of chronic adaptations required to observe stress buffering effects.

A common problem in this area has been limited sample size that can often lead to studies with insufficient statistical power. This issue was, however, addressed in a recent large randomised trial consisting of 149 healthy, young sedentary participants who were randomised to a twelve week exercise program followed by a further four weeks of sedentary de-conditioning (Sloan et al., 2011). Participants performed various stressors before and after the intervention, and after detraining, including a public speaking task, mental arithmetic, and the Stroop Color-Word task,

although there was no indication of any stress-buffering effects following exercise training. However, the study protocol did not include any objective measure of adherence to the training regime. In addition, participants were tested three times during the course of the study which may lead to an habituation effect that may be difficult to quantify in the absence of a control group. Therefore, the finding of a lack of an exercise training effect on CV reactivity may be due to the methodological limitations of the study.

In the present study, the lack of a PA withdrawal effect on blood pressure reactivity may be explained by the post-exercise hypotension hypothesis (Hamer, 2006). This suggests that an acute bout of exercise is associated with lower blood pressure eight to twelve hours following exercise. Blood pressure responses to mental stress are indeed attenuated after an acute bout of exercise (Hamer et al., 2006) but in this study participants were instructed not to undertake any form of exercise for at least 16 hour prior to stress testing. This requirement was implemented in order to avoid a carryover hemodynamic or mood enhancing effect of acute exercise. Therefore, the attenuated stress-induced blood pressure responses could be explained by the fact that participants are likely to be in the post-exercise window period when they encounter a stressor, and asking them to refrain from exercise for 16 hour prior to mental stress exposure might have cancelled out the blood pressure effect. This would suggest that acute exercise is more important than chronic training effects and may explain why there was no difference in stress reactivity after withdrawal.

An alternative explanation may be that PA abstinence in habitually active individuals leads to vascular and autonomic changes or adaptations which are not immediately reflected in heightened blood pressure reactivity. Indeed, it has been

shown that resting blood pressure returns to pre-aerobic training levels after a couple of weeks of detraining (Meredith et al., 1990). However, since the beneficial effects of chronic PA is likely to result from accumulated bouts of acute training sessions (Hamer, 2006) it may be feasible to hypothesized that blood pressure reactivity to stress is not affected by two weeks of PA withdrawal.

MHPG levels did not increase significantly in response to stress in the two PA conditions. Following PA withdrawal there was no increase post-stress but a steady decline to 45 minutes. In the PA maintenance phase, baseline levels were lower with a near significant increase 20 minutes after stress and return close to baseline levels at 45 minutes post-stress. The lack of a post-stress increase in the PA withdrawal phase may indicate a possible ceiling effect, in that already heightened baseline levels made it difficult to detect a further increase. This is indicative of an increased baseline sympathetic activity following PA withdrawal compared to the PA maintenance. However, an heightened sympathetic drive resulting from PA withdrawal can only be speculative in the absence of validated cut-off values for MHPG levels that indicate hyperactivity (such as blood pressure). The change in baseline MHPG levels between PA conditions was associated with the change in negative mood following PA withdrawal but only marginally. This suggests that the heightened resting sympathetic activity in individuals withdrawn from their regular PA regime may be linked to greater distress due to exercise abstinence. There were also correlations between MHPG AUC and inflammatory and blood pressure reactivity indicating that higher catecholamine output during stress testing were related to higher CV and immune reactivity lending more support to the validity of this marker of sympathetic activation. However, it is important to emphasise that

some of the correlations observed between MHPG and mood and other markers were only marginally significant and of small magnitude.

Salivary cortisol responses to mental stress, which is an index of the HPA axis reactivity, were also assessed. Overall, cortisol responses to the stress tasks were modest. Nonetheless, it was observed that compared to PA maintenance, cortisol was heightened at 20 minutes post-stress during PA withdrawal testing. The total level of cortisol exposure during the stress sessions was indexed as the area under the curve (AUC). Although this measure was heightened following PA withdrawal compared to PA maintenance, the difference did not reach statistical significance. Previous work that investigated associations of fitness levels with stress-induced cortisol responses has generally found lower cortisol levels in fitter participants (Rimmele et al., 2007; Rimmele et al., 2009). However, it is difficult to draw firm conclusions from those studies due to the cross-sectional nature of the design and because some of the participants were highly trained athletes. Therefore, the finding of heightened cortisol responses to stress following PA withdrawal may add more experimental support to a biological model wherein the health protective effects of regular PA are in part mediated through HPA regulation and cortisol release.

Inflammatory activity was measured in response to stress (IL-6) and as baseline change following PA withdrawal (CRP). Although CRP and IL-6 levels were slightly higher following PA withdrawal compared to the PA maintenance, the difference did not reach statistical significance. Recent work using a similar PA modification model has found that most inflammatory markers are not affected by a two week PA abstinence (Kop et al., 2008; Lund et al., 2010). Therefore, by using a randomized, cross-over method that overcomes problems of between subjects variability, this study further supports previous findings that short-term PA withdrawal does not alter

systemic levels of CRP. However, the lack of an effect could also be due to the fact that participants were young and fit, and CRP levels are generally low in this group, which makes finding an effect of PA withdrawal difficult. It may be possible that by employing a different sample such as active older individuals or participant with an inflammatory condition, an effect of PA withdrawal on inflammatory markers may become apparent.

The IL-6 stress reactivity response showed null results. There was no IL-6 increase at 45 minute post stress in both testing sessions, and no effect of PA withdrawal on the stress-induced response. There might be several reasons for this null effect. The stress-induced inflammatory response may already be attenuated in young and fit individuals and the stressors used in this study may not have been challenging enough to produce a marked IL-6 response. In addition, blood samples were drawn pre and post stress only. Although some reports have suggested that the IL-6 stress response may be detectable at 45 minutes post stress, further IL-6 increases for up to two hours post-stress have also been reported in the literature (Brydon, Edwards, Mohamed-Ali, & Steptoe, 2004).

For example, Von Kannel (von Kannel et al., 2006) has shown that with the Trier Social Stress Test maximum levels of IL-6 are reached at 105 minute post-stress in a sample of middle age men. In a cross-sectional study of young and healthy women, Brydon showed a 37 per cent stress-induced increase in IL-6 at 45 minutes using a stress protocol similar to the one used in this study, although no further blood samples were collected post 45 minutes (Brydon et al., 2008). Therefore, it is possible that the lack of a stress-induced IL-6 response observed in this study is attributable to a combination of the relatively mild stress protocol, the sampling time lag, and the participants' high level of fitness and relatively young age. Indeed, the

time lag of the IL-6 response to behavioural stimulation may vary by age, SES, gender and other factors which may make it difficult to detect a stress-induced increase when only one post-baseline sample is drawn.

Due to the fact that adherence to the PA withdrawal intervention protocol was mixed, secondary analyses were performed using two different methods. In the first method, participants with less than a 25 per cent reduction in daily CPM relative to their PA maintenance were excluded. The second method consisted of the use of mood disturbances caused by PA withdrawal to index the effectiveness of the intervention. Changes in mood following PA withdrawal were therefore used as a predictor continuous variable in several linear regression models. The most consistent finding from these secondary analyses was that the null effect of PA withdrawal on systolic and diastolic blood pressure, systemic vascular resistance and HRV responses to stress was confirmed. However, there was a near significant PA condition by time interaction for the HR response suggesting heightened HR responses following exercise withdrawal. Yet, within-subject comparisons of stress-induced HR reactivity and recovery indicated that the interaction could be explained by lower resting HR in the maintenance phase. Analyses involving mood change instead showed an association between HR reactivity and mood. The HR reactivity to the stress tasks after PA withdrawal was heightened in more distressed participants after adjusting for reactivity during PA maintenance. Therefore, the results of this secondary analysis suggests that an effect of PA withdrawal on HR can only be observed when using mood disturbance to index the effectiveness of the PA withdrawal protocol.

Interestingly, there was a borderline significant stress-induced IL-6 increase in the PA withdrawal testing session when participants with poor adherence were

excluded. Consistent with this finding, greater mood changes predicted heightened IL-6 reactivity during PA withdrawal after adjusting for IL-6 reactivity during PA maintenance. Negative mood independently accounted for 10 per cent of the variance in stress-induced IL-6. This indicates that an effect of PA withdrawal on IL-6 reactivity in this study may only be detectable in subgroup analysis. Therefore, it seems that overall the effects are greater in those participants that were more distressed by the intervention. Adherence to the study protocol and participant selection is crucial in this kind of experimental studies, and may also explain why other studies did not find an effect of exercise training on CV reactivity for example (Sloan et al., 2011).

Strengths of this study include the use of a single-blind, cross-over design which minimizes inter-individual variability, the standardized cardiovascular fitness assessment, and the recruitment of highly active and healthy participants. A further strength is the use of accelerometers to measure PA participation objectively and thus not only relying on self-report data. Strengths and weaknesses of the study design and protocol will be discussed in greater depth in Chapter six. However, some limitations of the study include the fact that it was not possible to test participants before randomization and then again after PA withdrawal and PA maintenance. In this way the comparisons might have been stronger but could have potentially biased the study because the administration of three repeated stress sessions over a month may lead to habituation or practice effects. Another limitation of the study is that PA participation before study entry was assessed by self-report as it would have been too cumbersome for participants to wear the accelerometer for an extra week. A non-stress control group was not employed. However, it has

previously been shown that with a non-stress control group, blood pressure and IL-6 remained stable over a 2 hour period (Brydon et al., 2005; von Kannel et al., 2006).

In summary, this randomized, cross-over study has found that two weeks of PA withdrawal does not alter blood pressure responses to mental stress. The heart rate stress reactivity response was heightened following PA withdrawal but only in secondary analysis using mood change to index the effectiveness of the intervention. The cytokine IL-6 was not affected by mental stress or PA withdrawal although a small post-stress increase following PA withdrawal was observed with the exclusion of participants with poor compliance to the study protocol. Furthermore, PA withdrawal induced negative mood predicted greater IL-6 reactivity independently of PA maintenance IL-6 reactivity. A small but consistent increase in stress-induced cortisol following PA withdrawal was also reported. Robust negative mood disturbances were observed following PA withdrawal that developed in a dose-response manner with reduction in daily level of physical activity. Overall, the study offers only partial support for the hypotheses laid down in the introduction.

CHAPTER5: ADIPOSITY AND STRESS REACTIVITY

5.1 INTRODUCTION

Obesity is an important risk factor for cardiovascular disease (CVD) mortality, certain cancers, and poor quality of life (Adams et al., 2006; Finucane et al., 2011). Being obese or overweight greatly increases one's chances of developing metabolic disturbances such as insulin resistance, dyslipidaemia, type 2 diabetes and atherosclerosis which are strong and independent risk factors for coronary heart disease morbidity and mortality (Hafner, 2007; Adams et al., 2006; Reaven, Abbasi, & McLaughlin, 2004). It has been proposed that the adipose tissue is an active endocrine organ that communicates with the central nervous system and other organs such as the skeletal muscle, and secretes several inflammatory and metabolic factors that contribute to a low grade systemic inflammatory state (Rocha & Libby, 2009; Li, Wang, & Miao, 2011).

5.1.1 Adipokines.

Adipokines is the term used to describe pro and anti-inflammatory cytokines that are mainly produced by the adipose tissue and include interleukin-6 (IL-6), IL-18, interleukin-1 receptor antagonist (IL-1ra), leptin, resistin, tumour necrosis factor alpha (TNF-α), C-reactive protein (CRP) and adiponectin (Eder, Baffy, Falus, & Fulop, 2009; Pou et al., 2007; Rexrode, Pradhan, Manson, Buring, & Ridker, 2003). Basal levels of some of these inflammatory markers are raised in individuals with

excess adipose tissue and in overweight people (Trayhurn & Wood, 2005) making this an important pathway mediating the effect obesity on metabolic functions and health outcomes (Caruso, Balistreri, & Candore, 2010; Gregor & Hotamisligil, 2011).

Psychosocial stress is another important risk factor for CVD (Dimsdale, 2008; Hjemdahl, Rosengren, Steptoe, 2012) and adiposity accumulation (Wardle, Chida, Gibson, Whitaker, & Steptoe, 2011) in which inflammatory mechanisms play a possible aetiological role (Li & Fang, 2004). Indeed, acute stress administered under controlled condition has been shown to activate a transcription factor resulting in the release of pro-inflammatory cytokines (Steptoe, Hamer, & Chida, 2007; Bierhaus et al., 2003; von Kannel, Kudielka, Preckel, Hanebuth, & Fischer, 2006) as well as eliciting robust and consistent cardiovascular (CV) and neuroendocrine responses (Hamer, Gibson, Vuononvirta, Williams, & Steptoe, 2006; von Kannel et al., 2006). In addition, these CV and neuroendocrine responses to acute mental stress have been shown to longitudinally predict risk factors including hypertension, increased intima media thickness and greater coronary artery calcification (Carroll, Phillips, Der, Hunt, & Benzeval, 2011; Chida & Steptoe, 2010; Hamer, O'Donnell, Lahiri, & Steptoe, 2010). Therefore, acute mental stress is a valuable experimental tool for investigating the mechanisms involved in the association between inflammatory responses and adiposity.

5.1.2 Adiposity and Stress-Induced Autonomic Responses.

Some evidence suggests that adiposity (waist to hip ratio [WHR] and waist circumference) and obesity (body mass index [BMI]), and obesity-related conditions such as insulin resistance are associated with disturbed autonomic reactivity to acute

mental stress. An early small study comparing insulin resistant overweight women with non-insulin resistant normal weight women showed exaggerated blood pressure responses to mental stress in the obese group which correlated with insulin levels (Sung, Wilson, Izzo, Ramirez, & Dandona, 1997).

Another small study of overweight women showed greater stress-induced blood pressure responses in individuals with central obesity compared to women characterized by peripheral obesity (Davis, Twamley, Hamilton, Swan, 1999). Greater systemic vascular resistance in overweight women was also observed in two cross-sectional studies (Seematter, Guenat, Schneiter, Cayeux Jaquier, Tappy, 2000; Agapitov, Correia, Sinkey, Dopp, Haynes, 2002), although in one of the study blood pressure responses did not differ between the obese and lean group (Agapitov et al., 2002). A large study of adolescent boys and girls found that waist circumference was positively associated with blood pressure reactivity independent of BMI and the effect was stronger in boys (Goldbacher, Matthews, & Salomon, 2005). This study is especially notable because it employed a larger sample size of healthy adolescents not at risk for CVD.

Further studies have also investigated neuroendocrine responses to mental stress in obesity, mainly by measuring the glucocorticoid cortisol as an index of HPA axis activation. In one study it was found that women with greater WHR secreted significantly more cortisol in response to mental stress and this response was consistent over 3 days of testing (Epel et al., 2000). This suggests that even in non-obese women greater central fat is related to larger stress-induced cortisol responses. However, another study performed with men (Ljung et al., 2000) found that men with greater WHR had lower cortisol responses to stress independent of BMI but larger hear rate (HR) responses and higher catecholamine excretion than

men with lower WHR. Furthermore, a more recent cross-sectional study comparing obese and non-obese (stratified by BMI) postmenopausal women found no difference in neuroendocrine (cortisol and ACTH) responses to a public speaking stress between the groups but HR and diastolic BP (DBP) were higher 15 minutes post-task in the obese group (Benson, Arck, Tan et al., 2009). Inconsistent findings clearly exist regarding CV and neuroendocrine reactivity to mental stress in obesity. Some of these inconsistencies may be accounted for by differences in the population under investigation as well as the different measure of adiposity used. Additionally, few studies controlled for physical activity participation which may confound associations with CV reactivity.

5.1.3 Adiposity and Stress-Induced Inflammatory Responses.

Recent investigations have also examined the association of pro-inflammatory responses and measures of obesity and adiposity. For example, In the study by Benson and colleagues (Benson et al., 2009), stress-induced IL-6 was higher in the obese group at both baseline and 45 minute post-stress but there was no evidence of an interaction effect between the groups. Furthermore, no group differences were observed in the stress-induced redistribution of circulating leukocyte immune cell subpopulation. A study of young women (Brydon, Wright, O'Donnell et al., 2008) showed that the adipokine leptin and IL-1ra stress response were positively and significantly correlated with waist circumference independent of BMI, age and smoking status. Delayed blood pressure recovery from stress was also associated with BMI and waist circumference so that the more obese women had slower post-stress recovery suggesting sustained sympathetic activation.

A recent quasi-experimental study comparing salivary cortisol responses to the Trier Social Stress Test (TSST, a marked social evaluative stressor (Kirschbaum, Pirke, & Hellhammer, 1993) in normal weight, obese and participants who had recently lost weight found some inconsistent results (Therrien et al., 2010). Men who had recently lost weight showed the smallest cortisol change from baseline to 20 minute post-stress (peak cortisol response), and the cortisol response was strongly and negatively correlated with the percentage change in weight loss. However, the non-obese men showed the greatest stress-induced cortisol change clearly pointing to a non-linear effect. There were no differences in cortisol responses reported in women. However, the weight loss in the reduced obese group was largely unsupervised and achieved through lifestyle changes including exercise.

Hence it is difficult to tease apart whether the observed effects were attributable to adiposity or to an unmeasured factor such as improvements in mood and self-esteem, or to an HPA axis adaptation to recent physical activity. Some of the discrepancies observed in these studies may be attributable to the different age group tested, the small sample size in some studies, differences in stress protocols and the failure to control for possible confounders such as physical activity or resting autonomic parameters.

5.1.4 Prospective Studies.

Inflammatory and adrenocortical responses to stress may therefore be biological pathways driving the relationship between obesity and CV risk factors, but the majority of the existing work has been cross-sectional making interpretation of results difficult. Limited longitudinal work has investigated the association of CV and

inflammatory responses to stress with the development of obesity or adiposity measure. This work has generally shown conflicting results.

In a large sample of healthy, middle age men and women drawn from the Whitehall II cohort, BMI and WHR were associated with impaired recovery in systolic blood pressure (SBP) and cardiac output (CO) in a linear fashion (Steptoe & Wardle, 2005). This impairment in SBP and CO stress recovery was independently associated with an increase in WHR at three years follow-up in men only. This study offers some support to a causative model in which disturbed CV responses to stress predict the development of central obesity. However, other studies do not support this hypothesis suggesting that it is a blunted CV response to mental stress that is associated with obesity. In the largest CV stress response study to date using the West of Scotland Twenty-07 (Ford, Ecob, Hunt, MacIntyre, & West, 1994) study cohort, the HR response to a 5 minute mental arithmetic task was negatively associated with BMI and WHR independent of a range of covariates (Carroll, Phillips, & Der, 2008). The BP response instead was positively associated with obesity but this association became non-significant after adjustment for covariates including age, social class, medication status, and resting blood pressure. Interestingly, HR reactivity was associated with a reduced likelihood of becoming obese after five years follow up (OR = 0.97, 95% CI 0.95 – 0.99) after controlling for a range of confounders including obesity at previous screenings.

No other stress-induced CV or immune parameters were measured in the West of Scotland Twenty-07 study, and CV stress recovery was not assessed. The authors argued that their finding cannot be explained by a ceiling effect of reactivity driven by higher resting levels since the results were adjusted for resting HR. Rather, they suggested (Phillips, 2011) that the lower sympathetic reactivity found in their

study may be explained by obesity-related elevations of the hormone leptin, which may be indicative of tissue resistance to the sympathetic nerve activation effect of this hormone (Trayhurn & Bing, 2006).

5.1.5 Leptin in Obesity.

Leptin is an important adipokine hormone involved in the production of the T-helper 1 cytokines and the suppression of T-helper 2 anti-inflammatory cytokines (Quilliot, Boahme, Zannad, Zielger, 2008). Leptin levels are markedly raised in the obese state especially in women. Therefore, elevations in plasma leptin may suggest that a leptin resistant obesity phenotype is inhibiting CV and autonomic reactivity to stress. Indeed, levels of leptin are proportional to body fat and it has been shown in a cross-sectional study of young women that high levels of resting leptin were associated with greater stress-induced HR and IL-6 increases (Brydon, O'Donnell Wright, Zachary, Wardle, Steptoe, 2008). This suggests a link between leptin and CV and inflammatory activation even in a non-obese sample which may further corroborate the notion that the visceral adipose tissue is important in mediating the activation of pro-inflammatory cytokines during stress.

5.1.6 Obesity and Depression.

In addition to disturbances in psychophysiological responses, obesity has also been associated with increased risk of depression and psychological distress, which might partly account for some of the inconsistencies in the psychophysiological area highlighted above. However, the data on obesity and depression are also

inconsistent. For example, a recent meta-analysis of prospective cohort studies suggest that people with greater BMI have an increased risk of depressive symptoms and depression (Luppino et al., 2010), but depression at baseline was also associated with increased odds of developing obesity. Another large scale prospective study has shown that obesity, and especially central obesity, during adolescence is associated with depression in adulthood (Herva et al., 2006).

Although some individual studies report no association between obesity and depression or depressive symptoms (Istvan, Zavela, & Weidner, 1992), another group of studies show greater BMI to be associated with reduced risk of future mental health problems and suicide (Magnusson, Rasmussen, Lawlor, Tynelius, & Gunnell, 2006). A negative association between overweight and obesity and depression has also been reported in two prospective studies of elderly individuals in a non-Western population (Wong, Leung, Leung, & Woo, 2011; Chang & Yen, 2011).

Recent Mendelian randomization studies, using adiposity-related genetic variants as un-confounded instrument variables for obesity have also produced inconsistent findings. For example, higher BMI attributable to genetic influences was associated with increased risk of depressive symptoms in men participating in the Whitehall II study (Kivimaki et al., 2011), but lower likelihood of psychological distress and antidepressant use in a study of Danish adults (Lawlor et al., 2011). Therefore, evidence to date seems to suggest that obesity or overweight lead to depression but depression may also lead to the development of obesity. This issue is likely to be further confounded by cultural differences since negative associations of obesity with depression have also been reported in non-western samples.

It has been argued that markers of inflammation may account for the positive association of chronic stress and depression with obesity (Hamer & Stamatakis,

2008), and increasing evidence suggests that pro-inflammatory cytokines and adipokines are implicated in the pathophysiology of depressive disorders (Miller, Maletic, & Raison, 2009; Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). It has been shown in one study that depressive symptoms promote expansion of adipose tissue with consequent release of IL-6 and leptin which may further contribute to a low grade inflammatory state (Miller, Freedland, Carney, Stetler, & Banks, 2003). However, that study argued against a model in which the inflammatory response arising from enlarged adipose tissue causes depression.

More recently, it has been shown that the hormone leptin was associated with depression in men with type 2 diabetes, after adjustment for covariates including BMI, medication use and glycated haemoglobin (Labad et al., 2011). In another study elevated serum leptin at baseline was a predictor of major depression over a five year follow-up period in women (Pasco et al., 2008). Therefore, enlarged adipocytes in visceral fat depots contribute to macrophage accumulation with subsequent release of inflammatory adipokines including IL-6, TNF-α and leptin. Increases in systemic levels of these inflammatory mediators may result in depressive symptoms (Shelton & Miller, 2010) but also in disturbances in the homeostatic balance and in impairments in autonomic responses to stress which may lead to the development of CV risk factors (Straznicky, Lambert, & Lambert, 2010).

5.1.7 Weight loss and Inflammation

Experimental studies have generally shown improvements in circulating inflammatory markers and hemodynamic profile after weight loss (Capuron et al., 2011; Selvin, Paynter, & Erlinger, 2007; Straznicky et al., 2010). Some evidence also suggests that even small reduction in visceral adiposity is associated with appreciable improvements in metabolic functions that can be achieved with minimal, or no weight loss (Ross & Bradshaw, 2009). At present, the mechanisms through which adiposity and stress responses are linked is not completely understood and it is unclear whether it is a heightened or a blunted stress reactivity that is associated with measures of adiposity and obesity.

Some evidence shows associations of stress responses with adiposity but the causal link has not been established. For example, adiposity may also cause heightened stress responses. No studies have previously examined the effect of weight loss on inflammatory and cardiovascular responses to mental stress in a healthy sample. The aim of this study is to examine the effect of experimentally induced weight loss (through caloric restriction alone) on inflammatory and cardiovascular responses to mental stress.

5.1.8 Hypotheses.

1) Weight loss achieved through caloric restriction will be associated with improvements in resting inflammatory markers (CRP, IL-6 and leptin) and lipids.
2) Cardiovascular and inflammatory responses to mental stress will be reduced after weight loss.

3) Reductions in leptin after weight loss will be associated with cardiovascular and inflammatory responses to stress after weight loss.
4) Weight loss will be associated with improvements in depressive symptoms which will be mediated though changes in adiposity or inflammatory markers.

5.2 METHODS

5.2.1 Study Design: Weight Loss & Stress Responses Study.

The Weight Loss and Stress Responses Study (WLSR) is a non-randomized study based on a nine week supervised weight loss intervention. The study did not employ a control group for several reasons. Firstly, the time constrain of this PhD project prevented me from designing and implementing a randomized controlled trial (RCT). Secondly, this study was originally designed as a smaller pilot/feasibility project in order to inform the design of a larger RCT.

Twenty-four women between the age of 18 and 45 years were recruited through newspaper adverts and intercollegiate emails for a study investigating the effect of a nine week caloric restriction diet on cardiovascular and inflammatory responses to mental stress. Participants attended the research laboratory at study entry for baseline anthropometric measures and psychophysiological testing. They returned after the nine week weight loss period for a repeated assessment. During the weight loss period participants attended a weekly nutritional counselling session with a Health Psychologist and received Slim-Fast meal replacements. The study design is illustrated in Figure 5.1.

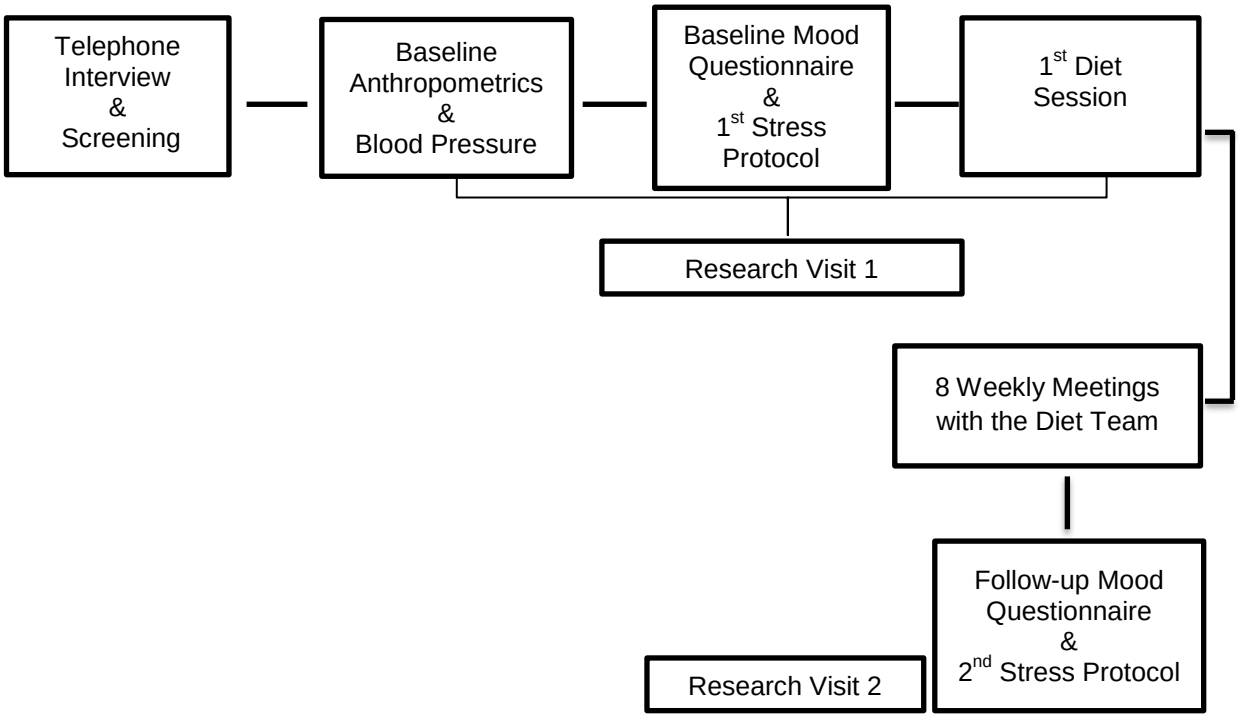


Fig-5.1: Overview of WLSR Study Design.

5.2.2 Study Sample Recruitment and Weight Loss Programme.

The study sample consisted of overweight or obese but otherwise healthy women with a body mass index (BMI) of between 27.5 and 42.5 kg/m^2 not currently taking part in any exercise programme or diet regime (i.e. Weight Watchers) and not on any regular medication (excluding the oral contraceptive). The recruitment selection process and response rate is shown in figure 5.2. Sixteen out of the 24 participants that enrolled completed the study (33.3 per cent attrition) and have complete data on all the outcome measures. One participant became pregnant halfway through the study and was therefore excluded from the analyses leaving a

final sample of 15. There were no differences in age, BMI and WHR between participants who completed the study and those who did not complete.

A Registered Clinical Dietician and a team of Health Psychologists at Weight Concern (Health Behaviour Research Centre) designed and delivered the weight loss programme. This individualized programme consisted of estimating participants' daily energy requirement using the widely employed Schofield equation method (Schofield, 1985). The equation model is as follow:

For women between 18 and 29 years basal metabolic rate (BMR) = 14.8 x body weight (W) + 487 standard error of estimate (SEE) = 120. For women between 30 and 59 years BMR = 8.3 x W + 846 SEE = 112.

The Schofield method is used to estimate BMR in adults and uses estimated errors factors (SEE) and activity factor values to adjust the equation for body type and activity levels. Six hundred calories were deducted from each participant estimated daily requirement for weight maintenance to create an energy deficit and induce weight loss. The daily calorie allowance consisted of two commercially available standardized meal replacement shakes (Slim-Fast) to provide about 450 calories, plus one healthy meal and snacks to make up the remaining number of calories.

As used in previous behavioural interventions to encourage weight loss (Heymsfield, Van Mierlo, Van der Knaap et al., 2003), information on meal planning, calorie counting, healthy snacking, distinguishing cravings from hunger, and methods of dealing with internal and external triggers were provided, discussed and

practiced during each session. Participants were asked to keep a food diary which would be reviewed during the weekly sessions.

Towards the end of the programme participants were given a Shape Up (Weight Concern) manual and provided with a tailored weight maintenance plan in order to encourage proper weight management once the study had terminated. Two post-study follow-up appointments were also offered to participants to provide on-going support. Weight Concern experts predicted that by following this weight loss plan participants were expected to lose between 0.5 and 1.0 Kg per week.

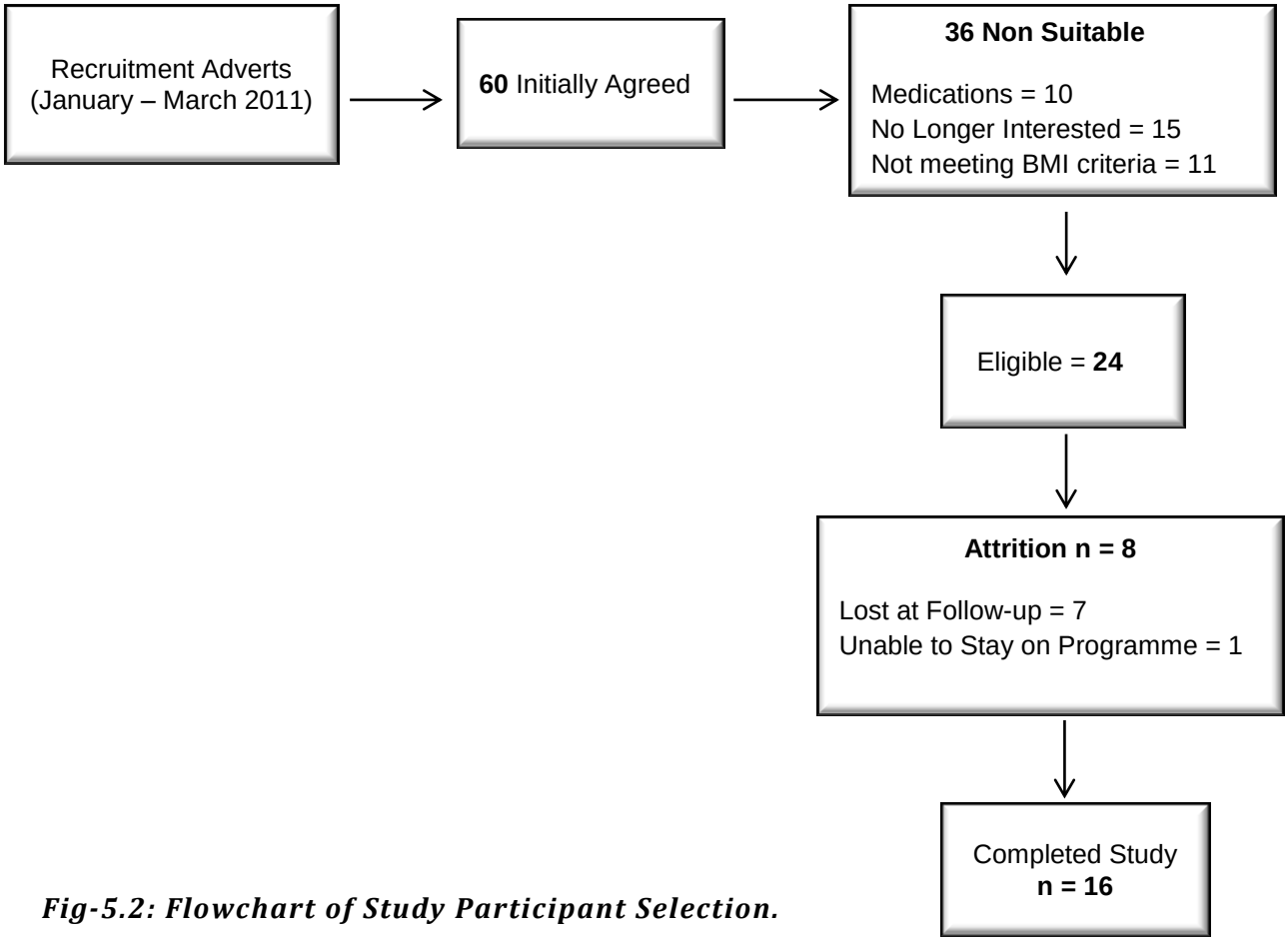


Fig-5.2: Flowchart of Study Participant Selection.

5.2.3 Materials

At the first research laboratory visit participants signed the informed consent form and were seen individually by a research nurse who measured baseline anthropometric variables and collected the first saliva sample. This same procedure was adopted at the follow-up visit nine weeks later.

5.2.3.1 Anthropometric Measures.

Waist circumference was measured twice to the nearest 0.1 cm using a metal anthropometric tape midway between the lower rib margin and the iliac crest. Hip circumference was measured at the level of the great trochanters to index a waist to hip ratio. Height was recorded according to standard protocol to the nearest 0.1cm using a Stadiometer and weight was measured using a digital Tanita scale with participant in light clothing. Fat mass and fat percentage was estimated using a Tanita body composition analyser. Body mass index (BMI) was calculated as the ratio of weight in kilogram and the square of the height in meters (Kg/m^2).

5.2.3.2 Blood Sampling Protocol.

At baseline (pre-weight loss) and follow-up (post-weight loss) two blood samples were obtained from the antecubital vein of the non-dominant forearm using standard aseptic techniques. Sample one was obtained after a 30 minute rest immediately before administering the stress protocol and sample two was collected 45 minute after the stress protocol.

Blood was drawn into EDTA coated vacuum tubes and serum separator vacuum tubes by a research nurse or the PhD candidate. EDTA samples were spun immediately at 2500g for 10 minutes at room temperature. Serum samples were left to clot for 30 minutes and spun at 2500g for 10 minutes. The resulting supernatant was harvested and kept in a -80 C freezer in 0.5ml aliquots.

5.2.3.3 Cardiovascular Measures.

Beat by beat blood pressure was monitored continuously during the two laboratory stress sessions with a Finometer Pro (Finapres Medical System, Amsterdam, The Netherlands). This device measures beat by beat pressure using a cuff attached to the middle finger of the non-dominant arm (see Chapter 4). Before the start of the stress protocol, the Finometer was initiated with the participant's characteristics (age, gender, height and weight) and calibrated according to standard protocol. A height sensor appropriately nulled during calibration accounts for differences in height between the finger and the arm.

The Beatscope software was used to derive the following cardiovascular variables from the Finometer raw data: Systolic (SBP) and diastolic (DBP) blood pressure; mean arterial pressure (MAP) and cardiac output (CO). Total peripheral resistance (TPR) was derived with the formula (TPR = MAP/CO x 80). An event button allows each time point to be marked and analysed. For each laboratory stress session the time points derived from the Finometer were: Baseline (the average recording of the last five of thirty minutes resting time); stress task 1 (5 minutes mean); stress task 2 (5 minutes mean); post stress recovery 1 (mean of 15-20 minutes post-stress); and recovery 2 (mean of 40-45 minutes post-stress).

5.2.3.4 Inflammatory Markers and Adipokines

Peripheral concentrations of Interlukin-6 (IL-6) and leptin were measured at rest and 45 minute after the stress protocol in each stress session. C-reactive protein (CRP) was measured at rest only. Samples were analysed in duplicate using Enzyme-linked immunoassorbent assay (ELISA) from R&D System (Oxford UK). The high sensitivity IL-6 assay has a limit of detection 0.016 pg/ml with a mean minimum detectable dose of 0.039 pg/ml and an intra and inter assay coefficient of variation (CV) of 7 and 7.2 per cent respectively. The CRP assay has a limit of detection of 0.005 ng/ml with a mean minimum detectable dose is 0.010 ng/ml and an intra and inter assay CV of 5.5 and 6.5 respectively. The minimum detectable dose of the leptin assay is typically less than 7.8 pg/mL with an intra and inter assay CV of 3.1 and 4.3 per cent respectively. Any one sample with a CV of >10% were re-assayed in triplicate and the mean of the two readings with the lowest CV was retained.

The device used to determine the optical density of the ELISA plates was the Molecular Devices (UK) Versa-Max Microplate Reader. The software used to analyse the optical density was the SoftMax® Pro 5 capable of reducing the raw absorbance data to a linear log/log parameter (IL-6 and leptin) and a 4 parameter logistic curve (CRP). The software is capable of generating a standard curve which is then used to compute the optical density values into a mean concentration value according to each ELISA kit protocol. All samples were processed and assayed by the PhD candidate.

5.2.3.5 Lipids analysis

Blood samples were collected into serum tube separators for lipids analysis. The variables for lipids were: triglycerides, total cholesterol, the total to high density lipoprotein (HDL) cholesterol ratio, and low density lipoprotein (LDL). Total cholesterol was assayed in a centrifugal analyser by the enzymatic colorimetric method. HDL cholesterol was measured after dextran sulfate–magnesium chloride precipitation of non-HDL cholesterol. LDL cholesterol was computed by using the Friedewald equation method. Lipid analyses were carried out at a validated biochemistry laboratory (The Doctor Laboratory, UK).

5.2.3.6 Mental Stress Protocol:

The stress protocol consisted of two 5 minute tasks designed to elicit mild stress responses, as described previously in Chapter 4: A public speaking scenario (PS) and a mirror drawing task (MT). These stressors are widely used in psychophysiology research and they have been previously used in our lab. These stress tasks were deemed to be suitable for the experimental design employed in this study because the responses from these tasks have been shown to be reproducible over repeated testing sessions with little habituation (Hamer et al., 2006)

5.2.3.7 Subjective Stress Appraisal and Mood Measures:

Subjective ratings of stress and anxiety were taken at five time points: end of rest period, after the PS and MT task, at recovery 1 (20 minutes post-stress) and at recovery 2 (45 minutes post-stress). In addition, task appraisal ratings were also taken at the end of each task. Task appraisal included the perceived level of difficulty, involvement and controllability. All subjective ratings were measured on a seven point Likert scale anchored at 0 (not at all) and 7 (very).

Mood was assessed using the 28 item General Health Questionnaire (GHQ-28) as previously described (Chapter 4). The GHQ-28 total score Cronbach's alpha at the baseline assessment was 0.85 and at the follow-up assessment 0.81. The total score and the depression subscale of the GHQ-28 were used as a measure of psychological distress and depressive symptoms. The mood questionnaire was administered at the baseline and follow-up research laboratory visit during the rest period, before the stress protocol.

5.2.4 Procedure.

Eligible participants were scheduled to attend the research laboratory on a weekday morning. Prior to their first visit, all participants received an appointment letter instructing them not to exercise for 24 hours prior to the appointment, not to consume any caffeinated drink, not to smoke, and to only have a light, low fat breakfast on the scheduled day. All participants signed an informed consent form before a research nurse measured their baseline anthropometric variables. Blood pressure was measured manually twice after a 30 minute rest period using an

automated UA-779 digital monitor. The mood questionnaire was completed by the participant during the rest period just before administering the stress tasks.

The psychophysiological stress protocol was then implemented. Briefly, after calibration of cardiovascular equipment and a 30 minutes rest period, the experimenter or the research nurse obtained blood and saliva samples and subjective ratings. The two stress tasks were administered in a random order with subjective ratings obtained after each task and a further saliva sample at the end of the tasks. A 45 minute post-stress recovery time then followed with subjective ratings and saliva samples obtained at 20 and 45 minute. The second blood draw was sampled at 45 minutes post-stress and the stress protocol ended. Participants received a light lunch and were then escorted to a research room to meet a member of the diet team from Weight Concern.

The follow-up laboratory visit was scheduled to take place on average nine weeks later at the same time as the baseline visit, and essentially followed the same protocol as in the baseline research session. Participants were tested in the psychophysiology laboratory by the same person in the two stress session, and all the diet meetings were held in an interview room adjacent to the psychophysiology laboratory.

All participants were reminded to avoid taking any medication in the week prior to testing. In case participants were ill or unwell on the testing day the appointment was rescheduled. Participants received an honorarium of £ 20.00 for taking part in the study. Figure 5.3 summarises the stress protocol and the measures collected.

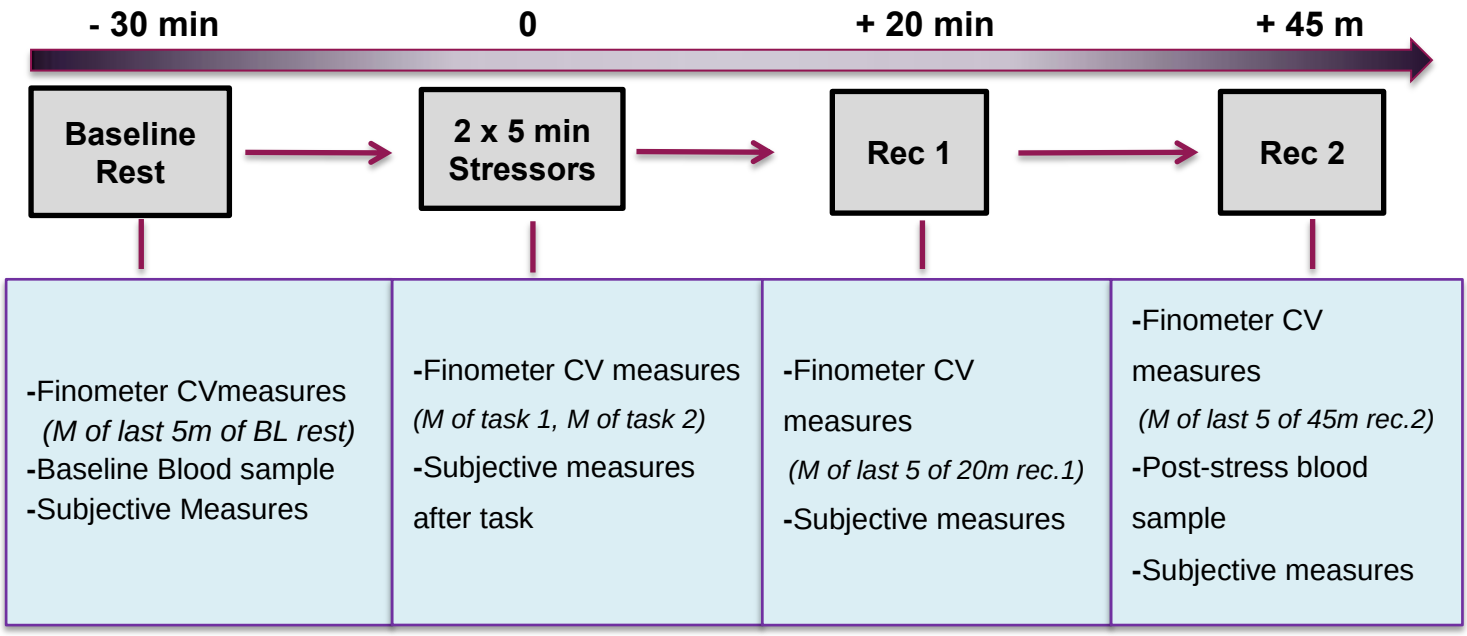


Fig-5.3: Summary of the measures obtained in the Weight Loss and Stress Responses Study

5.2.5 Statistical Approach.

Throughout the chapter the term "baseline" refers to the pre-weight loss stress testing session and "follow-up" refers to the post-weight loss stress session. "Rest" or "resting" refers to any measurement value taken before implementing the stress protocol when the participant is sitting down quietly. The assumption of normal distribution of the data was checked by examining the distribution and kurtosis/skewness values. Where appropriate the data were transformed using log or square root transformation.

The main stress-induced analyses were carried out using repeated measure ANOVA with stress session (baseline and follow-up) and time (rest, stress and recovery) as within-subjects factors using the Greenhouse- Geisser correction for degree of freedom in cases where the assumption of sphericity of the data was

violated. Main effect and interaction effects were explored using paired t-tests. Weight loss was calculated as absolute and relative change from baseline.

Change scores (Δ) were calculated as the difference between stress and rest values so that higher scores indicate higher reactivity. Pearson zero-order correlation and partial correlation was used to examine associations between stress-induced scores and change in leptin between baseline and follow up. Data are presented as means ± standard deviation (SD) or standard error of the mean (SEM).

5.3 RESULTS

5.3.1 Baseline characteristics of study sample

Table 5.1: Baseline characteristics of study sample (n = 15).

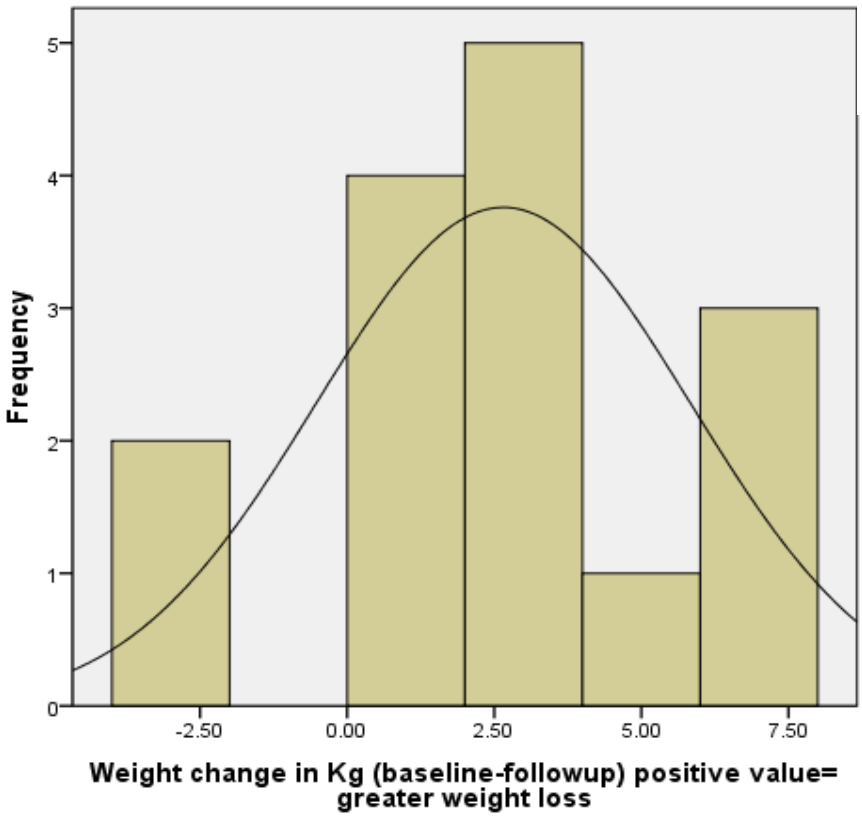
Variable	Mean ± SD	Range
Age (**years**)	30.54 ± 7.53	19.08 – 43.17
BMI (**kg/m^2**)	33.42 ± 3.99	27.70 – 42.50
WHR	0.79 ± 0.07	0.65 – 0.92
Waist circumference (**cm**)	91.30 ± 10.89	74.00 – 110.00
Fat Mass (**kg**)	38.52 ± 10.22	27.10 – 64.60
SBP (**mmHg**)	109.9 ± 9.98	95.00 – 129.00
DBP (**mmHg**)	78.7 ± 8.55	67.50 – 96.00
CRP (**mg/l**)	2.97 ± 3.06	0.33 – 9.39
Cholesterol (**mmol/L**)	4.88 ± 0.88	3.70 – 6.70

SBP = Systolic blood pressure, DBP = Diastolic blood pressure, WHR = waist to hip ratio, CRP = C-reactive protein.

Participants lost at follow-up (n = 8) did not differ from participant who completed the study (n = 16) in age, BMI and WHR (all P-values = ≥ 0.68). The mean follow-up time was 9.33 ± 0.96 weeks. Mean weight loss was - 2.65 ± 3.18 Kg (see figure 5.4) and the mean proportional weight change was - 3.25 ± 3.65 per cent. Mean BMI

change between baseline assessment and follow-up was - 1.01 ± 1.22 Kg/m^2 and the change in fat mass was - 3.59 ± 2.31 Kg. Data on the amount of weight lost by non-completers was available for seven participants only and the mean loss was 2.46 ± 2.65 Kg.

Fig-5.4: Distribution of weight loss body mass in Kg (n = 15).



5.3.2 Change in Resting BP and CRP levels After Weight Loss.

Resting SBP and DBP were measured manually following a 30 minute rest before administering the stress protocol. Following the weight loss intervention resting SBP did not change significantly from baseline (109.9 ± [SEM] 2.57 mmHg

vs. 107.76 ± 1.67 mmHg, p = 0.30). Resting DBP decreased significantly after weight loss (78.70 ± 2.21 mmHg vs. 74.66 ± 2.14 mmHg, p = 0.02). However, this reduction in DBP did not correlate significantly with reduction in fat mass (r = 0.36, p = 0.18), WHR (r = 0.29, p = 0.28), waist circumference (r = 0.13, p = 0.65) or body mass (r = 0.07, p = 0.80).

The mean CRP level at baseline was 2.97 ± [SD] 3.06 mg/L and 2.84 ± 2.91 mg/L at follow-up. Since CRP values were skewed, in order to test for differences in resting values after weight loss a log transformation was applied. A paired t-test showed that there was no significant difference between baseline and follow-up resting CRP levels ($t_{14} = 0.65$, p = 0.52).

5.3.3 Stress-Induced CV Responses.

In order to examine differences in reactivity (and recovery) to mental stress after weight loss it is important to examine whether subjective engagement or involvement in performing the two stress task is comparable across the two testing sessions. This was measured using a Likert scale that asked participants to rate the perceived difficulty and involvement of each task in each stress session. There was no difference in participant's ratings of speech task difficulty (M rating 5.06 ± [SEM] 0.37 vs. 4.73 ± 0.44, p = 0.57) and tasks' engagement (M rating 5.20 ± [SEM] 0.31 vs. 5.40 ± 0.36, p = 0.56) from the baseline compared to follow-up testing session.

In the mirror drawing task, task involvement also did not differ between the two testing sessions (Mean rating 6.46 ± [SEM] 0.16 vs. 6.07 ± 0.28, p = 0.11). However, perceived difficulty was slightly, and nearly significantly, lower at the follow-up stress

session (Mean rating 4.66 ± 0.41 vs. 3.87 ± 0.40, p = 0.07). Overall, participants' involvement in performing the stress tasks did not differ within the two stress testing sessions although in the mirror drawing task there was a trend towards lower perceived difficulty ratings in the post-weight loss stress session.

The tasks elicited marked increases in the CV parameters examined. SBP and DBP responses to the mirror (MT) and public speech (PS) tasks were highly correlated (r = 0.83) and were therefore averaged to create a mean stress score. CV responses were analysed using repeated measure ANOVAs with stress session (baseline and follow-up) and time (rest, mean stress, recovery 1 and recovery 2) as within-subject factors applying the Greenhouse-Geisser correction for degree of freedom when the assumption of sphericity was violated. Main effects and interaction effects were further explored using within-subjects t-test. Stress-induced blood pressure activity was measured continuously in the two stress testing sessions using a Finometer as described in the Method section.

5.3.3.1 Systolic Blood Pressure (SBP).

Results for SBP showed a significant main effect of time ($F_{1.60, 22.49} = 36.19$, p = <0.001) and stress session ($F_{1, 14} = 9.15$, p = 0.009) as well as a stress session by time interaction ($F_{3, 42} = 3.48$, p = 0.02) suggesting that SBP responses differed significantly across the two stress testing sessions. Resting SBP was significantly higher at baseline (M difference 4.79 ± [SEM] 1.77 mmHg, p = 0.01) compared to follow-up, as was the mean task stress value (M difference 8.58 ± [SEM] 2.12 mmHg, p = 0.001).

Fig-5.5: Systolic Blood Pressure Reactivity During Baseline (pre-weight loss) and Follow-up (post-weight loss) Psychophysiology Stress Testing. Values are means ±SEM, n=15

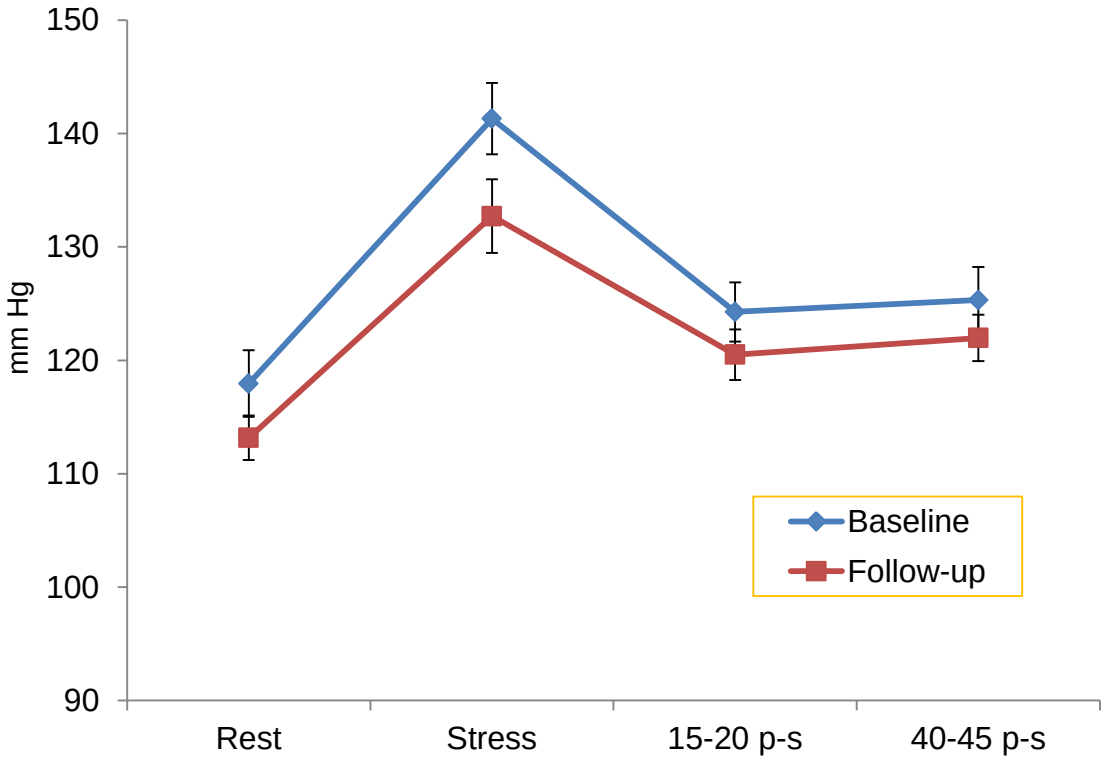


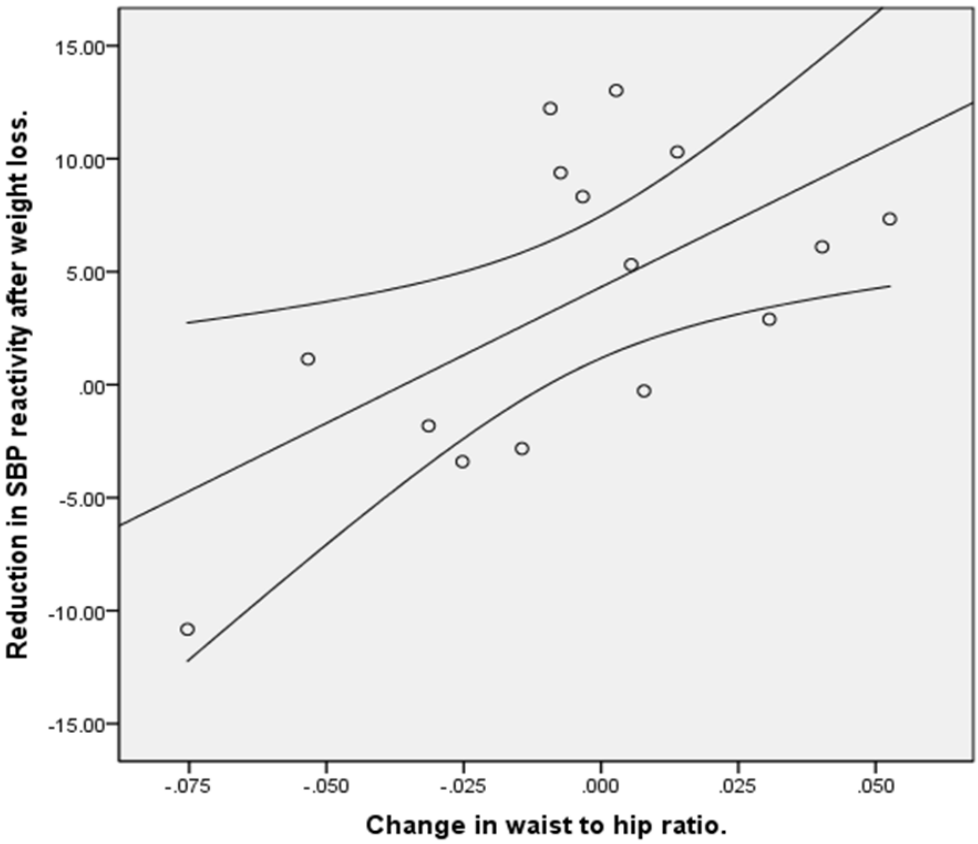
Figure 5.5 illustrates systolic blood pressure responses to mental stress during baseline and follow-up testing. A task reactivity score was computed as the mean difference between resting and stress values so that higher scores represent greater stress reactivity. At the baseline stress session reactivity was 23.34 ± 2.78 mmHg and at follow-up it was 19.55 ± 2.68. A paired t-test showed that the difference in reactivity following weight loss was significant ($t_{14} = -2.18$, $p = 0.04$).

The two stress recovery values (at 20 and 45 minute post-stress) were higher at baseline compared to the follow-up testing session (indicating poorer recovery from

stress) but this difference was not statistically significant (M difference recovery 1 [15-20m post-stress] = 3.77 ± [SEM] 2.16 mmHg, p = 0.10; M difference recovery 2 [40-45m post-stress] = 3.34 ± [SEM] 2.00 mmHg, p = 0.11).

Pearson zero-order correlation analysis was used to examine whether the change in SBP reactivity ($\Delta SBP_{baseline} - \Delta SBP_{follow\text{-}up}$) following weight loss (higher values indicates greater attenuation) correlated with reduction in obesity and/or adiposity measures. ΔSBP-reactivity was related to reduction in: WHR (r = 0.60, p = 0.02) and waist circumference (r = 0.52, p = 0.04) but not reduction in fat mass (r = -0.01, p = 0.96) or BMI (r = 0.23, p = 0.41). These results suggest that the attenuated systolic reactivity to mental stress following weight loss is likely due to changes in measure of central obesity (see figure 5.6).

Fig-5.6: Association of ΔSBP reactivity between baseline and follow-up with ΔWHR post weight loss n = 15. Greater ΔWHR positively associated with greater reduction in SBP.



In order to investigate whether the lower reactivity observed at the follow-up stress session may be accounted for by differences in resting values, a proportional reactivity score was calculated. In the baseline session the reactivity per cent value was 20.25 ± [SEM] 2.67 whereas in the follow-up stress session the value was 17.37 ± [SEM] 2.42.

This difference was analysed with a related t-test that showed a near significant higher reactivity of 2.88 per cent (SEM 1.53, $p = 0.08$) at baseline compared to the follow-up testing session. This shows that the lower resting SBP after weight loss accounts only in part for the attenuated reactivity to mental stress observed after weight loss.

5.3.3.2 Diastolic Blood Pressure (DBP).

Analyses of stress-induced DBP responses revealed a main effect of time ($F_{1.724, 24.14} = 46.52$, $p = <0.001$), and stress session ($F_{1, 14} = 6.22$, $p = 0.02$) and a near significant stress session by time interaction ($F_{3, 42} = 2.37$, $p = 0.08$). The DBP reactivity to and recovery from mental stress is illustrated in figure 5.7.

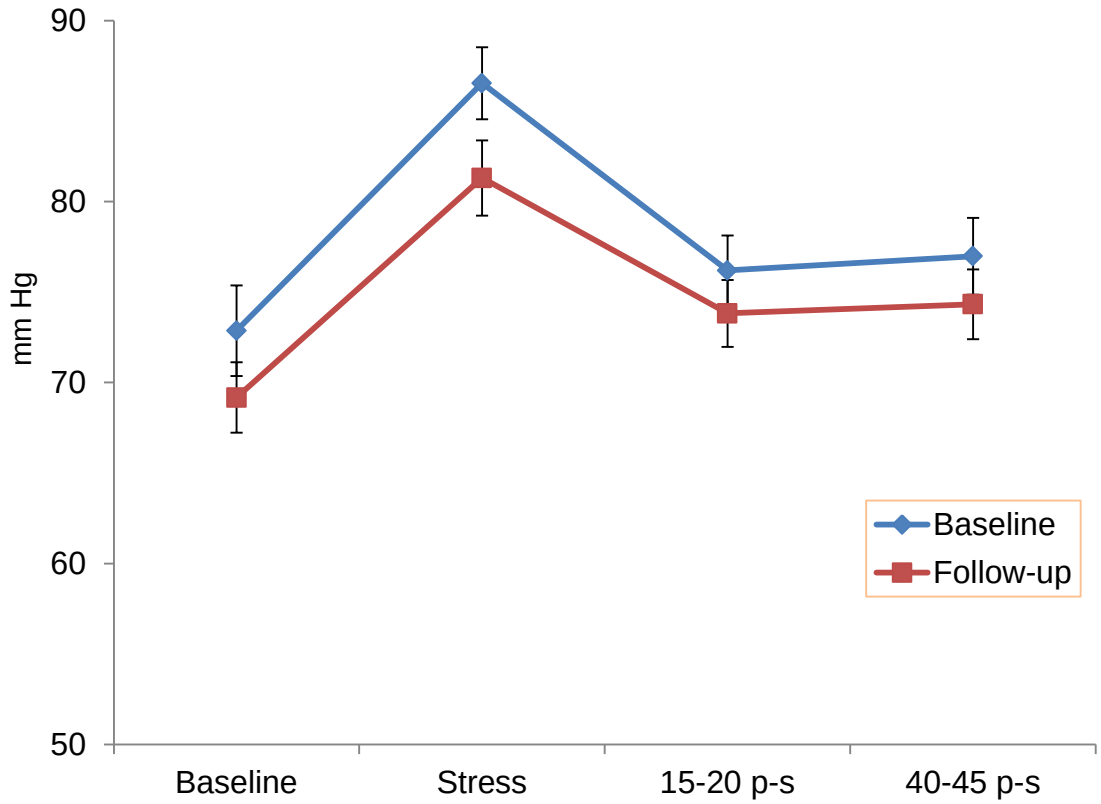


Fig-5.7: Diastolic Blood Pressure Reactivity During Baseline (pre-weight loss) and Follow-up (post-weight loss) Psychophysiology Stress Testing n = 15.

Within-subjects analyses of DBP showed significantly higher resting DBP at baseline compared to follow-up (M difference 3.69 ± [SEM] 1.58 mmHg, p = 0.03) and during stress (M difference 5.23 ± [SEM] 1.28 mmHg, p = 0.001), but not at recovery 1 (M difference 2.37 ± [SEM] 1.69 mmHg, p = 0.18) or recovery 2 (M difference 2.64 ± [SEM] 1.71 mmHg, p = 0.14).

The stress reactivity scores were again compared within the two stress testing sessions. At baseline the reactivity score was 13.67 ± 1.49 mmHg, and 12.13 ± 1.60 at follow-up but the difference was not statistically significant ($t_{14} = -1.31$, p = 0.21). Pearson correlation analysis was again used to determine whether the attenuated

post-weight loss diastolic reactivity (∆DBP-react) could be explained by changes in obesity and/or adiposity parameters. Similarly to SBP, ∆DBP-react was related to reduction in waist to hip ratio (r = 0.52, p = 0.04) and waist circumference (0.50, p = 0.05), but not to fat mass change (r = - 0.06, p = 0.82) or BMI (r = 0.14, p = 0.63).

Since there was a significant change in resting DBP, a proportional stress reactivity change score was calculated and compared within the groups. Per cent stress reactivity was 19.6 ± [SEM] 2.51 and 18 ± 2.59 in the baseline and follow-up testing session respectively but the difference was not statistically significant (p = 0.41). This indicates that the attenuated reactivity observed post weight loss is largely accounted for by lower resting DBP baseline values.

5.3.3.3 Total Peripheral Resistance (TPR).

TPR was analysed in the same way since there was a strong correlation between TPR reactivity to the MT and PS stress tasks (r = 0.87). There was a main effect of time ($F_{1.48, 20.77} = 11.98$, p = 0.001) but not a main effect of stress session ($F_{1, 14} = 0.04$, p = 0.82) nor a stress session by time interaction ($F_{3, 42} = 0.62$, p = 0.60) indicating a significant change in TPR over time that did not differ between the weight loss groups at the baseline and follow up stress sessions. The TPR activity during the stress sessions is depicted in figure 5.8.

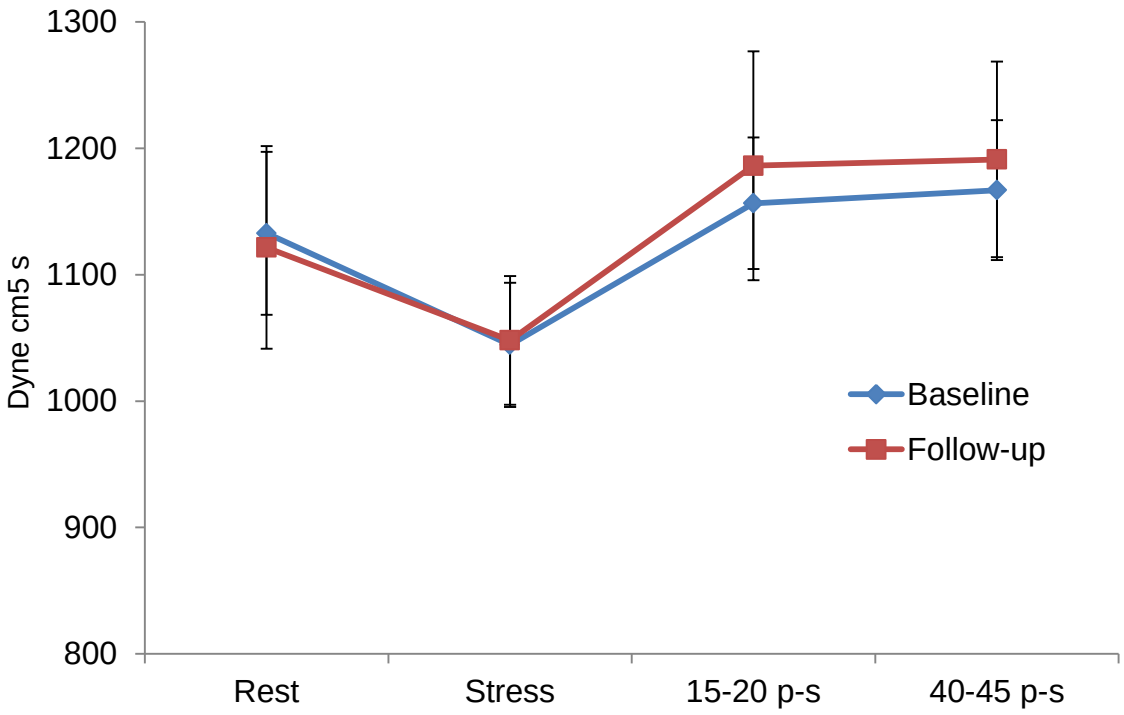


Fig-5.8: Total Peripheral Resistance at the Baseline and Follow-up Stress Session n =15.

5.3.3.4 Heart Rate (HR).

For HR responses to mental stress, the correlation between responses to the MT and the ST stress tasks were high (r = 0.94) and were therefore averaged to produce a mean stress task value. The HR means ± SEM values during the baseline and follow-up stress testing session are illustrated in figure 5.9

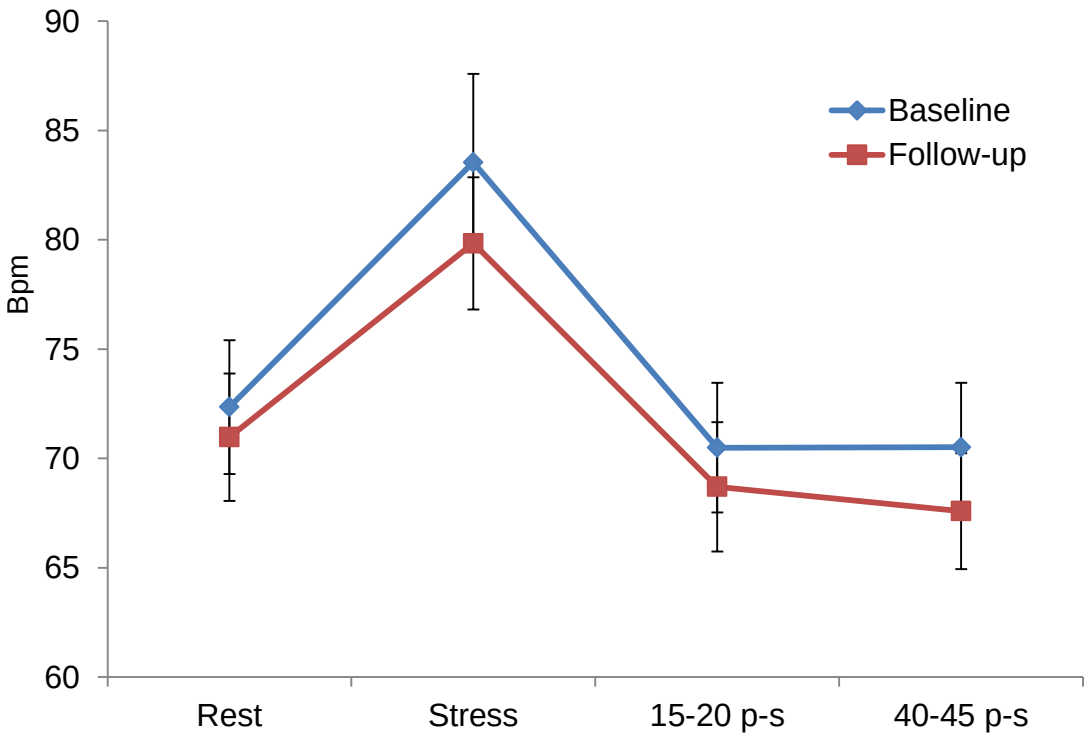


Fig-5.9: Heart Rate at the baseline and follow-up stress session. Weight loss and stress responses study n =15

The main effect of time was significant ($F_{1.28, 18.01} = 36.39$, $p = <0.001$), but there was no main effect of stress session ($F_{1, 14} = 0.82$, $p = 0.38$) or stress session by time interaction ($F_{1.8, 25.22} = 0.83$, $p = 0.43$) suggesting that the dynamic of the HR responses did not differ across the two testing sessions.

The stress reactivity score at baseline was 11.20 ± [SEM] 2.04 bpm and 8.86 ± 2.28 bpm at follow-up. However, when this task reactivity scores were compared using a within-subjects t-test the results indicated that the attenuated reactivity at follow-up was not statistically significant ($t_{14} = -1.16$, $p = 0.26$).

Likewise, the stress recovery score (computed as the mean difference between resting and 45 minute post-stress values) was calculated and compared in order to

investigate the effect of weight loss on recovery from stress. The mean difference in HR stress recovery from baseline to follow-up (-1.55 ± [SEM] 1.38 bpm) was not statistically significant ($t_{14} = -1.12$, $p = 0.28$).

5.3.4 Stress-Induced Inflammatory Responses.

Pro-inflammatory IL-6 responses to mental stress were evaluated for all participants. At baseline, resting IL-6 averaged 1.43 ± [SD] 1.18 pg/ml (range 0.44 to 5.42) increasing to 1.49 ± 1.33 pg/ml 45 minute post-stressor (a 4.19 per cent increase). At follow-up, resting IL-6 averaged 1.33 ± 0.75 pg/ml (range 0.53 to 3.28) increasing to 1.46 ± 0.80 pg/ml 45 minutes post-stress (a 10.5 per cent relative increase). Figure 5.10 depicts the IL-6 response to mental stress at the baseline and follow-up stress sessions.

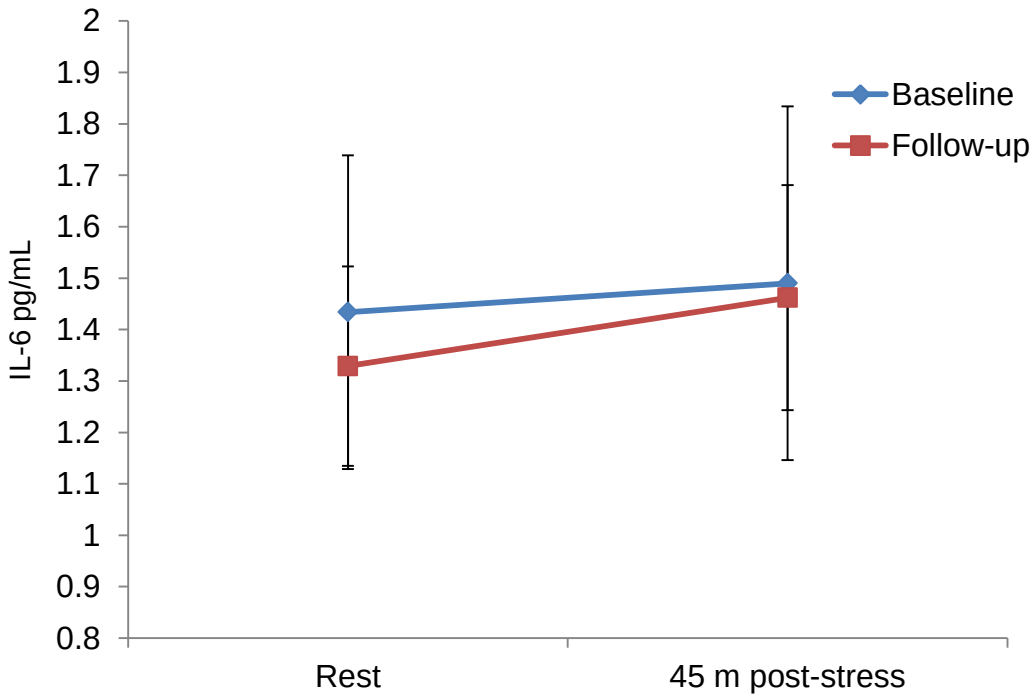


Fig-5.10 Stress-induced IL-6 responses in the 2 stress sessions n = 15.

The result of the within-subjects ANOVA test revealed no main effect of time ($F_{1, 14} = 2.79$, $p = 0.11$) or stress session ($F_{1, 14} = 0.12$, $p = 0.73$), and a non-significant stress session by time interaction ($F_{1, 14} = 0.25$, $p = 0.62$) suggesting no differences in stress-induced IL-6 responses from baseline to follow-up.

A mean IL-6 stress reactivity score was computed as the mean difference between resting and 45 minute post-stress values so that higher scores represent greater reactivity to stress. The scores were compared between baseline and follow-up in order to examine differences in reactivity before and after weight loss. Baseline reactivity was 0.06 pg/mL (SD 0.23) while follow-up reactivity was 0.13 pg/mL (0.45), and a paired t-test showed no statistically significant difference between baseline and follow-up reactivity ($t_{14} = 0.5$, $p = 0.62$). Because the distribution of IL-6 data tended to be skewed, these analyses were repeated using square root transformed IL-6 values but results did not change.

Correlational analysis showed that follow-up IL-6 reactivity did not correlate with changes in the adiposity related measures WHR (r = 0.09, p = 0.74), fat mass (r = -0.21, p = 0.44) or BMI (r = - 0.17, p = 0.53).

5.3.5 Associations of Leptin with Inflammatory and Cardio Metabolic Risk Factors.

Resting leptin values were normally distributed and ranged from 12.06 to 72.30 ng/mL (Mean 41.40 ± [SD] 19.84 ng/mL) at baseline, and 5.18 to 77.54 ng/mL (Mean 29.65 ± 19.21 ng/mL) at follow-up. Stress-induced leptin response values are shown in figure 5.11.

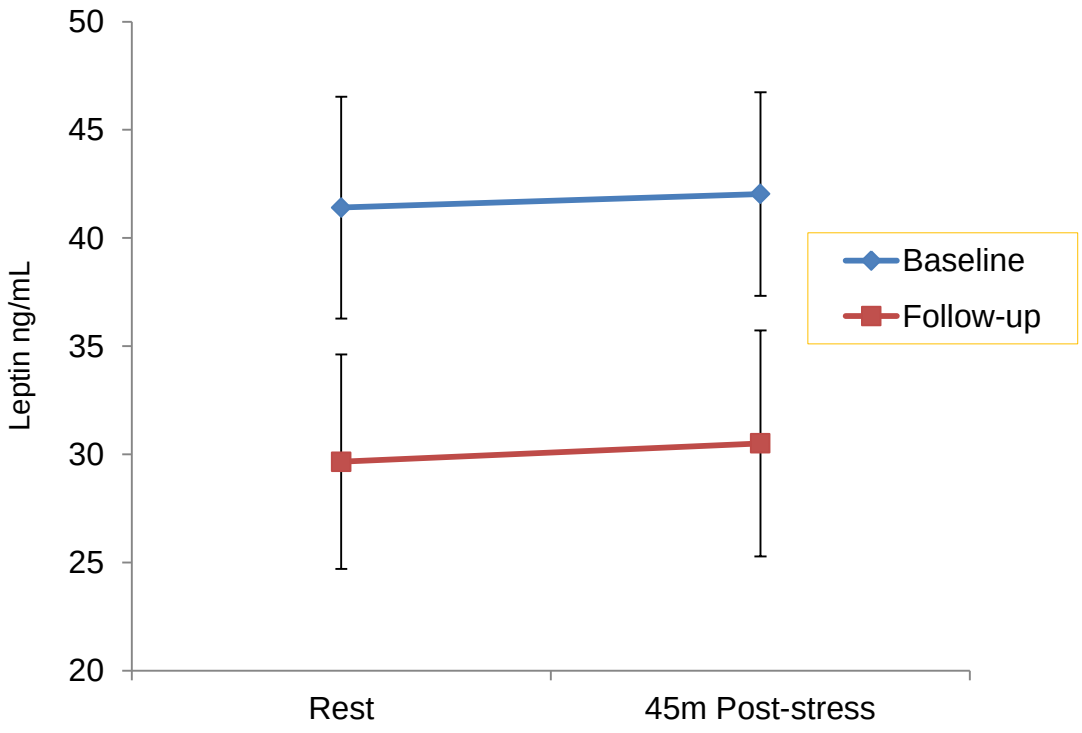


Fig-5.11: Stress-induced leptin responses in the two stress sessions n = 15

Resting leptin levels at baseline were highly correlated with baseline BMI (r = 0.87, p = <0.001), waist circumference (r = 0.68, p = 0.005), per cent fat mass (r = 0.66, p = 0.008), CRP (r = 0.66, p = 0.01) and basal IL-6 (r = 0.56, p = 0.03) but not with age (r = 0.2, p = 0.48) and resting SBP (r = 0.13, p = 0.64). Resting levels of CRP and IL-6 at baseline were also positively correlated (r = 0.64, p = 0.01).

A within-subject ANOVA showed that the main effect of time was not significant ($F_{1, 14}$ = 2.60, p = 0.13) indicating no increase in response to stress but a significant main effect of stress session ($F_{1, 14}$ = 13.46, p = 0.003) suggesting differences in leptin levels between the baseline and the follow-up stress sessions. The stress session by time interaction was not significant ($F_{1, 14}$ = 0.05, p = 0.83).

Following the weight loss intervention resting serum leptin markedly decreased (M difference = – 11.75 ± [SEM] 3.25 ng/mL, p = 0.003). The change in leptin (Δleptin) was positively and significantly correlated with reductions in BMI (r = 0.64, p = 0.01) and body mass (r = 0.65, p = 0.008), but only marginally with reduction in waist circumference (r = 0.43, p = 0.10) and not with change in WHR (r = 0.08, p = 0.76) and fat mass (r = 0.2, p = 0.50) measured at the follow-up visit.

In order to examine whether this reduction in leptin following weight loss correlated with stress-induced autonomic responses, correlation analyses were run with the stress reactivity values (difference between stress and rest values [Δ]) of the follow-up stress session. There were no significant correlations between weight loss induced reductions in leptin and ΔSBP (r = 0.03, p = 0.92), ΔDBP (r = 0.04, p = 0.89), ΔHR (r = 0.14, p = 0.63) and ΔTPR (r = - 0.06, p = 0.81).

5.3.5.1 Leptin and Inflammatory responses

The same method was used to examine the correlation of reduction in leptin with stress-induced IL-6 responses (difference between 45m post-stress and rest values) at the follow-up stress session. Leptin reduction was negatively correlated with ΔIL-6 ($r = -0.62$, $p = 0.01$; shown in figure 5.12) suggesting that a greater reduction in leptin was associated with an attenuated IL-6 response to stress after weight loss.

When controlling for ΔIL-6 during the baseline stress session the correlation between reduction in leptin and ΔIL-6 at the follow-up stress session was slightly reduced ($r = -0.53$, $p = 0.06$). This suggests that independently of the baseline (pre-weight loss) ΔIL-6 stress reactivity, a greater reduction in leptin is associated with an attenuated IL-6 response to stress at follow-up.

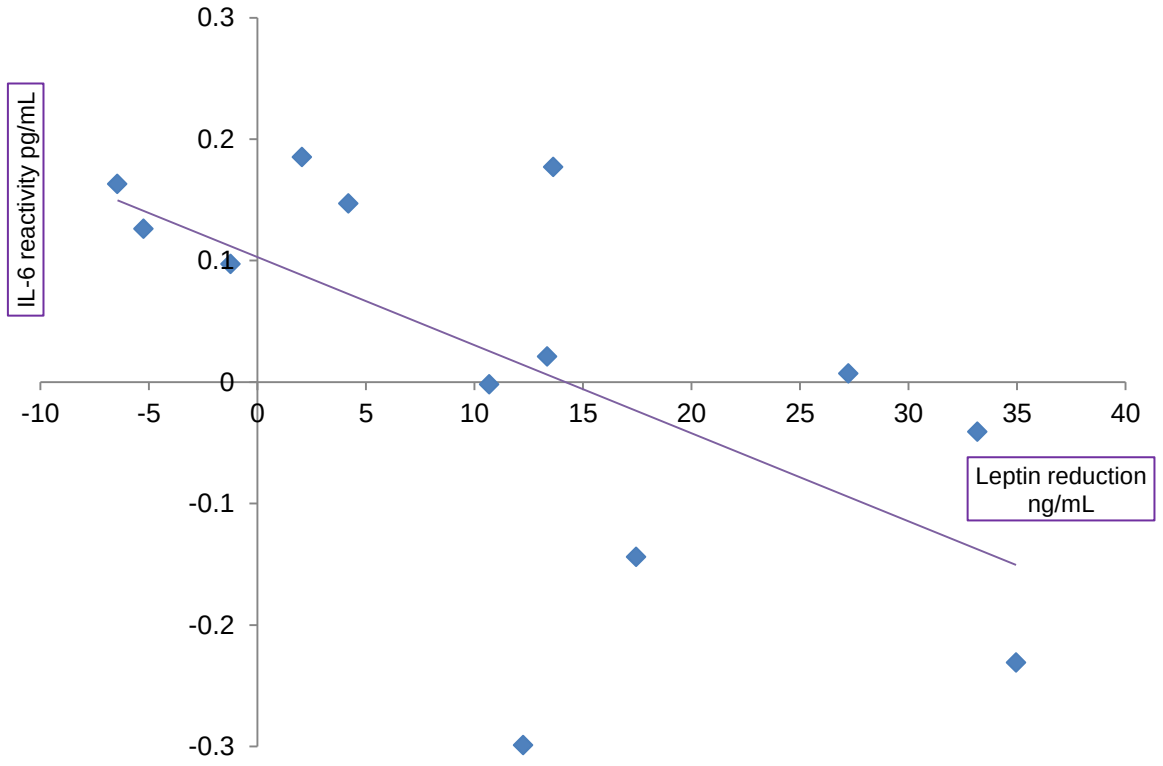


Fig-5.12 Association of leptin reduction following weight loss with stress-induced IL-6 at the follow-up stress testing.

Since reactivity values may be influenced by resting levels, this analysis was repeated with further adjustment for resting IL-6 levels at follow-up. Results indicated that controlling for stress-induced ΔIL-6 at baseline testing and resting IL-6 at follow-up testing, weight loss induced reductions in leptin were still associated with ΔIL-6 ($r = - 0.58$, $p = 0.05$).

This finding was further explored by modelling the change in waist circumference and/or fat mass into the model. Since leptin is mainly produced by adipocytes, the association between leptin and attenuated IL-6 responses observed above may be due to a loss in adiposity. The reduction in leptin may therefore be secondary to changes in adiposity which then drives the attenuated IL-6 responses to stress.

When changes in waist following weight loss was controlled for the association remained virtually unchanged ($r = - 0.56$, $p = 0.05$). The same results were obtained when changes in BMI was entered instead of waist circumference ($r = - 0.66$, $p = 0.02$). However, when changes in fat mass was controlled for, the association became not significant ($r = - 0.54$, $p = 0.07$). These data suggest that a reduction in leptin after weight loss was associated with attenuated IL-6 responses but the association is in part explained by reductions in adiposity.

5.3.6 Stress-induced Lipid Responses

As illustrated in table 5.2, resting cholesterol did not significantly change after weight loss ($p = 0.27$). Likewise, baseline triglyceride levels ($p = 0.39$) and the total to high-density lipoprotein cholesterol ratio (3.28 ± [SEM] 0.18 mmol/L vs. 3.37 ± 0.22, $p = 0.35$) did not change after weight loss.

Table-5.2 Summary of resting lipid values before and after weight loss n=15

Lipids	Baseline (M ± SD)	Follow-up (M ± SD)
Triglycerides	1.06 (0.38)	1.15 (0.54)
Tot cholesterol	4.88 (0.88)	4.72 (0.88)
LDL-cholesterol	2.86 (0.79)	2.75 (0.78)
HDL-cholesterol	1.53 (0.35)	1.44 (0.26)

LDL = low-density lipoprotein HDL = high-density lipoprotein

Lipids data were normally distributed. There was a significant main effect of time for cholesterol ($F_{1, 14} = 6.91$, $p = 0.02$) but not of stress session ($F_{1, 14} = 1.16$, $p = 0.30$) nor a stress session by time interaction ($F_{1, 14} = 0.035$, $p = 0.85$) suggesting that cholesterol levels increased after stress by the same magnitude in both stress testing visits. For LDL cholesterol the main effect of time was significant ($F_{1, 14} = 4.69$, $p = 0.048$) but again there was no main effect of stress session ($F_{1, 14} = 0.74$, $p = 0.40$) nor a stress session by time interaction ($F_{1, 14} = 0.10$, $p = 0.75$).

5.3.7 Weight Loss and Changes in Mood Symptoms.

Changes in mood symptoms following weight loss were measured using the GHQ-28 which consists of four subscales as well as a total score. Table 5.3 shows the mean scores on the GHQ subscales before and after the weight loss

intervention. Overall, there was an improvement in mood after weight loss as evidenced by a 6.63 point difference in the GHQ total score.

This difference was more pronounced in the depression subscale with a change of 3.38 points. The social dysfunction scale changed by 1.9 point but only approached significance while the somatic symptoms and the anxiety /insomnia scale did not change significantly.

Table-5.3: Weight loss and stress responses study, GHQ-28 mean scores n =15.

Scale	Baseline Pre-weight loss	Follow-up Post-weight loss	P
Social dysfunction	8.36 ± 2.74	6.40 ± 2.89	0.08
Anxiety/insomnia	6.93 ± 4.04	5.86 ± 3.92	0.38
Somatic symptoms	4.81 ± 3.20	4.59 ± 4.08	0.85
Depression	8.31 ± 4.68	4.93 ± 4.01	**0.001**
GHQ-Total score	28.42 ± 9.66	21.78 ± 10.31	**0.01**

In order to examine whether improvements in the GHQ depression scale and the total score was related to adiposity measures, zero-order Pearson's analysis was used. Improvements in GHQ total score were not related to reduction in WHR (r = - 0.17, p = 0.53), BMI (r = - 0.07, p = 0.81), body mass (r = - 0.074, p = 0.79) and fat mass (r = - 0.06, p = 0.82). In the depression subscale there were no significant correlations in any of the obesity measures.

Spearman Rho correlation was employed to examine whether changes in GHQ depression and GHQ total score following weight loss were related to changes in resting levels of leptin, CRP and IL-6 between baseline and follow-up. The GHQ total score change was not related to reduction in leptin (rho = 0.16, p = 0.57), CRP (rho = 0.1, p = 0.74) and IL-6 (rho = - 0.45, p = 0.10). Similar results were obtained when changes in the depression subscale was used. These findings suggest that improvements in mood symptoms after weight loss are not related to changes in obesity and adiposity measure or reductions in inflammatory markers and leptin.

Further correlations were used to examine whether these improvements in mood might be related to stress-induced CV and inflammatory responses at the follow-up stress testing session. However, neither the GHQ total score change nor depression change correlated with any of the stress-induced variables (blood pressure, heart rate, systemic vascular resistance and IL-6) suggesting that improvements in depressive symptoms following weight loss are not related to CV or inflammatory responses to mental stress.

5.3.8 Secondary Exploratory Analysis.

In order to carry out an exploratory subgroup analysis, participants whose fat mass did not change by at least two kilos were excluded (n = 3). Therefore, the remaining sample included 12 participants. However, results did not change from the main analyses and there were no further differences in any of the stress-induced parameters examined.

5.4 DISCUSSION

The aim of this study was to examine the effects of experimentally induced weight loss on cardiovascular, neuroendocrine and inflammatory responses to standardized mental stress. At present, there is conflicting evidence on the association of obesity with cardiovascular stress responses. Some studies have reported that obesity is associated with blunted and others heightened reactivity to mental stress (Benson et al., 2009; Carroll et al., 2008; Epel et al., 2000; Steptoe & Wardle, 2005). Some of these inconsistencies may be attributable to variations in the stress protocols employed, study design, the markers under investigation, and in the population studied. For example, stress tasks used to elicit autonomic responses vary in the type of responses they elicit with public speaking protocol being more effective in eliciting a myocardial response while protocols like the TSST stimulate a marked HPA axis response. In addition, stress-induced inflammatory responses in obesity have received little attention and in general there is a lack of experimental work in this area.

The nine week weight loss intervention resulted in modest reductions of body mass and fat mass. The reduction in waist circumference and in the waist to hip ratio was also small. It has been suggested that substituting a main meal with commercially available meal replacements results in a reduction of food intake and significant weight loss over a two week period (Levitsky & Pacanowski, 2011). A previous small weight loss intervention study that used a protocol similar to this study found that participants randomized to a structured meal replacement plus weekly support and physical activity advice group lost 8.5 per cent of body weight after eight

weeks (Anderson, Raymond Reynolds, Bush, Rinsky, & Washnock, 2011). However, that study included a physical activity component, in addition to intensive nutritional counselling, which we did not want to use in our study. Thus, this may be the reason why this study achieved a more modest change in weight (3.07 per cent). It has been shown that when exercise training is combined with diet it may only add a modest but significant additional weight loss (Shaw, Gennat, O'Rourke, Del Mar, 2006). Furthermore, exercise alone even without weight loss is associated with improvements in CV risk markers (Ross & Bradshow, 2009). Therefore, adding a physical activity component may result in additional reduction in weight that is likely to obscure the associations between adiposity and stress responses.

Resting diastolic, but not systolic, blood pressure was significantly reduced following weight loss but this reduction did not correlate significantly with loss of fat mass or weight loss. Although the size of the correlation was 0.36 it did not reach significance level probably because of the low statistical power. Likewise, resting levels of CRP, IL-6 and lipids were not significantly reduced following the weight loss intervention. As expected and in line with previous studies, resting leptin levels at study entry were highly correlated with adiposity measures and inflammatory markers, and absolute levels were comparable with other studies. For example, resting leptin level averaged 37.9 ng/ml in a large sample of women with a mean BMI of 32.5 kg/m^2 (Rock et al., 2010), and 36.7 ng/ml in a study of overweight older women with type 2 diabetes (Labad et al., 2011).

Following the intervention leptin levels dropped by an average of 28.4 per cent, and this reduction was highly correlated with changes in weight, BMI and fat mass, a finding that is consistent with data from a recent weight loss study (Sumithran et al., 2011). This suggests that it was the loss in adiposity that induced the reduction in

leptin levels rather than other non-specific factors. A previous small scale prospective study of overweight or obese middle age healthy men and women using a very low calorie dietary formulation that achieved a 14 per cent weight loss showed that fasting leptin levels decreased by 64 per cent at the 10 week follow-up (Sumithran et al., 2011). However, in another study of obese women randomized to a free prepared meal replacement and support group the six month change in resting leptin was 38 per cent (Rock et al., 2010). These latter two weight loss interventions were of longer duration and used either a very low calorie diet and weekly support sessions or free prepared meals and weekly support sessions. Therefore, those studies used an intensive and costly protocol including medical supervision with the provision of food. In this study we used a partial meal replacement strategy coupled with weekly supervision aimed at addressing and managing barriers to weight loss and adherence to the programme plus cognitive behavioural skills such as self-monitoring, stimulus control and response substitution in order to facilitate weight maintenance after the intervention.

Overall, the results indicate that despite an overall modest reduction in obesity and adiposity measures, the intervention was successful in modifying some cardio-metabolic risk factors. This result may be in line with recent evidence showing that improvements in obesity-related metabolic dysfunctions can be achieved despite minimal or no weight loss (body mass) but through lifestyle modification (i.e. healthy diet) (Ross & Bradshaw, 2009). Indeed, these changes may be achieved through reduction in visceral fat depots that can be better measured using imaging techniques such as DEXA.

The psychophysiology stress protocol was implemented at baseline before the weight loss intervention, and again after an average of nine weeks in order to

evaluate whether adiposity would be associated with autonomic stress responses. A significant difference was observed in the systolic and diastolic blood pressure reactivity to mental stress between sessions. Participants showed attenuated blood pressure reactivity after weight loss compared to baseline values and this difference was only in part accounted for by an attenuation in post-weight loss resting systolic pressure. Systolic blood pressure recovery from mental stress although lower after weight loss did not reach statistical significance. The attenuated diastolic blood pressure reactivity instead could be wholly accounted for by lower resting values after weight loss. No further differences in stress reactivity and recovery were observed in the other cardiovascular parameters examined.

We did not employ a non-weight loss control group and thus it is impossible to be certain that it was adiposity driving the attenuated reactivity and not other factors such as habituation to the tasks or familiarity with the experimenter or natural variability. However, the reduction in reactivity between the two stress sessions was positively and strongly related to reduction in adiposity in the predicted direction. In addition, we have previously demonstrated strong reproducibility of these responses over two repeated stress sessions, with no changes in blood pressure reactivity (Hamer et al., 2006). This further supports the notion that obesity is likely to result in heightened cardiovascular stress responses, but weight loss, especially loss of central fat mass, may result in attenuated responses.

Few studies have examined the effects of experimentally induced weight loss on cardiovascular reactivity. Weight loss through four months exercise training and diet, but not diet alone, improved an impaired vasodilatation response to mental challenge in obese children (Ribeiro et al., 2005). In another intervention that examined the effects of a six month weight loss programme on stress reactivity in obese,

hypertensive participants, absolute BP levels were reduced during mental stress after weight loss although BP reactivity was unchanged compared with controls (Georgiades et al., 2000). Another study that has addressed this issue was a non-randomized 12-week low calories weight loss programme carried out with overweight men (Torres & Nowson, 2007). It was found that weight loss had an effect on resting blood pressure but not on reactivity to stress compared to a non-weight loss control group. In addition, post-stress recovery was enhanced in the weight loss group but this was in part accounted for by lower resting levels. However, Torres and colleagues' study included participants on anti-hypertensive drugs and the results were not adjusted for medication use and resting blood pressure differences making the results difficult to interpret. Therefore, we have shown that reduction in adiposity following weight loss achieved through caloric restriction is associated with reduced blood pressure responses to mental stress.

There were no significant differences in resting levels of IL-6 and CRP following weight loss indicating that the intervention did not have an effect on basal levels of pro-inflammatory markers. A meta-analysis of the effect of weight loss achieved through lifestyle interventions on CRP reported a weighted effect size of $r = 0.3$ between weight change and decline in resting CRP levels (Selvin et al., 2007). However, the weight loss induced reduction in IL-6 is generally much smaller and not consistently observed (Byers & Sedjo, 2011; Heinonen et al., 2009). The lack of an effect observed in this study might be attributable to the small weight change achieved and the fact that our participants were overweight or obese but otherwise healthy. Moreover, physical activity participation was not encouraged throughout the duration of the study but only after the follow-up stress session in order to rule out possible confounding effects. It may be the case that the inclusion of participants

with greater obesity and a larger sample size may facilitate the detection of differences in inflammatory markers.

IL-6 did not significantly increase at 45 minute post-stress in either the baseline or the follow-up stress session. The lack of stress reactivity is likely due a combination of insufficient power to detect a significant increase, the relatively mild mental stress tasks, and the timing of the second blood sample; peak post-stress IL-6 responses may be detected a hour or more after behavioural stimulation (von Kannel, et al., 2006). Similarly, leptin did not increase post-stress but resting levels markedly decreased in proportion to fat mass loss between the baseline and the follow-up session. It is difficult to understand whether the lack of a leptin response indicates a state of leptin desensitization that after weight loss has improved or a ceiling effect on reactivity driven by higher resting level pre-weight loss. It may also be that, as argued above, the stress protocol was not robust enough to elicit a detectable inflammatory response at 45 minute, which might have been present at 60 minute or later in this population.

However, post-hoc analyses on weight loss induced reduction in basal leptin and stress-induced inflammatory changes at follow-up showed some interesting findings. In partial correlation analysis, a reduction in resting leptin was associated with an attenuated IL-6 stress response at follow up independent of resting levels. This relationship also remained significant when adjusted for the IL-6 stress response at the pre-weight loss session suggesting that the association cannot be accounted for by baseline IL-6 reactivity. Yet, when the change in fat mass post-intervention was added to the model, the association became non-significant suggesting that adiposity might at least in part be driving the association between leptin and post-stress IL-6 change.

There are two possible interpretations for this finding. Since the observed reduction in leptin was mainly driven by loss of adiposity, this implies that the reduction in leptin may be a more precise measure of reduction in adiposity in this study than waist circumference or WHR. In this way the negative correlation between IL-6 and leptin (shown in Figure 5.12) may be accounted for by loss of adiposity which was better captured by a reduction in leptin. This notion may be supported by the observation that the leptin association with IL-6 was reduced to non-significance when fat mass change was entered into the model. Alternatively, the reduction in leptin might have partially restored the normal autonomic sympathetic – leptin balance which is thought to be disturbed in the obese state (Quilliot, et al., 2008). Consequently, this restoration of normal sympathetic function may have activated the cholinergic anti-inflammatory pathway resulting in an attenuated IL-6 response to stress.

Previous work has shown that high resting leptin is cross-sectionally associated with greater stress-induced IL-6 responses, reduced heart rate variability and greater HR response to stress (Brydon, O'Donnell Wright, Zachary, Wardle, Steptoe, 2008). Since leptin levels are proportional to adipose tissue, and appear to be elevated in overweight and obese women, an association between weight loss induced change in leptin and IL-6 stress change may support the hypothesis that adiposity promotes greater stress-induced inflammatory responses that may resolve with weight loss. Indeed it has been shown that obesity related autonomic dysfunction is reversible even with a modest amount of weight loss leading to an improvement in cardiac autonomic modulation and enhanced neural sympathetic responsiveness (Straznicky et al., 2010). However, this hypothesis is speculative since the observed relationship was only present in correlational analysis and not in the main analysis. In addition,

we did not observe any association between changes in leptin and stress-induced blood pressure and HR, and we did not employ a measure of autonomic balance such as heart rate variability. Nonetheless, this finding suggests an intriguing relationship between adiposity, leptin and the inflammatory pathway.

Mood symptoms measured with the GHQ showed an improvement after weight loss which was driven mainly by changes in the depression subscale. However, this improvement in mood did not correlate with changes in weight or adiposity measures, or with post-weight loss reduction in resting pro-inflammatory markers. In fact, there was a trend pointing towards a correlation between greater changes in depression and lower change in IL-6 which suggests that the mechanisms underlying the improvements in mood is not a loss of adiposity or an attenuation in inflammatory factors. This mechanism is likely to be attributable to a non-specific effect of the weight loss intervention. The intervention protocol included weekly nutritional counselling sessions with Health Psychologists that served to motivate and monitor participants, reviewing goals, and generally used psychological techniques to facilitate weight loss.

It is therefore conceivable that since the improvements in mood did not correlate with the outcomes under investigation, the observed mood effect is largely explained by the social support and counselling element included in the weight loss protocol. Indeed a recent meta-analysis of the effect of weight loss on symptoms of depression (Fabricatore et al., 2011) reported that although weight loss interventions were generally associated with improvements in depressive symptoms this could not be fully explained by weight loss. The authors argued that factors such as learnt cognitive behavioural strategies led participants to master skills such as self-mastery and self-efficacy that were then responsible for the improvement in symptoms of

depression. Thus, in order to disentangle the effects of weight loss from social support it would have been desirable to collect further follow up data several months after the intervention or having employed a non-weekly support control group.

In the Introduction it was argued that inflammatory factors might mediate the bi-directional association between obesity and depressed mood, and it is generally accepted that major depression is associated with raised level of certain pro-inflammatory cytokines (Dowlati et al., 2010). The present study did not find any evidence of associations between depressive symptoms and changes in CRP and IL-6. However, on average, there were no significant changes in resting levels of CRP and IL-6 post-weight loss and therefore it was not possible to examine this issue. In addition, this study was performed in a small, healthy sample without history of mental illness, thus it was not possible to examine clinically relevant changes in mental health.

Leptin has also been proposed to have a role in mood regulation in obese individuals which may be mediated through tissue resistance to leptin in some obese individuals. However, the brain regions and the intracellular transduction pathways involved have not been identified (Lu, 2007; Lu, Kim, Fraser, & Zhang, 2006). Therefore, although some cross-sectional studies have found associations between leptin levels and depression (Labad et al., 2011) the causative role of leptin in depression is still under investigation. In particular, the role of leptin in mediating or moderating the effect of weight loss on depression in humans has not been experimentally investigated (Lutter et al., 2008).

This study has several limitations that need to be highlighted. Firstly, there was a notable drop-out rate, although participants that failed to complete the study did not differ on key characteristics compared with the final analytic sample. In fact, data for

body mass loss of the participants that were lost at follow-up indicate that the mean loss registered at the last attended nutrition session was comparable to that of the completers. It follows that a further weakness of this study was sample size. The small sample might have reduced the statistical power to find an effect of weight loss on IL-6 reactivity for instance or heart rate. Indeed, a post-hoc power calculation suggested that this study achieved a 46 per cent power to detect a difference in stress reactivity. Nonetheless, some of the associations found were remarkably robust. A further limitation is that this was not a randomized study with a parallel group. Due to time constrains setting up this study using a non-weight loss control group and random allocation to conditions would not have been possible and consequently causal associations and firm conclusions cannot be drawn.

In summary, this study used a supervised calorie restriction intervention to test the effects of weight loss on psychophysiological stress reactivity in overweight and obese women. The weight loss programme resulted in modest reduction in body mass and fat mass. Systolic and diastolic blood pressure responses to mental stress were attenuated after weight loss and this difference in reactivity correlated with reduction in waist and waist to hip ratio. However, lower basal blood pressure after weight loss accounted in part for the attenuated reactivity especially for diastolic blood pressure. Weight loss induced reductions in leptin were related to an attenuated pro-inflammatory IL-6 reactivity independent of basal IL-6. This study has provided partial support for the hypotheses outlined in the Introduction.

CHAPTER 6: DISCUSSION

Two studies have been carried out that investigated the effect of physical activity on mood and daily stress, the cortisol diurnal rhythm, and autonomic and inflammatory stress-induced reactivity. One study has also been completed that examine the effect of weight loss on stress-induced autonomic and inflammatory reactivity and mood. The key aims of this work were to investigate underlying biological mechanisms explaining links between physical activity, adiposity, and stress with relevance to CVD risk.

Several methodologies have been employed in this thesis. A naturalistic approach was used in study one whereas study two and three employed an experimental method. There are advantages and disadvantages in both approaches. Specifically, the naturalistic approach allows to examine relevant associations in everyday life under natural conditions without the constrain of the laboratory. Ambulatory measures provide valuable information on in the participant's typical environment but the range of markers that can be assessed is limited compared to more extensive possibilities that are available in the clinic or laboratory. The experimental method allows to investigate the links between psychosocial factors and biological responses under controlled conditions with a more extensive range of measure available to the researcher.

The major problem with this method is that the stimuli used are somewhat artificial and may not correspond to real life stressors. However, combining the different approaches and methodologies is likely to strengthen the evidence. For example, the magnitude or duration of a given response to stress in the laboratory may be related to future ambulatory blood pressure. Likewise, the amount of

objective ambulatory physical activity recorded during a week may be related to physiological responses to mental stress in the laboratory. In this way one may be more confident about the associations between certain psychosocial or behavioural characteristics and a given risk factors of health outcomes.

6.1 Study I

This study was a cross-sectional analysis of self-reported frequency of habitual physical activity, mood, and diurnal cortisol carried out in a relatively large sample of full-time employed, office-based women from London and Budapest. The primary hypothesis stating that women engaging in greater levels of moderate to vigorous physical activity (MVPA) would score lower on a questionnaire measure of depressive symptoms (Centre for Epidemiological Study Depression CES-D) was accepted. Specifically, after adjusting for age, BMI and between country differences, participants in the two higher tertiles of self-report MVPA had lower depressive symptoms compared to the lowest tertile. I also hypothesised that greater participation in MVPA would be associated with lower daily ratings of total stress (stress and feeling in control) derived from an aggregated Ecological Momentary Assessment (EMA) daily rating during a work day and a weekend day. This hypothesis was partially accepted because daily stress was associated with physical activity only during the weekday and not the weekend.

The second hypothesis stated that higher self-reported MVPA would be negatively associated with several measures of the diurnal cortisol cycle assessed during a work day and a weekend day. This hypothesis was only partially confirmed.

Specifically, there was a modest difference in evening cortisol concentration between the highest and lowest tertile of habitual MVPA on the work day only. There was also a significant difference in the work day total cortisol output indexed as the area under the curve. The two most active groups demonstrated significantly lower cortisol output compared to the least active group independent of age, BMI, smoking and sampling country. This difference was not found for the leisure day. The magnitude of the cortisol awakening response did not significantly differ between the MVPA groups in either cortisol sampling day. Likewise, there were no significant between group differences in the diurnal slope of cortisol decline in either the working day or the leisure day.

These findings suggests that cortisol level during the day and evening appear to be lower in more active women after adjusting for age, BMI, smoking status and sampling country. Lower cortisol levels during the day may therefore be an important biological pathway driving the association between regular physical activity and health outcomes.

6.2 Study II

Study two was a randomized, cross-over experimental study design to investigate the effect of physical activity on autonomic and inflammatory responses using a physical activity withdrawal model in healthy, regularly active individuals. The first hypothesis stated that two weeks withdrawal from regular exercise would cause changes in negative mood and depressive symptoms which would also be associated with the reduction in daily physical activity. This hypothesis was

accepted. Compared to a two week of physical activity maintenance (the control condition), withdrawal from regular physical activity resulted in marked negative changes in depressive symptoms and psychological distress. Importantly, the objectively measured changes in daily level of physical activity over the two weeks were associated with the changes in negative mood in a linear fashion.

The results indicate that the physical activity withdrawal intervention was successful in inducing psychological distress and changes in negative mood, and that this mood disturbance was in large part attributable to a reduction in daily level of physical activity. The hypothesis further predicted that alterations in resting levels of inflammatory cytokine IL-6 and CRP following withdrawal would also be associated with changes in negative mood. There were no significant associations between changes in resting inflammatory markers and changes in mood following exercise withdrawal and therefore the hypothesis was not accepted.

The second hypothesis which stated that autonomic, neuroendocrine and inflammatory responses to mental stress would be enhanced following two week physical activity withdrawal compared to two week physical activity maintenance was generally not supported. Specifically, a near-significant higher inflammatory reactivity following withdrawal was observed only when participants with poor compliance to the physical activity withdrawal protocol were excluded. In addition, this greater IL-6 reactivity was associated with changes in mood following withdrawal which was independent of the IL-6 reactivity during PA maintenance stress testing.

The heart rate reactivity during physical activity withdrawal testing was also significantly associated with mood disturbance independent of heart rate reactivity at maintenance stress testing. A modest difference in stress-induced cortisol was also observed at 20 minutes post-stress, indicating that participants had higher cortisol in

the withdrawal phase compared to exercise maintenance. Furthermore, this hypothesis also predicted that subjective stress would be greater and task appraisal less favourable following physical activity withdrawal compared to the maintenance phase. This hypothesis was however not accepted.

These results seem to suggest that by using an experimental model to manipulate physical activity, heart rate and inflammatory IL-6 responses to mental stress were greater in individuals who were more distressed as a result of exercise withdrawal. However, these associations were only apparent in secondary analysis prompting the need to replicate these findings in future well-controlled studies.

6.3 Study III

This study was designed to experimentally manipulate adiposity levels and compare stress-induced autonomic and inflammatory responses before and after weight loss. The first hypothesis stating that weight loss would be associated with lower levels of resting inflammatory markers was only partially accepted because CRP and IL-6 did not significantly change. However, there was a decrease in the hormone leptin and this decrease correlated with changes in BMI and waist to hip ratio.

The second hypothesis predicted that autonomic responses to mental stress would be attenuated after weight loss. This hypothesis was only partially supported. Specifically, systolic blood pressure stress reactivity was attenuated independently of the resting value and the magnitude of this attenuation correlated with the change in the waist to hip ratio following weight loss. There were no other differences in reactivity or recovery in cardiovascular or inflammatory responses.

The third hypothesis stated that reductions in leptin would be associated with cardiovascular and inflammatory responses to mental stress at follow-up stress testing. This hypothesis was partially accepted. The change in leptin was not associated with stress-induced cardiovascular reactivity but it was positively and significantly associated with IL-6 reactivity independently of pre-weight loss reactivity and resting IL-6.

Finally, the fourth hypothesis stating that the weight loss would result in improvements in depressive symptoms mediated by loss of adiposity or resting inflammatory markers was not accepted. Although mood significantly improved following weight loss, it did not correlate with any measure of adiposity or inflammatory markers.

These results indicate that following a modest reduction in adiposity, blood pressure reactivity to stress is reduced. In addition, the weight loss-induced change in resting leptin appears to be partly driving an attenuation in pro-inflammatory responses to stress. However, the lack of a non-weight loss control group precludes any firm conclusion and certainty about the significance of the observed post-weight loss blunted reactivity. For example, although the changes in autonomic stress reactivity were correlated with reductions in adiposity, there may be a possibility that the attenuated reactivity is driven by an habituation effect to stress or some other non-specific confounding factors.

6.4 Implications and Importance of Findings

This thesis has shown that regular physical activity is associated with a small reduction in daily cortisol output and lower bedtime cortisol levels. This association was only reported during the working day sampling. Attenuated cortisol secretion

during the working day, when stress is typically higher than the leisure day, may be an important mechanism partly mediating the effect of physical activity on cardiovascular risk factors. For example, elevations in diurnal cortisol have been shown to contribute to endothelial dysfunction, hypertension and to a switch towards cardiac sympathetic predominance (Brunner et al., 2002; Broadley et al., 2005), and raised evening cortisol was a predictor of cardiovascular mortality in a recent study (Kumari, Shipley, Stafford, & Kivimaki, 2011). Indeed, the latter study was the first to demonstrate that a flatter decline in cortisol driven by high evening levels was predicted mortality for cardiovascular causes independent of several covariates.

Depressive symptoms and aggregated rating of daily stress were also lower in the most active participants although some of these associations were observed in the work day only. I demonstrated a direct association between physical activity and mood symptoms by using an experimental exercise withdrawal design. The positive effect of regular physical activity on mood and psychological wellbeing is generally accepted (Rethorst, Wipfli, & Landers, 2009). However, the evidence for a causal association comes mainly from exercise trials. These interventions often require participants to exercise in groups and have therefore a certain degree of social contact. It may be argued that the positive effect of exercise may in part be mediated by improvements in non-specific factors such as self-efficacy, self-esteem or social support. The use of an exercise withdrawal design overcomes these limitations. A worsening in mood symptoms and psychological distress in these healthy participants which was associated with objective reduction in daily level of physical activity developed after two weeks of exercise abstinence.

The participants who developed negative mood showed enhanced heart rate and pro-inflammatory IL-6 responses to mental stress but this enhanced reactivity

was not observed in the main analysis but only in secondary analysis. In contrast, the systolic blood pressure reactivity of participants who lost weight was reduced compared to the reactivity observed pre-weight loss. However, an attenuated inflammatory reactivity could only be observed in correlational analysis. Therefore, this thesis has provided only limited experimental evidence for an association of physical inactivity and adiposity with exaggerated stress-induced autonomic and inflammatory responses.

6.5 Methodological Issues and Limitations.

The strengths and weaknesses of the studies carried out in this thesis have already been highlighted in previous chapters. However, it is important to discuss some of the methodological issue arising from the use of the experimental design further.

6.5.1 Physical Activity Withdrawal Design.

This experimental paradigm has been used as a model of physical inactivity and to induce mood disturbance in healthy, fit and regularly active individuals. This model can be used to experimentally investigate effects of exercise on mood and underlying psychobiological mechanisms under stimulated conditions such as mental stress. However, the sampling technique is central to the success of this experimental design. Participants were only eligible if they engaged in at least three-weekly moderate to vigorous or higher intensity exercise sessions for one hour or

more in total. Since this level of physical activity may only be achieved by a minority of the population, the extent to which the results obtained by using this model can be generalized is uncertain.

Yet, this issue needs to be balanced against the alternative model used to investigate the effect of physical activity on various psychological and biological outcomes. Active exercise intervention trials are the alternative model that has been used in this kind of research. This design also suffers from methodological shortcoming. For example, exercise trials need larger number of participants in order to account for attrition rates, greater manpower and monetary costs. In addition, the duration of the exercise intervention may need to be considerably longer as chronic physiological exercise adaptations are unlikely to be observed over short trials lasting 3 – 6 months, when modest gains in cardiorespiratory fitness (<5%) are often observed.

The mood disturbance and depressive symptoms which are reliably induced by the physical activity withdrawal paradigm are interpreted to represent the effect of physical activity on mood and general wellbeing. I measured adherence to exercise withdrawal using objective activity measures, which is a major strength of the study design. It may, however, be argued that withdrawing from a supposedly pleasurable activity such as exercise or sport participation may result in mood alterations which might not be related to the effect of physical activity itself but to other factors including social contact. Indeed, although a previous investigation reported a correlation between changes in inflammatory markers after exercise withdrawal and mood (Poole, Hamer, Wawrzyniak, & Steptoe, 2011) an effect of inflammatory activity on exercise withdrawal induced negative mood has not been demonstrated.

This could indicate that the mechanism driving the effect of physical activity on mood and wellbeing in a physical activity withdrawal paradigm may not be physiological but attributable to other non-specific factors such as social contact or the withdrawal of an enjoyable activity. This issue is however also relevant in exercise intervention trials since these studies require a great deal of contact time, social interactions, and other factors such as subjective expectations. There is some evidence that may argue against the proposition that the mood changes experienced during physical activity withdrawal are a result of merely negative feelings arising from withdrawal of social contact during exercise. For example, anti-depressive effects of exercise are comparable in home based compared to group based exercise settings (Blumenthal et al., 2007). Therefore, although I cannot discount the possibility that the mood change in this study may have partly been caused by the withdrawal of a pleasurable activity, it seems very unlikely that the negative mood experienced as a result of physical activity withdrawal in this regularly active sample is attributable to non-specific effects such as social contact.

Some of the effects observed in this study, for example negative mood and inflammatory reactivity or heart rate reactivity, were only demonstrated in secondary analysis using change scores and regressions techniques. Being a post-hoc analysis, this method represents a weaker source of evidence compared to the planned, pre-hoc experimental analysis. The need to carry out secondary analysis arose because there was evidence that the manipulation of physical activity that is central to the experimental design might have not worked for all participants. For example, objective reduction in daily physical activity data showed great variation in adherence to the protocol. Therefore, there was a need to carry on secondary analysis that offers less reliable evidence due to its main correlational nature.

6.5.2 Weight Loss Intervention

The nine weeks weight loss regime resulted in a small overall weight reduction and a higher than expected drop-out rate (estimated at 29 per cent in a meta-analysis) (Franz et al., 2007). The most likely reason for the small weight change is that the intervention did not stimulate the anticipated reduction in caloric intake because of over-compensation. For example, the participants may have overcompensated during their evening meal or with their snacking.

However, the data available on the amount of weight loss lost by non-completers seem to be comparable to that of study completers, thus it is unlikely that attrition biased the results. Participants on the weight loss study generally demonstrated improved mood following the intervention although this was not related to weight loss. Thus, these effects might have been accounted for by having weekly contact with the investigators, which may have also impacted on other aspects of the study outcomes.

I chose to recruit women in order to limit the between gender variability of inflammatory responses. Moreover, since this study was designed as a feasibility study that could inform a larger randomised controlled trial, it was originally decided to recruiting women only. Indeed, the sample size for this study was small and it is possible that in future studies including men as well as women the attrition rate may be lower.

The lack of a non-weight loss control group means that the reduction in blood pressure reactivity to stress observed from baseline to follow-up although correlated with reduction in adiposity is not strictly derived from an experimental manipulation. Furthermore, some secondary cardiovascular parameters such as cardiac output were estimated using the Finometer device algorithm (see 4.3.3.9) rather than

measured directly. This method, although widely used in stress reactivity research, may be less accurate than other more objective methods but are deemed to be less cumbersome for the participant. This means that caution is needed in interpreting the results and that replication of the findings using a control group is warranted.

6.6 Suggestions for Further Research.

Future studies examining the association between physical activity and diurnal cortisol may benefit from the use of accelerometer devices. At present, the majority of ambulatory physical activity research is based on self-report measures. In addition, studies of ambulatory physical activity and cortisol may be confounded by the acute effect of exercise on the HPA axis and therefore the accelerometer could be used as a further check of adherence to sampling protocol. Likewise, incorporating repeated momentary measures of stress and general mood into research protocols would prevent some of the problems associated with questionnaire measures of mood such as recall bias. It would also be desirable to conduct larger studies that include men as well.

Receptor or tissue sensitivity to the effect of glucocorticoids such as cortisol may be an important mediator of the effect of physical activity on immunological processes but this is currently an under researched area. The exercise withdrawal paradigm could therefore be employed to investigate the effect of physical inactivity on tissue sensitivity to cortisol. In addition, further studies that employ the exercise withdrawal design will benefit from the application of more advanced psychoneuroimmunological techniques. For example, since the stressors used did

not elicit marked pro-inflammatory responses, it would be advantageous to use an ex-vivo challenge model in which blood samples drawn before and after the stressors are challenged with an inflammatory stimulus such as lipopolysaccharide (Rohleder, Wolf, & Wolf, 2010). In this way the ensuing pro-inflammatory response can be measured and compared between the physical activity withdrawn and the control group.

Further work should aim to investigate the effect of physical activity and adiposity on stress reactivity in a single randomized study. In this way it would be possible to examine whether these two factors are associated with stress independently. For example, overweight or obese men or women could be recruited and tested in the psychophysiology laboratory at baseline before being randomly assigned to either a reduced calories weight loss, an exercise intervention or an advice only control group. When stress testing is repeated at follow-up one will be able to test which factor is more important in reducing CV reactivity. In addition, by adopting such design it may be possible to test the effect of weight loss on mood. In study three I showed that weight loss was associated with improvements in depressive symptoms which were unrelated to adiposity. Hence it is unclear whether improvements in depressive symptoms after weight loss are due to non-specific effects such as social contact or to loss of adiposity with subsequent improvements in the inflammatory profile.

6.7 Conclusions

Between forty to sixty per cent of all cardiovascular events can be explained by traditional risk factors including smoking, lipids, hypertension, physical inactivity and family history. Other factors such as cardiovascular and inflammatory activation

during psychosocial stress have been proposed to account for the development of cardiac risk factors. Psychophysiological stress testing in a controlled laboratory setting represents a way to investigate the contribution of physiological reactivity to risk factors and the mediating or moderating role played by physical activity and adiposity. Evidence has shown that these autonomic responses are prospectively associated with risk factors including hypertension (Chida & Steptoe, 2010) as well as the progression of subclinical atherosclerosis plaques (Hamer, Endrighi, Venuraju, Lahiri, & Steptoe, 2012).

Previous work suggested that higher physical activity levels and low adiposity may act as a buffer against sustained or exaggerated autonomic responses to stress. However, much of this work has been observational highlighting the need for more experimental evidence. The present series of studies has provided only weak experimental evidence for an association between modifiable risk factors such as physical activity and adiposity and responses to mental stress. Regular physical activity may also contribute to better health outcomes through regulation of inflammatory and cardiovascular responses to acute or chronic stressors but more experimental evidence is needed to confirm this hypothesis. Furthermore, adiposity levels may negatively affect cardiovascular and inflammatory responses to stressful or challenging situations which may also be mediated through leptin regulation. Even though heightened autonomic responses in obesity may be an important pathway in the association between obesity and the development of cardiovascular risk more research is warranted before a direct association between adiposity levels and up-regulation of autonomic responses is firmly established.

In summary, physical activity and adiposity seems to be associated with several psychobiological mechanisms that could be potentially important for various health

outcomes. The studies presented in this thesis have contributed to the psychophysiology literature by evaluating autonomic and inflammatory responses to acute stress before and after experimental manipulation of physical activity and adiposity. In addition, this thesis has contributed to current knowledge on the association between diurnal cortisol profile and physical activity.

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Appendix 1: Physical Activity Withdrawal Study Information Sheet

SCHOOL OF LIFE AND MEDICAL SCIENCES

UNIVERSITY COLLEGE LONDON

Volunteer Information Sheet V.1 15/01/09 (Confidential)

(The Exercise Withdrawal and Stress Study)

This study has been approved by the NHS Research Ethics Committee. We are interested in studying how the cardiovascular and immune systems respond to physical activity. The study is part of a larger programme of research into biological aspects of stress in relation to cardiovascular disease risk funded by the British Heart Foundation.

Who can take part?

This study will be carried out with healthy, non-smoking men and women aged 18-35 years who exercise regularly. We will not be able to include anyone who is diabetic, or has a liver, kidney, heart, lung, blood or skin disease. We also cannot include you if you have been taking any medicine or pills prescribed by a general practitioner over the past month (oral contraceptives are allowed). You need to be exercising at least three times a week for 30 minutes per session of vigorous, intense activity, and to have maintained this level of activity for at least the past six months.

What will I have to do?

You will come to the laboratory on three occasions over a total of 4 weeks. The appointments will be at UCL (1-19 Torrington Place), and will take place on a weekday morning or afternoon. At least three hours before the session we want you to avoid eating a large meal, drinking coffee or alcohol, because these may affect the validity of the study. In addition, please do not exercise for 12 hours before your appointment because, again, this could affect the results. If you become unwell beforehand or are suffering with an infection, please contact us to reschedule your appointment.

The first visit is for an assessment and will last approximately 1 hour and 10 minutes. During this visit we will measure your height, weight, give you a questionnaire and ask you to cycle on an exercise

bike for 16 minutes to assess your fitness level. At the end of this session you will also be told whether you will be expected to maintain your regular exercise regime or withdraw from it for the 14 day interval between appointments. If you are asked to maintain your regime, you will carry on doing at least three 30-minute intervals of vigorous, intense exercise per week. If you are asked to withdraw from your exercise regime, you will need to stop any physical activity that is of low, moderate or vigorous intensity or greater during the two weeks. You should continue your daily routines as normal (excluding any exercise sessions), and not compensate for your lack of regular exercise. You will also be asked to wear a tiny device fitted to your belt called an Actigraph, which will measure your activity levels to check that you are adhering to the exercise instructions. This is a randomised study, so we do not know which condition you will be in first.

The second appointment will be 14 days later and will last approximately 2 hours and 15 minutes. During this session you will be asked to complete a set of short questionnaires which assess your exercise habits, general stress levels, mood and physical health, and we will take a small blood sample from your arm. You will then be asked to perform two 5 minute tasks; a speech task and a hand-eye-co-ordination task. These tasks do not require any special skills and most people find them interesting and even fun to do. After the tasks you will rest comfortably for 45 minutes and we will take another small blood and saliva sample. At the end of the visit we will ask you to switch to the condition to which you were not originally randomised (i.e. if you maintained regular exercise you will now stop exercising for two-weeks and vice versa). You will again be fitted with an Actigraph which will monitor your activity levels for the following 2 weeks.

Finally, we will ask you to come back for a third appointment at the end of the second 2 week interval. In this final visit we will again give you the questionnaires, tasks and take two small blood and saliva samples as we did previously. This last visit will last approximately 2 hours and 15 minutes

You will be provided with an honorarium sum of £80 for taking part in the study.

What will happen to the blood sample and other information?

All the information we obtain from this study about you, including your name, will be confidential and will only be used for research purposes. The data will be stored in accordance with the data protection act. Since data will be combined, it will not be possible to identify any individual within published results. Only those people directly involved in this study will have access to the blood samples. When the study is complete, your sample will be destroyed in accordance with UCL biohazard disposal policy.

What will happen to the results from this study?

This study is being undertaken in part fulfilment of a Ph.D. project that investigates behavioural and lifestyle factors that are relevant to cardiovascular health and has been funded by the British Heart Foundation. We wish to report our findings in academic/health related journals and present them at scientific meetings and conferences. You will not be identified in any report or publication arising from the study.

Are there any advantages/benefits from taking part?

We cannot promise the study will help you directly but the information collected from you and other participants will help to improve our understanding of the effects of lifestyle and exercise on health. At the end of the study we can give you feedbacks on your blood pressure, weight, BMI and fitness test score.

Are there any disadvantages from taking part?

We consider there to be minimal disadvantages e.g. the inconvenience of attending /completing the questionnaires. We realise that not everyone likes having a blood test but this is a crucial part of the study. The procedure for obtaining the blood sample may cause a little discomfort. Blood will be taken by a trained phlebotomist who will follow procedures and take appropriate precautions to minimise any discomfort.

What if there is a problem?

Complaints: If you have any concerns or wish to complain about any aspect of the way you have been approached or treated as part of this study, you should initially contact Dr. Hamer who will do his best to answer your questions. His contact details are provided at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this through the Research Governance Sponsor of this study, University College London (UCL). Please write to Joint UCL/UCLH Biomedical Research Unit, R&D Directorate (Maple House), Rosenheim Wing, Ground Floor, 25 Grafton Way, London WC1E 5DB quoting reference 08/0284. All communication will be dealt in strict confidence.

Harm: Every care will be taken to ensure your safety during the course of the study. However, UCL has insurance arrangements in place for no-fault compensation in the unlikely event that something unforeseen goes wrong and on the balance of probabilities, harm is attributed as a result of taking part in the research study.

What if I change my mind during the study?

If at any point for any reason you do not want to carry on, then you may stop. There are no consequences of withdrawal from the study.

Thank you for taking the time to read this information sheet! We hope you are able and willing to take part in this study.

If you have any questions, please contact Romano Endrighi on 020 76798328 (voice mail 020 76791804 r.endrighi@ucl.ac.uk). Postal address: Psychobiology Group, Research Dept of Epidemiology and Public Health, 1-19 Torrington Place London, WC1E 6BT

Further information about the work carried out in the Psychobiology Group can be found at www.ucl.ac.uk/psychobiology

Appendix 2: Condition Allocation Letters (Exercise Withdrawal Study)

PSYCHOBIOLOGY GROUP
RESEARCH DEPT OF EPIDEMIOLOGY AND
PUBLIC HEALTH

Dear Participant:

You have been allocated to the EXERCISE WITHDRAWAL group.

Please read these instructions carefully and keep them safe. It will tell you exactly what you will have to do in the next 14 days, starting from now, in order to successfully complete the study.

- Please refrain from doing your usual exercise regime, including all aerobic exercise, resistance training and weight lifting at the gym or outdoors.
- Please do not take up any physical activity to replace the exercise you would normally be doing. It is very important that you adhere to this rule. For your convenience we have compiled a list of activities that you may do during a typical day:
    1. Do take the bus (or tube) instead of walking to go to university or work
    2. Do not cycle
    3. Do use the lift rather than walking up and down the stairs
- Try to be sedentary as much as possible!

Your activity levels will be monitored for the duration of the study to check how well you are complying with these rules. You will be given a small device called an Actigraph. Please make sure you keep this device strapped snugly round your waist, with the pouch positioned against the side of your hip. You can wear it above or below your clothes, it does not necessarily have to touch your skin. Keep the Actigraph positioned the correct way up (see sticker on Actigraph).

It is important that you wear this device during waking hours, but DO NOT get the Actigraph wet as it is not waterproof!

In the meantime if you have any questions, call or email Mark Hamer 020 7679 5969/ m.hamer@ucl.ac.uk or Romano Endrighi 020 7679 8328 r.endrighi@ucl.ac.uk

Thank you for taking part in this research.

PSYCHOBIOLOGY GROUP
RESEARCH DEPT OF EPIDEMIOLOGY AND
PUBLIC HEALTH

Dear Participant:

You have been allocated to the EXERCISE MAINTENANCE group.

Please read these instructions carefully and keep them safe. It will tell you exactly what you will have to do in the next 14 days, starting from now, in order to successfully complete the study.

- Please carry on doing your daily activities as usual.
- Carry on doing your usual exercise regime, including all aerobic exercise, resistance training, sport or weight lifting as you normally do, and certainly more if you so wish.

Your activity levels will be monitored for the duration of the study to check how well you are complying with these rules. You will be given a small device called an Actigraph. Please make sure you keep this device strapped snugly round your waist, with the pouch positioned against the side of your hip. You can wear it above or below your

clothes, it does not necessarily have to touch your skin. Keep the Actigraph positioned the correct way up (see sticker on Actigraph).

It is important that you wear this device during waking hours, but DO NOT get the Actigraph wet as it is not waterproof!

In the meantime if you have any questions, call or email Romano Endrighi 020 76798328/ r.endrighi@ucl.ac.uk or Mark Hamer 020 7679 5969 m.hamer@ucl.ac.uk

Thank you for taking part in this research.

Appendix 3: Weight Loss Study Information Sheet

UNIVERSITY COLLEGE LONDON

SCHOOL OF LIFE AND MEDICAL SCIENCES

Volunteer Information Sheet V. 3 04/02/11 (Confidential)

Adiposity, Stress and Immunity Study (Weight loss Study)

You are being invited to take part in a research study. Before you decide to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

We believe that a person's weight (body fatness) may be linked to the way he or she respond to psychological stress, and this might predispose towards the development of chronic conditions such as cardiovascular disease. In this study we are trying to find out whether people who lose weight react differently to stress. This study is part of a larger programme of research that investigate how psychological and lifestyle factors may be related to cardiovascular disease and is funded by the British Heart Foundation.

Who can take part?

The research study will be carried out with healthy, non-smoking women aged 18-45 years who are overweight (this will be ascertained by a short interview), but who would like help and support in managing their weight safely and effectively. We will not be able to include anyone who is diabetic, or has a liver, kidney, heart, lung, blood or skin disease. We also cannot include you if you have been taking any medicine or pills prescribed by a general practitioner over the past month (oral contraceptives are allowed).

What will I have to do if I decided to join the study?

The study consists of two parts, (lasting 8 weeks in total) and we can only enrol you if you agree to participate in both parts:

1. An 8 week supported weight loss programme based on daily meal replacements with regular contact time and advice.

2. Two laboratory testing sessions in which you will complete two mild mental stress tasks during each session and a questionnaire. Throughout these laboratory sessions we will be taking a number of measures, which include: blood pressure, saliva and blood samples. A chemical that is produced during stress called cortisol can be measured in the saliva and various chemicals in the blood will be measured as a marker of immune function.

The weight loss programme

Research has shown that a meal replacement diet combined with advice and support is a safe and effective method for weight loss. You will be given two commercially available meal replacement shakes per day for eight weeks and will be allocated a Health Psychologist researcher who will work out a detailed strategy that will help you plan and manage your third meal and snacks as well as providing ideas for helping you to stick to the dietary plan. An important component of the weight loss programme is practical support which we will offer on a weekly basis in the form of face to face contact with Health Psychologist researchers who are supervised by a Registered Dietician experienced in weight management. At the beginning of the study you will agree the dates for your sessions with your allocated expert, which may also be reviewed throughout the duration of the study. We expect each session to last between 30 minutes to a hour and the timing of these will be flexible to accommodate your other commitments. At the end of the eight week weight-loss programme, we will also offer you two follow-up appointments and provide you with strategies to help in maintaining weight loss after you have stopped taking the meal replacements.

The laboratory testing session

This part of the study will be carried out twice: once at the very beginning of the weight loss programme on the same day that you come in to see us and again at the end of the programme, which will be about eight weeks later. The session takes approximately 3 and a half hours, starting on a weekday morning or afternoon. During the session you will be asked to perform two 5-minute tasks; a speech scenario and a hand-eye coordination task whilst your blood pressure is recorded by a small cuff attached to your middle finger. These tasks do not require any special skills and most people find them interesting or even fun to do. We will also measure your height, weight and fat percentage before these sessions and take two small blood samples (about 3 tablespoons) and five saliva samples. During these laboratory sessions we will also ask you to complete a questionnaire about general stress, mental and physical wellbeing, and physical activity levels. We will provide some refreshments at the end of each session.

The questionnaires are to provide research data, not to diagnose medical or mental health issues. If any of the questions raise concerns for you then we recommend that you speak to your GP

Where will the study take place?

If you agree to take part in the study, we will arrange an appointment for you to attend at the Department of Epidemiology and Public Health, University College London (1-19 Torrington Place).The study (both the laboratory tests and the weight loss support) will take place in our research rooms at UCL.

We will reimburse your travel expenses where applicable. In addition, if you request it, we can give you a written record of your blood pressure, weight, body mass index and (non-fasting) lipid profile (cholesterol and triglycerides) and give you an extra copy to take to your GP if you so wish. We will, however, not get in contact with your GP under any circumstances.

What will happen to the blood sample and other information?

We want to emphasise that all results obtained will be strictly confidential and will only be used for medical research purposes. All information about you will have your name and address removed so that you cannot be recognised from it. You are free to withdraw from the study at anytime without the need to give any reasons and there will be no consequences if you decide to withdraw or not to take part. In that case we will destroy your blood and saliva samples and the data we have obtained from you.

Blood samples will be analyzed in our laboratory for biological markers that we believe are affected by both stress and weight loss and therefore potentially relevant in chronic conditions such as cardiovascular disease.

When the study is complete, your samples will be destroyed in accordance with UCL biohazard disposal policy.

What will happen to the results from this study?

This study is being undertaken in part fulfilment of a Ph.D. project that investigates psychosocial, behavioural and lifestyle factors that are relevant to cardiovascular health and has been funded by the British Heart Foundation. We wish to report our findings in academic/health related journals and present them at scientific meetings and conferences. You will not be identified in any report or publication arising from the study.

Are there any advantages/benefits from taking part?

By taking part in this weight loss programme you will be supported and receive advice to overcome possible barriers to weight loss. Our team will decide with you how many sessions of contact time you will need. We recognize that some people may find it difficult to maintain the weight loss achieved during the study once the meal replacements are stopped. We will provide you with strategies to help maintain any weight you have lost and offer two follow-up contact

appointments to help you deal effectively with this. The information collected from you and other participants will help to further our understanding of how weight may affect the way people respond to stress and ultimately improve the management of chronic condition such as heart disease.

Are there any disadvantages from taking part?

We consider there to be minimal disadvantages e.g. the inconvenience of attending /completing the questionnaires. We realise that not everyone likes having blood samples taken, but this is a crucial part of the study. The procedure for obtaining the blood sample may cause a little discomfort or small bruising. Blood will be taken by a qualified nurse who will follow procedures and take appropriate precautions to minimise any discomfort.

What if there is a problem?

We do not expect you to suffer any adverse effects from this study.

Complaints: If you have any concerns or wish to complain about any aspect of the way you have been approached or treated as part of this study, you should initially contact Dr. Hamer who will do his best to answer your questions. His contact details are provided at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this through the Research Governance Sponsor of this study, University College London (UCL). Please write to Joint UCL/UCLH Biomedical Research Unit, R&D Directorate (Maple House), Rosenheim Wing, Ground Floor, 25 Grafton Way, London WC1E 5DB quoting reference 09/0417. All communication will be dealt in strict confidence.

Harm: Every care will be taken to ensure your safety during the course of the study. UCL does not have insurance arrangements in place for no-fault compensation and, in the unlikely event that something unforeseen goes wrong and on the balance of probabilities harm is attributed as a result of taking part in the research study, negligence will have to be proved.

Contact for further Information

If you have any questions, please contact: Psychobiology group, Department of Epidemiology and Public Health, University College London, 1-19 Torrington Place, London, WC1E 6BT. Telephone 020 7679 8328.

Dr. Mark Hamer (020 7679 5969)-m.hamer@ucl.ac.uk

Web-site: www.ucl.ac.uk/psychobiology.

Email: uclweight.loss@gmail.com

Thank you for taking the time to read this information sheet! We hope you are able and willing to take part in this study.

- If you have any questions regarding the laboratory testing session please contact Romano Endrighi

on 020 7679 8328 (voice mail 020 7679 1804) – r.endrighi@ucl.ac.uk

- For questions regarding the weight loss programme contact Helen Croker on 020 7679 5634 (voice mail 020 7679 8354) h.croker@ucl.ac.uk or Sarah Young on 020 7679 1614 s.e.young@ucl.ac.uk

Appendix 4: Day-Tracker Study Information Sheet

UCL PSYCHOBIOLOGY GROUP
DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH

The Day-Tracker Study

The Biology of Everyday Life

PARTICIPANT INFORMATION SHEET (Confidential)

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

We are trying to understand how our activities and emotions relate to biological function in everyday life. We believe that positive wellbeing is associated with good health, while more negative emotional states may contribute to ill health. In previous work, we have found that different behaviours and emotions have distinct biological profiles. In this study, we would like to build on our previous findings with some new measures. This research study is part of an international collaboration, funded in part by the National Institute on Aging in the USA, and in part by the World Health Organisation. The study is being carried out in UCL by Professors Andrew Steptoe, Jane Wardle and Sir Michael Marmot from the Department of Epidemiology and Public Health.

Who can take part?

This study is being carried out with healthy women aged 18 to 65 years old who are working full-time. Volunteers should not pregnant, or be on any regular medicines or medications except for oral contraceptives or hormone replacement treatment. If you have suffered from a serious illness such as heart disease or cancer over the past two years, you will not be suitable for the study.

What will happen during the study?

The study involves taking measurements over two 24 hour periods, one during the week and the second on Friday to Saturday. These two study periods will not follow directly one after another but should take place within 10 days of each other. After the first 24 hour study period, you will be asked to complete a questionnaire. This questionnaire includes measures of lifestyle factors such as smoking and physical activity, and measures of work stress, financial strain and social support. This information will help us interpret the biological results we collect. It is completely confidential, and results will not be available to anyone outside the study group and will only be used anonymously.

On the first 24-hour study day, you will need to come to the Department of Epidemiology and Public Health situated in 1-19 Torrington Place after work (between 4 and 6pm). When you arrive in the building, one of our team members will take you to an office on the 3rd floor. If you happen to have a cold or flu or have had to take any medicines shortly before, please get in touch so that we can reschedule the appointment.

We will first of all measure your height and weight. To do these measurements, you will need to be barefoot. Next, we will fit you with a small electronic device that will measure your heart rate over the next 24 hours. This involves using two adhesive pads to stick electrodes on the left side of your chest, just above the heart. This device is not uncomfortable, and once it has been fitted you should not be able to feel it.

We will also ask you to give us some samples of saliva over the next 24 hours, so that we can measure levels of stress hormones. The saliva samples are taken by chewing gently on a cotton roll for two minutes, then putting the wet cotton roll into a test tube. We want to collect two saliva samples in the evening, then 5 more over the next day. After you have been shown how to take the saliva samples, you can go off and spend the evening, night, and the next day as normal. You will be able to bath or shower as normal while you are wearing the heart monitor.

We will ask you to return to the Department 24 hours later. At that point, we will collect the heart monitor and test tubes from you, and then ask you to complete a computerised interview called the 'Day Reconstruction Interview'. This involves providing details of what you were doing over the previous 24 hours, and how you felt at different times of the day and evening. It is quite a detailed procedure which will take 30-60 minutes to complete. The computerised questionnaire can also be done from your home or other personal computer before you come to the office.

The second 24-hour study is exactly the same, except that it will start on Friday after work, and go on until the early evening on Saturday. You will not need to come in on Saturday, but can take of the chest electrodes yourself and keep the samples until the following Monday. After you have finished the measurements on Saturday, we would like you to complete the computerised interview as before. If you have a computer at home, you can do it there, or else back at UCL on Monday.

What if I change my mind during the study?

If at any point for any reason you do not want to carry on, then you may stop. There are no consequences of withdrawal from the study, other than forfeiting the honorarium payment (see below).

What happens to the information?

All the information we get from this study about you, including your name, will be confidential and will only be used for research purposes. The data will be collected and stored in accordance with the Data Protection Act. The data we collect from all volunteers will be combined, and it will not be possible to identify any individual within published results.

What happens at the end of the study?

Provided you have completed all the parts of the study successfully we will give you an honorarium of £60. When the study is complete and all the results are analysed, we will send you a summary of our findings.

Can I take part if I am pregnant?

There are no risks to taking part in the study because you are pregnant. However, because pregnancy has effects on some of the hormones that we will be measuring, we do not wish pregnant women to participate.

We hope you are able and willing to take part in our study. If you have any questions, please contact Nina Grant, Psychobiology Unit, Department of Epidemiology and Public Health, 1-19 Torrington Place, London WC1E 6BT. Tel. 020 7679 1702 (internal 41702). E-mail: nina.grant@ucl.ac.uk

PUBLICATIONS:

Hackett, R., Hamer, M., Endrighi, R., Brydon, L., & Steptoe, A. (2012). Loneliness and stress-related inflammatory and neuroendocrine responses. *Psychoneuroendocrinology, http://dx.doi.org/10.1016/j.psyneuen.2012.03.016 [in press]*

Jackowska, M., Dockray, S., Endrighi, R., Hendrickx, H., & Steptoe, A. (2012). Sleep problems and heart rate variability over the working day. *Journal of Sleep Research doi: 10.1111/j.1365-2869.2012.00996.x. [Epub ahead of print]*

Hamer, M., Endrighi, R., Venuraju, SM., Lahiri, A., & Steptoe, A. (2012). Cortisol responses to mental stress and the progression of coronary artery calcification in healthy men and women. *Plos one, 7(2)e31356*

Endrighi, R., Hamer, M., & Steptoe, A. (2011). Associations of trait optimism with diurnal neuroendocrine activity, cortisol responses to mental stress, and subjective appraisal in healthy men and women. *Psychosomatic Medicine, 73,(8) 672-8.*

Brown, J., Dockray, S., Endrighi, R., Grant, N., & Steptoe, A. Resilience mediates the effects of psychosocial stress on questionnaire measures of affect and wellbeing, but not daily measures of affect. *Under review.*

Hamer, M., Endrighi, R., & Poole, L. Physical activity, stress reduction and mood: insight into immunological mechanisms. *In Psychoneuroimmunology: Methods and Protocols [Forthcoming Edited Book].*

CONFERENCE ABSTRACTS:

Some of the research outlined in this thesis, as well as other work carried out at the Psychobiology Research Group at UCL has been presented and discussed at the following international scientific meetings:

Endrighi, R., Steptoe, A., & Hamer, M. "The Effect of Experimental Reduction in Daily Physical Activity on Stress-Induced Cardiovascular, Neuroendocrine and Subjective Responses". Psychosom Med V. 74:3 A-88. Oral presentation, *70th Annual Scientific Meeting of the American Psychosomatic Society, March 14–18, 2012, Athens, Greece.*

Endrighi, R., Hamer, M., Young, S., & Steptoe, A. "Effect of Weight Loss on Cardiovascular Stress Responsivity". Psychosom Med V. 74:3 A-73. Poster presentation, *70th Annual Scientific Meeting of the American Psychosomatic Society, March 14–18, 2012, Athens, Greece.*

Endrighi, R., Hamer, M., & Steptoe, A. "Examining the Associations of Physical Activity and HPA Axis Function". Oral presentation, *42nd Annual Meeting of the International Society for Psychoneuroendocrinology, August 4-6, 2011, Berlin, Germany.*

Endrighi, R., Steptoe, A., & Hamer, M. "Mood Disturbance Following Exercise Withdrawal is Associated with Greater Inflammatory Responses to Stress" Psychosom Med V. 73:3 A-110 (APS Scholar Award). Oral presentation, *69th Annual Meeting of the American Psychosomatic Society, March 9-12, 2011, San Antonio, TX, USA.*

Endrighi, R., Steptoe, A., & Hamer, M. “The Effect of Exercise Withdrawal on Mood and Inflammatory Responses to Mental Stress”.. Poster presentation, *6th Annual Scientific Meeting of the UK Society for Behavioural Medicine, December 14-15, 2010, Leeds, England, UK.*

Endrighi, R., Poole, L., Hamer, M., & Steptoe, A. “Physical Activity, Adiposity, Stress-Induced Inflammation and Cardiovascular Disease Risk” Poster presentation, *2nd Biological Psychology, University of Dresden Spring School “The ABC of Stress: Biological Assessment, Basics and Consequences”, March 18-21, 2009, Dresden, Germany.*