

**The role of psychosocial wellbeing and biological stress processes in linking type II
diabetes and cardiovascular disease**

Ruth Hackett

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Student Declaration

I, Ruth Hackett, 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.

Signed:

Date:

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Abstract

Type II diabetes increases the risk of cardiovascular disease. Evidence suggests that psychosocial stress is involved in both conditions but the biological pathways involved are poorly understood. This PhD investigated the role of psychosocial wellbeing and stress-related biological processes in diabetes. Studies 1 and 2 used acute laboratory stress testing to assess biological stress responses in people with diabetes. Studies 3 and 4 used data from a large population dataset to assess associations between cortisol and diabetes.

Study 1 tested the notion that people with diabetes experience stress-related disturbances across multiple biological systems, coupled with heightened life stress. A comparison of laboratory stress responses in people with diabetes and matched controls was conducted. The results suggested that people with diabetes have dysregulated biological responses to stress and increased exposure to life stress.

Study 2 assessed whether hostility (a psychosocial factor) exaggerated the pattern of disturbances in stress responsivity seen in Study 1, looking at the diabetes group alone. The findings suggest that high hostile individuals with diabetes have heightened inflammatory stress responses and blunted cortisol stress responses in comparison to low hostile individuals.

Studies 3 and 4 assessed neuroendocrine disturbances in diabetes using Whitehall II study data. Study 3 assessed whether daily cortisol output differs between people with and without diabetes cross-sectionally. The findings suggested that people with diabetes have a flatter slope in cortisol output combined with heightened evening cortisol concentrations.

Following on from this Study 4 used a prospective approach to assess whether components of daily cortisol output are linked to future diabetes in an initially healthy sample. The results suggested that raised evening cortisol levels are predictive of new onset diabetes over a 9 year follow-up period.

In combination, these studies contribute to the literature linked diabetes with poor psychosocial wellbeing and stress-related alterations in biological processes.

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List of abbreviations

ADA American Diabetes Association

ARIC Atherosclerosis Risk In Communities

AUC Area under the curve

BMI Body mass index

BP Blood pressure

BPM Beats per minute

CAD Coronary artery disease

CAR Cortisol awakening response

CES-D Center for Epidemiologic Studies Depression scale

CHD Coronary heart disease

CI Confidence interval

CRP C-reactive protein

CVD Cardiovascular disease

DBP Diastolic blood pressure

ELISA Enzyme linked immunosorbent assay

ELSA English Longitudinal Study of Ageing

FPG Fasting plasma glucose

FU Follow-up

HbA1c Glycated haemoglobin

HDL High density lipoprotein

HPA Hypothalamic pituitary adrenal

HR Hazard ratio

HRV Heart rate variability

IGT Impaired glucose tolerance

IFG Impaired fasting glucose

IL Interleukin

IL-1Ra Interleukin-1 receptor antagonist

LASA Longitudinal Ageing Study of Amsterdam

LDL Low density lipoprotein

LOT-R Life Orientation Test-Revised

MESA Multi-Ethnic Study of Atherosclerosis

MI Myocardial infarction

NESDA Netherlands Study of Depression and Anxiety

NHANES National Health and Nutrition Examination Survey

OGTT Oral glucose tolerance test

OR Odds ratio

RCT Randomised control trial

RR Relative risk

SAM Sympathetic-adrenomedullary

SBP Systolic blood pressure

SD Standard deviation

SES Socio-economic status

T2D Type 2 diabetes

TNF- α Tumour necrosis factor- α

WHO World Health Organisation

1. Literature Review

1.1. Introduction

This chapter will provide a literature review describing the role of psychosocial well-being and biological stress factors in type 2 diabetes (T2D) and cardiovascular disease (CVD). Firstly, the pathophysiology of both conditions will be described and research highlighting the increased risk of CVD in people with diabetes will be presented. Following this, the idea that psychosocial stress factors may play a role in linking T2D and CVD will be introduced, and observational literature in the area will be discussed. Thereafter, an overview of the potential biological pathways through which stress may contribute to the excess risk of CVD in T2D will be presented. Finally, the limitations of work to date will be described, highlighting the gaps which this PhD sets out to address.

Note: Some of the literature included in this chapter has been published in Hackett, R., A., & Steptoe, A., (accepted). Psychosocial Factors in Type 2 Diabetes: Effect on Diabetes Risk and Cardiovascular Complications. *Current Cardiology Reports*.

1.2. Definition and description of diabetes

Diabetes mellitus is a group of metabolic disorders of multiple aetiologies characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2014). Deficient insulin action on target insulin-sensitive tissue is the basis of the abnormalities in carbohydrate, fat and protein metabolism in diabetes. Deficient insulin action can be caused by impairment in insulin secretion and/or reduced tissue responsiveness to insulin. Frequently these abnormalities

co-occur and it is often unclear which disturbance is the primary cause of hyperglycaemia.

The characteristic symptoms of marked untreated hyperglycaemia include weight loss, polyuria (excessive urination), polydipsia (excessive thirst), polyphagia (excessive hunger), and blurred vision. Susceptibility to infection and growth impairment may also accompany the onset of diabetes. Chronic hyperglycaemia is life-threatening as it can result in ketoacidosis or non-ketotic hyperosmolar syndrome (American Diabetes Association, 2014).

1.2.1. Classification of diabetes

The majority of cases of diabetes fall into two broad categories: type I diabetes and type II diabetes (American Diabetes Association, 2014). Discussion of other specific types of diabetes such as gestational diabetes, genetic defects in β -cell function or insulin action, diseases of the exocrine pancreas (e.g. pancreatitis), endocrinopathies (e.g. Cushing's syndrome), drug, chemical or infection induced diabetes are beyond the remit of this thesis.

1.2.1.1. *Type I diabetes*

Type I diabetes (previously known as insulin dependent diabetes or juvenile-onset diabetes) accounts for 5-10% of cases of diabetes (American Diabetes Association, 2014; International Diabetes Federation, 2015). The vast majority of cases of type I diabetes result from a cellular-mediated autoimmune destruction of the β -cells of the pancreas. The rate of β -cells destruction is variable, ranging from rapid (mostly in infants and children) to slow (mainly in adults). Type I diabetes usually occurs in

childhood and adolescence but the disease can affect people of any age. Autoimmune destruction of the β -cells is caused by mutations in multiple genes and is also related to environmental factors (e.g. viral infection). Destruction of the β -cells leads to an absolute insulin deficiency. Thus, in this form of diabetes, patients are dependent on replacement insulin therapy for survival.

1.2.1.2. *Type II diabetes*

Type II diabetes (T2D), formerly referred to as non-insulin dependent diabetes or adult onset diabetes accounts for 90-95% of cases of diabetes (American Diabetes Association, 2014; Holman, Young, & Gadsby, 2015; International Diabetes Federation, 2015). The pathogenesis of T2D is complicated and specific aetiologies are unclear. However, autoimmune destruction of β -cells does not occur and often individuals with T2D do not need replacement insulin treatment to survive. The condition encompasses individuals who have insulin resistance with a relative insulin deficiency, as well as those who have predominately an insulin secretory defect with insulin resistance. Frequently patients present with a combination of varying degrees of insulin resistance and impairment in insulin secretion. It is often uncertain which disturbance, if either alone, is the main cause of hyperglycaemia (American Diabetes Association, 2014).

Glucose metabolism is regulated by a feedback loop to ensure glucose homeostasis and the maintenance of glucose concentrations within a narrow range (Kahn et al., 1993). This feedback loop is dependent on communication between the β -cells and insulin-sensitive tissues, in which the magnitude of the β -cell response is affected by tissue sensitivity to insulin. Insulin mediates the uptake of glucose, amino

acids, and fatty acids by insulin-sensitive tissues. These tissues, in turn, feedback information to the β -cells about the amount of insulin they require. If insulin resistance is present the β -cells increase insulin output to maintain normal glucose homeostasis. Only when β -cells are unable to release sufficient insulin do plasma concentrations of glucose begin to rise (Kahn, Cooper, & Del Prato, 2014).

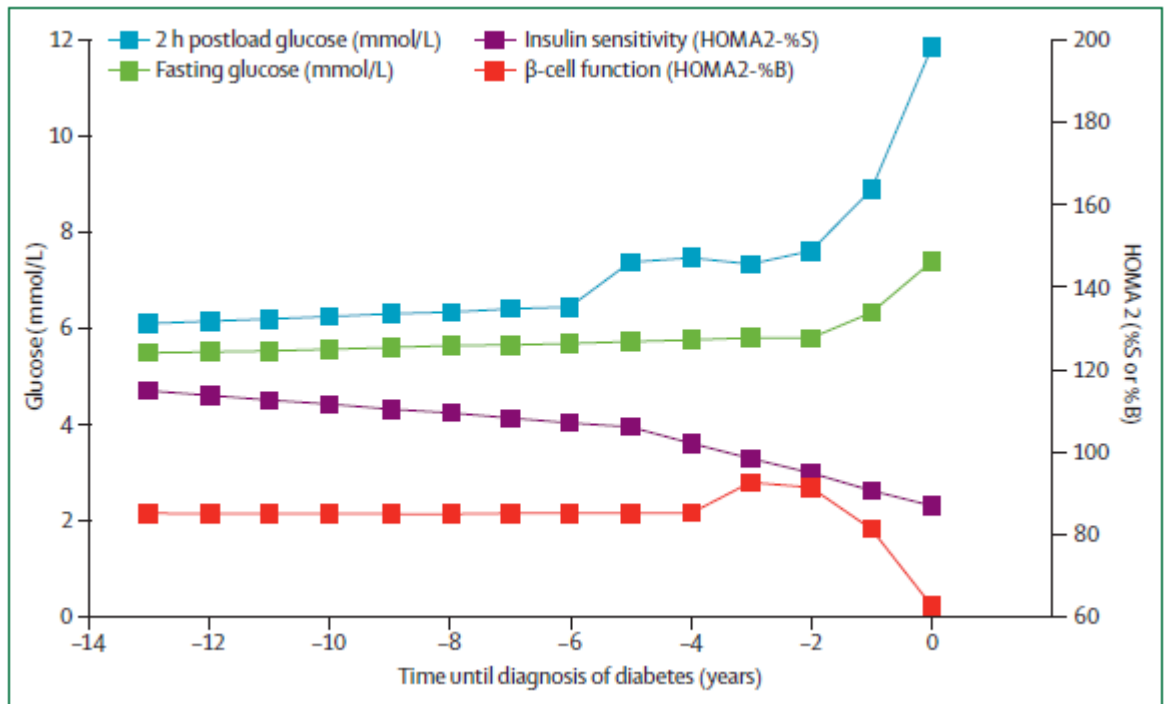
These abnormalities represent a continuum in which the scale of reduction in β -cell function establishes the magnitude of the increase in glucose concentrations. Moreover, the continuous reduction in β -cell function is affected by “glucotoxicity”, as hyperglycaemia itself can further impair β -cell function (Robertson, Harmon, Tran, & Poitout, 2004). This cycle of hyperglycaemia leads to further progressive deterioration of β -cell function and accounts for the transition from impaired glucose tolerance (IGT) to T2D.

The multi-stage model of diabetes development proposed by Weir & Bonner-Weir corresponds to the above described glucose metabolism feedback loop (Weir & Bonner-Weir, 2004). In this model progression to diabetes has definable stages characterised by changes in β -cell function. In the first “compensatory” stage insulin secretion increases to maintain normal glucose levels in the face of insulin resistance. During the second stage “stable adaption” glucose concentrations start to rise as β -cells are unable to fully compensate for insulin resistance. Individuals can remain in stage 2 for many years. However, at some critical point β -cell function becomes inadequate and glucose concentrations increase relatively rapidly through the “transient unstable period” of stage 3 to the overt diabetes of stage 4.

The importance of increasing hyperglycaemia, insulin resistance and impaired insulin secretion in the pathogenesis of T2D has also been observed in epidemiological studies. Evidence from longitudinal studies with repeated measures of glucose

concentration, insulin sensitivity and insulin secretion suggest that the development of T2D is a continuous process (Ferrannini et al., 2004; Mason, Hanson, & Knowler, 2007; Sattar et al., 2007; Tabák et al., 2009). Tabák et al., investigated trajectories of glucose, insulin sensitivity and insulin secretion in over 6500 initially healthy individuals from the Whitehall II cohort (Tabák et al., 2009). Figure 1.1 shows the trajectories of glucose and insulin parameters over time in individuals who go on to develop T2D and is taken from a review paper by the same first author (Tabák, Herder, Rathmann, Brunner, & Kivimäki, 2012).

Figure 1.1 Fasting and 2 hour post load glucose, insulin sensitivity and β - cell function before the diagnosis of diabetes



Source: Tabák, Herder, Rathmann, Brunner, & Kivimäki, (2012)

In this analysis of the Whitehall II cohort, fasting glucose and post-load glucose were found to be higher among participants who developed diabetes 13 years before disease onset (Tabák et al., 2009). Glucose values increased in a linear fashion until 2-6 years before T2D diagnosis when abrupt elevations in glucose concentrations were observed in the incident diabetes cases. In the control participants who did not develop T2D glucose parameters increased slightly over time (not shown on figure) but no abrupt rises in concentrations were detected. Looking at insulin parameters, those who developed T2D had lowered insulin sensitivity at baseline and showed a marked decrease in sensitivity in the 5 years before onset. Participants who developed T2D had elevated insulin secretion until three to four years before diagnosis when steep declines were observed in the diabetes cases. Conversely, the controls did not experience a change in insulin parameters over time (not shown on figure). A similar pattern of changes preceding diabetes development has been observed in other studies (Ferrannini et al., 2004; Festa, Williams, D'Agostino, Wagenknecht, & Haffner, 2006; Mason et al., 2007; Sattar et al., 2007).

1.2.2. Diabetes diagnostic criteria

The diagnosis of diabetes is based on glucose criteria, either fasting plasma glucose (FPG), the 75g oral glucose tolerance test (OGTT) or glycated haemoglobin (HbA1c) a marker reflecting average blood glucose concentrations over the previous 2-3 months (American Diabetes Association, 2014; WHO, 2016). The cut points presented in Table 1.1 were selected as they are associated with an inflection point for microvascular complication of diabetes (The International Committee, 2009).

Table 1.1: Criteria for diabetes diagnosis

HbA1c \geq 6.5% /48 mmol/mol

OR

8 hour fasting plasma glucose (FPG) \geq 7.0 mmol/l (126 mg/dl)

OR

2-h plasma glucose \geq 11.1mmol/l (200 mg/dl) during an OGTT

OR

A random plasma glucose \geq 11.1 mmol/l (200 mg/dl) in the presence of classic diabetes symptoms

1.2.2.1. *Pre-diabetes*

T2D can frequently go undiagnosed for many years as hyperglycaemia develops gradually. Therefore intermediate states of hyperglycaemia that are higher than normal but do not meet the diagnostic criteria for diabetes have been defined (American Diabetes Association, 2014; WHO, 2016). These states are significant as individuals with glucose concentrations in this range have an elevated risk of diabetes (Morris et al., 2013; Tabák et al., 2012). It is estimated that 70% of people with pre-diabetes will eventually go on to develop overt diabetes (Tabák et al., 2012). Obesity is the biggest modifiable risk factor for progression from pre-diabetes to diabetes, and lifestyle modification is suggest to reduce risk by 40%–70% (Tabák et al., 2012). The criteria for pre-diabetes are presented in Table 1.2.

Table 1.2: Categories of increased risk for diabetes*

HbA1c 5.7–6.4%/ 38.8 - 46.4 mmol/mol

8 hour FPG 6.1mmol/l (110 mg/dl) to 6.9 mmol/l (125 mg/dl)**

2-h plasma glucose 7.8 mmol/l (140 mg/dl) to 11.0 mmol/l (199 mg/dl) during an OGTT

*For all tests the risk is continuous becoming disproportionately greater at higher ends of the range

** The American Diabetes Association (ADA) has chosen a lower cut off of 5.6mmol/L or 100mg/dl. For the other measures the ADA and WHO criteria are the same.

1.3. Diabetes prevalence

Diabetes is major public health challenge both globally and nationally. According to reports by the International Diabetes Federation and the World Health Organisation (WHO) over 8% of the world population (415-420 million people) currently have diabetes, with prevalence expected to rise to 10.4% (642 million) by 2040 (International Diabetes Federation, 2015; WHO, 2016). In the UK, diabetes is a rapidly growing problem. Recent data suggest that 3.3 million adults, representing 6.2% of the population had diabetes in 2014 (Holman et al., 2015). It is expected that 5 million people will have the condition by 2025, with T2D accounting for 90% of cases (Holman, Young, & Gadsby, 2014). The prevalence of undiagnosed diabetes is also a significant concern. Worldwide, 193 million cases (46.5%) of diabetes are currently undiagnosed (International Diabetes Federation, 2015). While, in the UK it is estimated that 549,000 individuals are unaware of their condition (Diabetes UK, 2015). The number of individuals with glucose criteria in the pre-diabetes range is additionally rising both globally and in the UK (International Diabetes Federation, 2015; Mainous,

Tanner, Baker, Zayas, & Harle, 2014). The International Diabetes Federation estimates that 1 in 15 individuals worldwide have pre-diabetes (International Diabetes Federation, 2015). Diabetes is the fourth or fifth leading cause of mortality in most high-income countries and as such represents a significant burden to public health systems (WHO, 2011). In 2015, health spending on diabetes represented 12% (USD673 billion) of global health expenditure and it may account for up to 20% of national healthcare budgets in some countries (International Diabetes Federation, 2015). Additionally, the indirect costs of diabetes such as a reduced labour force and lowered economic productivity are considerable (Seuring, Archangelidi, & Suhrcke, 2015). The UK it is estimated that 10% of the NHS budget is spent on diabetes. The direct and indirect costs of diabetes care totalled £23.7 billion in 2012 and are predicted to rise to £39.8 billion by 2035 (Hex, Bartlett, Wright, Taylor, & Varley, 2012).

The rapid increase in diabetes prevalence has been attributed to population ageing and rising obesity rates (Danaei et al., 2011; International Diabetes Federation, 2015). In 2013, there were almost a billion people globally over the age of 60 and this figure is expected to rise to 1.5 billion by 2035. As the number of older people has increased there has been a concomitant rise in the number of individuals over 60 living with diabetes (International Diabetes Federation, 2014). Obesity is a potent risk factor for T2D (Guh et al., 2009). Worldwide rates of overweight and obesity have increased substantially over the past 30 years (Ng et al., 2014). According to recent estimates 60% of the adult population in the UK are overweight and 25% are obese (OECD, 2014).

1.4. The complications of diabetes

Chronic hyperglycaemia is linked with long-term damage, dysfunction and failure of various organs. The harmful effects of diabetes are separated into microvascular

(damage to the small blood vessels) and macrovascular (damage to the large blood vessels) complications. Microvascular complications include retinopathy (damage to the eyes) leading to blindness, nephropathy (damage to the kidneys) leading to renal failure and neuropathy (damage to the nerves) leading to impotence, foot ulcers and amputation. The macrovascular complications of diabetes include various cardiovascular diseases (Fowler, 2008).

1.4.1. CVD: Pathophysiology and prevalence

Cardiovascular disease (CVD) is an umbrella term for a group of disease affecting the circulatory system. The most common forms of CVD are coronary heart disease (CHD) (also known as ischemic heart disease or coronary artery disease (CAD)) and stroke. The primary pathological process which underlies CHD is atherosclerosis, a life-long chronic inflammatory process in which fatty deposits (atheroma) cause progressive narrowing of the coronary arteries leading to impaired blood flow to the cardiac muscle. The majority of strokes also stem from atherosclerosis whereby the arteries to the brain are impaired (Libby, Ridker, & Hansson, 2011). CVD is the leading cause of death worldwide and as such represents a major public health challenge. According to the most recent estimates from the WHO, 17.5 million people died from CVD in 2012, accounting for 31% of deaths globally (WHO, 2015). CVD is one of the leading causes of death in the UK. In 2014, around 155,000 deaths were attributed to CVD, of which 45% and 25% were due to CHD and stroke respectively. In the UK, mortality from CVD has been falling since the early 1970s (Townsend, Bhatnagar, Wilkins, Wickramasinghe, & Rayner, 2015). This decline is thought to be attributable to reductions in smoking, improved hospital treatment and better management of blood pressure (BP) and cholesterol (Smolina, Wright, Rayner, & Goldacre, 2012). Despite

downward trends in mortality the economic costs of CVD are vast. In 2009, the overall cost of CVD to the UK economy was an estimated £19 billion. Approximately 46% of this figure was due to direct health care costs, 34% to productivity losses and 20% to informal care of CVD patients (Townsend et al., 2012). Current cost data for the UK as a whole is not available. However, the most recent figures for England estimate that £6.8 billion was spent on treating CVD in 2013 (Bhatnagar, Wickramasinghe, Williams, Rayner, & Townsend, 2015).

1.4.2. CVD: Mortality and morbidity in T2D

Cardiovascular disease (CVD) is a major cause of mortality and morbidity in individuals with T2D. Results from a meta-analysis of 102 prospective studies indicate that people with diabetes have a two-fold excess risk of CVD compared with controls. This association was independent of traditional CVD risk factors such as smoking, body mass index (BMI), BP and lipids (Emerging Risk Factors Collaboration et al., 2010). Contemporary evidence from a longitudinal population study of 1.9 million people provides more evidence that there is a strong association between T2D and incident CVD (Shah et al., 2015); however, it is worth noting that the magnitude of the reported relationship varied between different CVD sub-types in this study.

Diabetes was previously thought of as “CHD risk-equivalent”, suggesting that people with diabetes without prior myocardial infarction (MI) have the same risk of a cardiac event as individuals without diabetes who had suffered a MI (Haffner, Lehto, Rönnemaa, Pyörälä, & Laakso, 1998). More recent meta-analytic results suggest that although diabetes greatly increases the risk of CHD it may not reach risk-equivalence for harmful cardiovascular outcomes (Bulugahapitiya, Siyambalapitiya, Sithole, & Idris, 2009). Moreover, in keeping with wider population time trends CVD rates have fallen

over the past decades among people with T2D (Barengo, Katoh, Moltchanov, Tajima, & Tuomilehto, 2008); however, the reduction has been less than in the rest of the population, thus the heightened risk of CVD in people with T2D persists (Fox et al., 2015; Gore et al., 2012).

In addition to heightened CVD risk, reductions in life-expectancy following a T2D diagnosis are primarily driven by CVD. According to a review of 97 prospective studies, individuals with diabetes die 6 years earlier on average than their counterparts without the condition, and approximately 58% of this survival difference is attributable to excess vascular deaths after adjustment for conventional CVD risk factors (Emerging Risk Factors Collaboration, 2011). Similar estimates of CVD driven excess mortality are presented in the latest International Diabetes Federation and WHO reports (International Diabetes Federation, 2015; WHO, 2016). In an authoritative pooled analysis of 91 longitudinal cohort studies, diabetes, stroke and MI were found to have equivalent associations with all-cause mortality (The Emerging Risk Factors Collaboration, 2015).

Outcomes following a cardiovascular event are substantially worse in people with T2D. In the UK, diabetes is thought to account for 44% of hospital bed days for CVD (Diabetes UK, 2015). Similarly in the US in 2010, hospitalization rates for MI and stroke were 1.8 and 1.5 times higher respectively, in people with T2D compared to those without the condition (CDC, 2014). In a trial of 13,608 ST-segment elevation MI patients, participants with diabetes had significantly higher rates of recurrent non-fatal MI and cardiovascular death than patients without diabetes (Wiviott et al., 2008). Meta-analytic results suggest that diabetes is approximately a third more strongly related to fatal than non-fatal MI (Emerging Risk Factors Collaboration et al., 2010). Similarly, in individuals with heart failure diabetes is an independent predictor of repeat

hospitalisation and sudden cardiac death (MacDonald et al., 2008). Diabetes increases the risk of recurrent stroke (van Wijk et al., 2005) and attenuates both cognitive and functional recovery (Newman, Bang, Hussain, & Toole, 2007). Myocardial revascularisation procedures (coronary artery by-pass graft or percutaneous coronary intervention) are also challenging in individuals with diabetes, compared to patients without the condition, people with diabetes have a substantially increased risk of mortality and adverse clinical outcomes following these procedures (Hlatky et al., 2009; Kuchulakanti et al., 2006).

Additionally, evidence from systematic reviews and meta-analyses indicate that pre-diabetes is associated with an increased risk of CVD (Emerging Risk Factors Collaboration et al., 2010; Ford, Zhao, & Li, 2010; Levitan, Song, Ford, & Liu, 2004). Indeed, it has been suggested that abnormal glucose regulation is more common than normal glucose metabolism in patients with CHD (Beckman, Paneni, Cosentino, & Creager, 2013). For example, one large study conducted an OGGT in CHD patients without known diabetes in 110 medical centres in 25 countries. Results from the study indicated that the majority of participants had previously unknown abnormal glucose metabolism. Of these, 18% were newly diagnosed with overt diabetes, 32% had IGT and 5% had impaired fasting glucose (Bartnik et al., 2004). Overall, the literature indicates that CVD and diabetes are strongly linked at all stages of the disease process.

1.5. CVD and T2D: A link beyond traditional risk factors

Lifestyle factors (smoking, poor diet, physical inactivity, excess alcohol consumption), clinical factors (obesity, hypertension, raised cholesterol) and psychosocial stress have been identified as modifiable risk factors for CVD development (WHO, 2011; Yusuf et al., 2004).

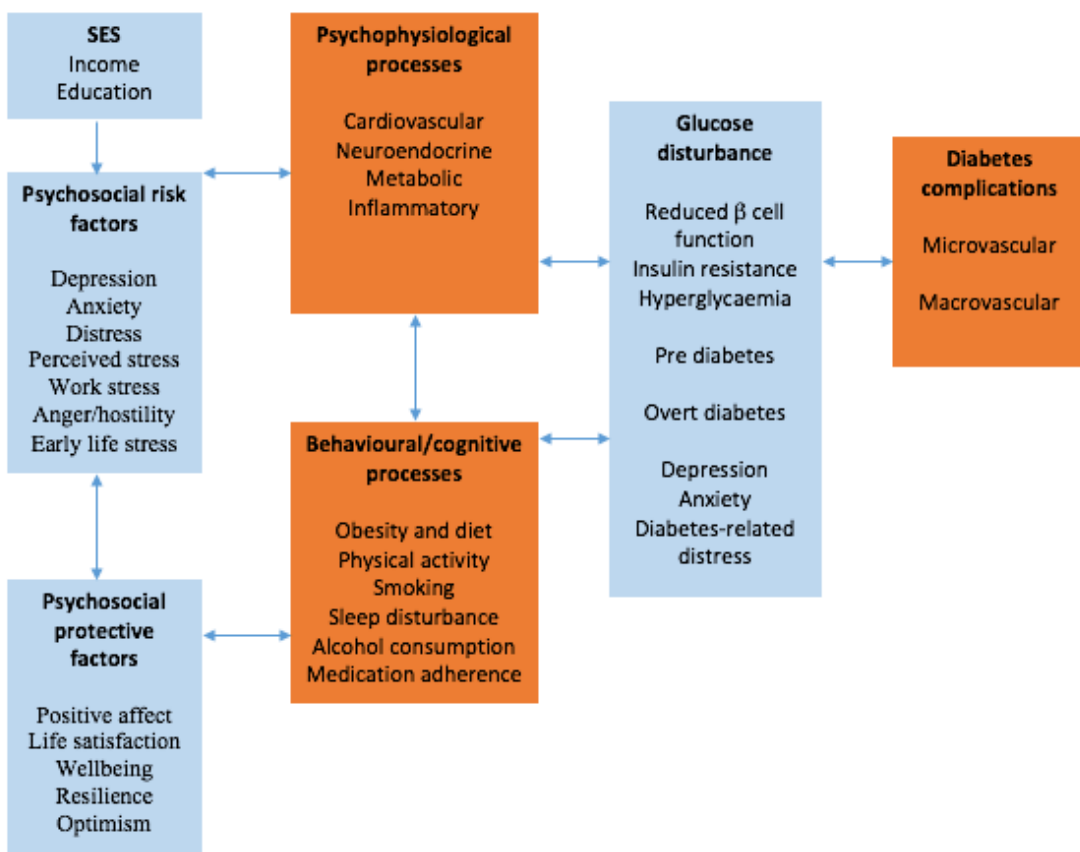
There is evidence that the link between CVD and T2D is not fully accounted for by behavioural and clinical risk factors. In meta-analyses showing greatly increased CVD morbidity and mortality in T2D, the reported associations were robust to adjustment for traditional CVD risk factors (Emerging Risk Factors Collaboration, 2011; Emerging Risk Factors Collaboration et al., 2010). With regards to intervention and prevention, intensive programs targeting lifestyle factors such as diet, physical activity and weight management have been shown to prevent T2D onset in people with and without pre-diabetes (Knowler et al., 2002; Li et al., 2008; Uusitupa et al., 2009). The Diabetes Prevention Programs are recognised as some of the most effective lifestyle interventions for preventing chronic disease. Since these interventions modify CVD risk factors such as high BMI and BP (Look AHEAD Research Group et al., 2013), they in turn should have an impact on CVD outcomes.

However, lifestyle interventions to reduce CVD in people with T2D have been largely disappointing. These trial results were reviewed in 2015 in a joint report from the American Heart Association and ADA (Fox et al., 2015). Evidence from prospective intervention studies such as the LookAHEAD trial (Look AHEAD Research Group et al., 2013) and the ACCORD trial (ACCORD Study Group et al., 2010) suggests that the modification of behavioural risk factors, such as weight loss and BP do not significantly lower the risk of adverse CVD outcomes in people with T2D. Another review of nearly 100 studies that looked at physical activity interventions and subsequent CVD in people with T2D, concluded that although effects have been seen in small studies, large randomised control trials (RCTs) have not found a protective effect (Koivula, Tornberg, & Franks, 2013). Some positive findings regarding diet have been reported in the Spanish PREDIMED RCT. This study found that participants with T2D who were randomised to a Mediterranean diet had a 30% reduced risk of CVD over 4.8 years

follow-up (Estruch et al., 2013). However, these results must be interpreted with caution as a similar effect of dietary change on CVD outcomes were not reported in the LookAHEAD trial or the Diabetes Prevention Programs (Knowler et al., 2002; Li et al., 2008; Uusitupa et al., 2009). It may be that the Mediterranean diet is superior to the low-calorie diet used in other trials, but further studies are required to test this assertion.

In light of this evidence, it appears that increased risk of CVD in people with T2D is not fully explained by traditional risk factors. Therefore, psychosocial stress offers another potentially modifiable pathway linking CVD and T2D. Observational evidence concerning the role of psychosocial stress in both conditions will be presented in the following sections. A conceptual model outlining the proposed pathways through which psychosocial stress, stress-related biological changes and lifestyle factors are linked to diabetes is presented in Figure 1.2 below.

Figure 1.2 Conceptual model



1.5.1. Psychosocial factors in CVD

Psychosocial stress factors can be divided into negative emotional disorders (e.g. depression and anxiety), personal traits (e.g. anger or hostility) and external stressors (exposure to stressful conditions). There is accumulating evidence that psychosocial stress plays a role in the pathogenesis of CVD (Dimsdale, 2008; Hjemdahl, Rosengren, & Steptoe, 2012; Steptoe & Kivimäki, 2013). Systematic reviews and prospective analyses (Everson-Rose & Lewis, 2005; Kuper, Marmot, & Hemingway, 2002) are in agreement that chronic stressors and psychosocial factors predict future CHD in initially healthy populations independently of standard risk factors. The following section provides a brief overview of the literature investigating the link between different psychosocial factors and CVD.

In the INTERHEART study the association between stress over the previous 12 months and CHD was assessed in 15,152 MI patients and 14,820 controls free of CHD. The participants in this study were recruited from 52 countries worldwide and stress was assessed using a composite score which comprised of stress at work and home, financial stress, major life events, lack of control and depression. The results of the study indicated that psychosocial stress increases the risk of MI almost threefold (Odds ratio (OR) = 2.67; 95% confidence interval (CI) 2.21 - 3.22) controlling for a range of traditional CVD risk factors. This association was seen in all age groups, in men and women and in all the countries assessed (Yusuf et al., 2004).

Prospective studies and meta-analyses have also investigated the association between individual psychosocial stress factors and subsequent CHD development. Work stress is the most widely studied external stressor. The work stress literature has been dominated by the 'demand-control' or 'job strain' conceptual model, in which a combination of highly demanding work and low decision latitude elicits stress in the

workplace (Karasek & Theorell, 1990). Kivimäki et al., conducted a meta-analysis of 13 prospective cohort studies investigating the link between job strain and CHD in initially healthy samples (Kivimäki et al., 2012). Results of the study suggest that employees who experience job strain have a 1.2 fold increased risk of CHD compared to their counterparts who do not experience stress in the workplace (95% CI 1.1 - 1.4). Social isolation is another external stressor that has been related to CHD. Meta-analytic results indicate that social isolation and loneliness are associated on average with a 50% excess risk of CHD (Relative risk (RR) 1.5; 95% CI 1.2 - 1.9) (Steptoe & Kivimäki, 2012).

Negative emotional disorders have also been researched as aetiological factors in CHD. Psychological distress (which captures symptoms of anxiety and depression, as well as social dysfunction and loss of confidence) has been prospectively related to increased CVD mortality in populations who were disease free at baseline (Russ et al., 2012). Depression and anxiety as independent constructs have additionally been related to subsequent CHD development. Nicholson, Kuper, & Hemingway, (2006) conducted a meta-analytic review of 21 prospective studies linking depression with future CHD. Over a mean follow-up period of 10.8 years depressed individuals were found to have 1.81 fold greater risk of future CHD compared to people without the condition (95% CI 1.53 - 2.15). A meta-analytic review of anxiety and incident CHD identified 20 studies with an average of 11.2 years follow-up (Roest, Martens, de Jonge, & Denollet, 2010). Results from this study indicate that anxiety is linked with subsequent CHD development (Hazard Ratio (HR) 1.26; 95% CI 1.15 - 1.38), as well as an increased risk of cardiac death (HR 1.48; 95% CI 1.14 - 1.92).

Personality factors have been implicated in CHD. Chida & Steptoe, (2009b) evaluated the association between anger and hostility in 25 prospective cohort studies. These investigators found that more hostile individuals and those with an angry

temperament had a 1.19 fold increased risk of subsequent CHD development (95% CI 1.05-1.35). Personal attitudes concerning the relationship between stress and health have also been evaluated in relation to CHD. For example, in a study of 7269 individuals from the Whitehall II cohort, individual belief that stress affects health was prospectively associated with a two-fold increased risk of CHD (HR 2.12; 95% CI 1.52 - 2.98) over 18 years of follow-up (Nabi et al., 2013). This association was robust to adjustment for a range of conventional CVD risk factors.

In addition to being associated with increased CVD risk over time in initially healthy populations, psychosocial factors can act more acutely as triggers of major cardiac events in individuals with underlying CAD. Evidence for emotional triggering of events derives from population based studies of hospitalisations and sudden deaths following major events such as earthquakes, terrorist attacks and important sporting events, as well as from clinical investigations with survivors of acute coronary events (Steptoe & Brydon, 2009). Patient studies indicate that a period of intense anger, emotional stress or depression can trigger acute coronary events. Case-crossover methods are the gold standard of research in this area. These involve comparison between a 'hazard period' before the onset of cardiac symptoms and a control period on the same individual. A meta-analysis of five case-crossover studies suggests that the pooled relative risk of acute coronary syndrome onset is elevated 2.48 fold (95% CI 1.75 - 3.51) when preceded by a period of acute emotional stress, anger or depression (Steptoe & Kivimäki, 2013). Similarly, a more recent meta-analysis (Mostofsky, Penner, & Mittleman, 2014) of nine case-crossover studies investigating anger outbursts and acute MI found that the onset incidence was elevated 2.43 fold (95% CI 2.01 - 2.90) within two hours of an intense period of anger. A dose-response relationship was observed in this analysis as greater anger was associated with a higher relative risk of an

acute event.

As well as playing a role in the aetiology of CHD and the triggering of cardiac events, prospective analyses have also linked psychosocial adversity with poorer prognosis in CHD patients. Work stress has been investigated in relation to repeat cardiac events. In a study of 972 of men and women who returned to work following an MI, individuals who reported job strain at initial return to work and 2 years later had twice the risk of recurrent CHD 2.2 years later than their counterparts who did not report job strain at work (HR 2.20; 95% CI 1.32 - 3.66) (Aboa-Éboulé et al, 2007). This association was robust to adjustment for 26 potential confounding factors including socio-demographic, cardiovascular health and conventional CVD risk variables. Another study (László, Ahnve, Hallqvist, Ahlbom, & Janszky, 2010) of 676 survivors of acute MI with a longer follow-up period of 8.5 years found that high levels of job strain predicted non-fatal MI as well as cardiac death (HR 1.73; 95% CI 1.06 - 2.83). Research has also investigated the impact of social support on prognosis in CHD patients. Barth et al., conducted a review of 20 prognostic studies of CHD patients (including MI patients and individuals who had undergone a percutaneous coronary intervention) (Barth, Schneider, & von Känel, 2010). Results of the study suggest that lower functional social support (lack of help and encouragement by an individual's social network) increases cardiovascular and all-cause mortality in CHD patients (RR 1.71; CI 95% 1.26 - 2.31). Perceived stress is another external stressor that has been associated with adverse health outcomes in CHD patients. In a study of 4204 MI patients, individuals who reported moderate or high stress had 12.9% increased 2 year mortality compared to 8.6% mortality for those reporting low stress levels. This association was independent of both socio-demographic as well as clinical risk factors.

Negative emotional disorders are suggested to influence health outcomes in

CHD patients. Depression is prevalent among CHD patients. According to a comprehensive review of the literature 20% of patients hospitalized following an MI meet the criteria for major depression, while approximately 1 of 3 patients have mild-to-moderate symptoms of depression (Thombs et al., 2006). Results from several meta-analyses indicate that depression post MI is associated with increased cardiovascular and all-cause mortality, as well as a greater risk of repeat cardiac events (Barth et al., 2010; Meijer et al., 2011; Nicholson et al., 2006). There is also evidence that anxiety is linked with poor prognosis in CHD patients. For example, anxiety has been found to prospectively increase the risk of cardiac events in patients with stable CHD (Moser et al., 2011) and meta-analytic results indicate that anxiety following an MI is associated with a 36% increased risk of adverse cardiac outcomes (Roest et al., 2010).

Personality traits have also been associated with adverse health outcomes in patients with CHD. Chida & Steptoe reviewed 19 prospective cohort studies investigating the association between anger/hostility in samples with existing CHD (Chida & Steptoe, 2009b). The results of this study suggest that anger or hostility is associated with a 1.24 increased hazard of adverse outcomes in CHD patients (95% CI 1.08 - 1.42).

In sum, the evidence in this area suggests that psychosocial stress factors contribute to CHD across the disease process from aetiology to prognosis and progression. However, it should be emphasised that the studies relating psychosocial factors with CHD are observational, and as such they cannot prove causality. CHD is associated with many risk factors (WHO, 2011; Yusuf et al., 2004) and the possibility of confounding arises when these factors are additionally associated with psychosocial stress (Steptoe & Kivimäki, 2012). Confounding by unknown or unmeasured factors offers an alternative explanation for the observed associations between exposure and

outcome. Residual confounding can also occur from known factors if they have been poorly measured. Heterogeneity in the measurement of psychosocial factors is another methodological limitation of this literature, as is the inconsistent use of covariates.

Specific reviews of the depression-CHD literature point to issues of publication bias in the field (Frasure-Smith & Lespérance, 2005; Kuper et al., 2009), as studies that report null findings are less likely to be published than those that report statistically significant associations (Song et al., 2010). This possibility could lead to an overestimation of the relationship between depression and CHD.

Furthermore, although the healthy cohort studies included this review had extended follow-up periods and excluded people with prevalent CHD at baseline the issue of reverse causality cannot be ruled out. It is possible in studies that relied on self-report measures of CHD that some of the participants included could have had subclinical atherosclerosis at baseline, as this condition develops slowly and progressively over the life course (Libby et al., 2011).

Despite these issues, the bulk of the evidence to date suggests that psychosocial factors are involved in the pathogenesis of CHD. Considering the strong links between CVD and T2D, it is not surprising that there has been interest in investigating whether psychosocial stress plays a role in T2D and whether this is related to CVD risk in this population. The following sections review evidence from prospective studies that have looked at the links between different psychosocial stress factors and T2D.

1.5.2. Psychosocial factors and T2D risk

The idea that stress is linked to diabetes is an old hypothesis. Since the 17th century, it has been postulated that stress plays a role in the pathogenesis of disease (Pouwer,

Kupper, & Adriaanse, 2010; Willis, 1678). A summary of prospective studies that have investigated the association between psychosocial factors and T2D risk can be found in Table 1.3. As can be seen from the table, several different types of psychosocial factor have been investigated.

Table 1.3 Prospective studies linking psychosocial factors with T2D risk

First author (date)	Population (sample size, follow-up (FU))	Design	Psychosocial measure	Association with T2D outcome (green = positive; orange= some red=none)
Knol (2006)	9 studies (n = 174,035; average FU 9.4 years)	Meta-analysis	Depression	RR: 1.37 (95% CI 1.14-1.63)
Mezuk (2008)	13 studies (n=6916 incident T2D cases; average FU 9.4 years)	Meta-analysis	Depression	RR: 1.60 (95% CI 1.37-1.88)
Demakakos (2014)	ELSA cohort (n=4238; 6 years FU)	Prospective cohort	Depressive symptoms using the 8-item Centre for epidemiologic studies-depression scale (CES-D)	OR: 1.53 (95% CI 0.80-2.93)
Golden (2008)	MESA cohort (n=5201; 3.2 year FU)	Prospective cohort	Depressive symptoms using 20 item CES-D	Relative hazard 1.10 (95% CI 1.02-1.19)
Pan (2010)	The Nurses' Health Study (women only; n=65381, incident T2D cases n=2844; 10 years FU)	Prospective cohort	Depressive symptoms using 5-item Mental Health Index	RR 1.17 (95% CI 1.05-1.30)
Engum (2007)	Norwegian cohort (n=37,291; 10 years FU)	Prospective cohort	Anxiety using the Hospital anxiety and depression scale, 7-items for anxiety	OR= 1.5 (95% CI 1.3-1.8) Did not control for depression.
Atlantis (2012)	NESDA cohort (n=2460; FU 2 years)	Prospective cohort	Anxiety using Composite Interview Diagnostic Instrument	OR=1.6 (95% CI 1.2-2.1)
Abraham (2015)	MESA cohort (n=5598; FU 11.4 years)	Prospective cohort	Anxiety using the Spielberger Trait Anxiety Scale	HR 1.16 (95% CI 0.87- 1.54)
Edwards (2012)	US adults (n=1920; FU 11 years)	Prospective cohort	Anxiety using the Diagnostic Interview Schedule	OR 1.00 (95% CI: 0.53- 1.89)
Farvid (2014)	1. Health Professional's FU study (n=30791 men) 2. Nurses' Health Study (n=68904 women) 3. Nurses' Health	3 prospective cohorts	Phobic anxiety symptoms using the 8-item Crown-Crisp index	1. No association in the male cohort after adjustment. <i>Associations in women</i> 2. Nurses' Health Study

First author (date)	Population (sample size, follow-up (FU))	Design	Psychosocial measure	Association with T2D outcome (green = positive; orange= some red=none)
	Study II (n=79960 women) Total n=12831 incident cases, 18-20 years FU			HR, 1.02 (95% CI 1.01–1.03) 3. Nurses' Health Study II HR, 1.04 (95% CI 1.02–1.05).
Demmer (2015)	1. NHANES cohort (n=3233; 17 year FU) 2. The Detroit Neighbourhood Study (n=1054; 18 year FU)	2 prospective cohorts	Anxiety using the Generalized Anxiety Disorder-7 item questionnaire	No association in the overall sample. Dividing the groups by sex, in both studies association found in women only. 1. Risk ratio = 2.19 (95% CI 1.17–4.09) 2. Risk ratio =1.62 (95% CI 0.61-4.32)
Mommersteeg (2012)	UK adults (n=9514; 18 years FU)	Prospective cohort	Psychological distress using the 12- item General Health Questionnaire	HR 1.33 (95% CI 1.10–1.61)
Virtanen (2014)	Whitehall cohort (n=5932; average FU 5.46 years)	Prospective cohort	Psychological distress using the 30- item General Health Questionnaire	No association in the overall sample, only in those at high risk of T2D at baseline OR 2.07 (95% CI 1.19–3.62)
Eriksson (2008)	Swedish adults (n=5227; 8-10 years FU)	Prospective cohort	Psychological distress using an index of 5 questions on anxiety, apathy, depression, fatigue and insomnia	Association only in males OR 2.2 (95% CI 1.2-4.1)
Nyberg (2014)	13 European studies (n=124,808; n=3703 incident T2D cases; 10.3 year FU)	Meta-analysis	Work stress defined by job strain	HR 1.15 (95% CI 1.06–1.25)
Kivimäki (2015)	19 cohort studies from the US, Europe, Japan & Australia (n=222,120, n=4963 incident cases; average 7.6 years FU)	Meta-analysis	Work stress defined by long working hours >55 hours a week	Association only in low SES groups RR 1.29 (95% CI 1.06- 1.57)
Novak (2013)	(n=7251 men; FU 35 years)	Prospective cohort	Perceived permanent stress (self-reported stress related to work or home life ongoing for >1 year)	HR 1.52 (95% CI 1.26–1.82)
Toshihiro (2008)	Japanese men (n=128; 3.2 years mean FU)	Prospective cohort	Perceived stress using 15-item stress in daily life questionnaire developed for Japanese individuals	HR 3.81 (95% CI 1.09-13.35)
Rod (2009)	Danish adults (n=7066; 10 year)	Prospective cohort	Perceived stress intensity on 7 point scale (1-item)	Association only in men OR 2.36 (95%

First author (date)	Population (sample size, follow-up (FU))	Design	Psychosocial measure	Association with T2D outcome (green = positive; orange= some red=none)
	FU)		and stress frequency on 7 point scale (1-item).	CI 1.22- 4.59)
Williams (2013)	Australian adults (n=3759; 5 year FU)	Prospective cohort	Perceived stress questionnaire 30 item	Outcome was abnormal glucose tolerance rather than overt T2D. Association only in women OR 1.72 (95% CI 1.07-2.76).
Kato (2009)	Japanese adults (n=55,826, n=1601 incident cases; 10 year FU)	Prospective cohort	Perceived stress based on 1 item 'How much stress to feel in daily life?' (3 point scale)	Overall association but effects were stronger among male (OR 1.39; 95% CI 1.15-1.65) than female participants (OR 1.25; 95% CI 1.01-1.56)
Wiernik (2016)	French adults (n=22,567, n=527 incident cases; 5.3 year FU)	Prospective cohort	Perceived Stress using the 4-item Perceived Stress Scale	Association only in those of low occupational status OR 1.39 (95% CI: 1.02–1.90)
Huang (2015)	7 studies, 4 prospective (n=87, 251, n=5879 incident cases)	Meta-analysis	Adverse childhood experiences	OR 1.32 (95% CI 1.16 - 1.51)
Virk (2012)	Danish cohort (n=1.9 million, n=45,302 who experienced pre-natal stress)	Prospective cohort	Pre-natal stress measured by mother's experience of bereavement	Incidence rate ratio 1.31 (95% CI 1.01–1.69)
Golden (2006)	ARIC cohort (n=11,615; 6 year FU)	Prospective cohort	Anger using the Spielberger Trait Anger Scale (10 item)	HR 1.34 (95% CI 1.10- 1.62)
Abraham (2015)	MESA cohort (n=5598; FU 11.4 years)	Prospective cohort	Anger using the Spielberger Trait Anger Scale	1. Trait anger HR 1.48 (95% CI 1.04-2.12). Attenuated after adjustment for waist circumference. 2. Analysis of the Spielberger anger reactivity sub-scale HR = 1.07 (95% CI 1.03-1.11) robust to all covariates
Crump (2016)	Swedish male military conscripts(n=1.5 million; average 25.7 FU)	Prospective cohort	Low stress resilience assessed by semi-structured interview	HR, 1.51 (95% CI 1.46-1.57)
Boehm (2015)	Whitehall cohort (n=7800; 13 years FU)	Prospective cohort	1. Life satisfaction measured by self-report satisfaction with 7 life domains. 2. Emotional vitality	No associations in overall sample. Sub-analyses by diabetes type (doctor diagnosed or

First author (date)	Population (sample size, follow-up (FU))	Design	Psychosocial measure	Association with T2D outcome (green = positive; orange= some red=none)
			measured using 3 items from the Short-form 36 3. Optimism measured using 1-item “Over the next 5–10 years, I expect to have many more positive than negative experiences” (6 point scale)	Whitehall assessment) people with high life satisfaction (OR=0.85; 95% CI=0.76-0.95) & emotional vitality (OR=0.86; 95% CI=0.77-0.97) were less likely to report doctor diagnosed diabetes.

ARIC= Atherosclerosis Risk in Communities Study; CES-D= Centre for Epidemiologic Studies-Depression scale; FU= Follow-up; ELSA= English Longitudinal Study of Ageing; HR= hazard ratio, MESA= Multi-Ethnic Study of Atherosclerosis, NESDA= Netherlands Study of Depression and Anxiety; NHANES = National Health and Nutrition Examination Survey; OR=odds ratio; RR= relative risk; T2D= type 2 diabetes; SES= socioeconomic status; 95% CI= 95% confidence interval

1.5.2.1. *Emotional distress*

In the present day, depression is the most commonly researched factor in studies of diabetes. Results from two meta-analyses of longitudinal studies indicate that depression is associated with 37-60% increased risk of developing T2D (Knol et al., 2006; Mezuk, Eaton, Albrecht, & Golden, 2008). More recently, a cross-sectional study of 37,403 Swedish twins detected a relationship of similar magnitude, where major depression was associated with 32% increased risk of T2D, but only in younger (40-55 years) and not older twins (>55 years) (Mezuk, Heh, Prom-Wormley, Kendler, & Pedersen, 2015). Prospective evidence also suggests that elevated depressive symptoms, as well as clinical depression are related to subsequent incidence of diabetes (Demakakos, Zaninotto, & Nouwen, 2014; Golden et al., 2008; Pan et al., 2010). The associations reported in these studies remained significant after controlling for T2D risk

factors such as, BMI, family history of diabetes, smoking, physical activity, diet and alcohol consumption. It should be emphasised that these studies do not prove causality, and alternative non-causal explanations are plausible (Tabák, Akbaraly, Batty, & Kivimäki, 2014). One possibility is that diabetes and depression share common etiological factors such as physical inactivity or inflammation that may not be completely eliminated by statistical adjustments. Alternatively, preclinical diabetes may increase the chances that an individual reports depression, resulting in a reverse causal process that is detailed later in this chapter.

Fewer studies have investigated whether anxiety is associated with T2D development. Engum (2007) investigated the association in a Norwegian population cohort of 37,291 people. Over 10 year follow-up individuals who reported symptoms of anxiety at baseline had an increased risk of developing T2D (OR= 1.5; 95% CI 1.3 - 1.8) (Engum, 2007). A limitation of this study was that anxiety and depression symptoms were not investigated separately, therefore the effect of anxiety on T2D independent of depression could not be assessed. Nevertheless, a similar association between anxiety alone and incident diabetes (OR=1.6; 95% CI 1.2- 2.1) was found in the Netherlands Study of Depression and Anxiety (NESDA) (Atlantis, Vogelzangs, Cashman, & Penninx, 2012).

Other research in the field has not found an association between anxiety and subsequent T2D. In the Multi-Ethnic Study of Atherosclerosis (MESA) cohort the association between trait anxiety assessed by questionnaire and incident T2D was assessed in 5598 individuals. However, no relationship was detected over 11.4 years follow-up (Abraham et al., 2015). Another US cohort study of 1920 people found no association between anxiety disorders assessed by diagnostic interview and new onset T2D over 11 years follow-up (Edwards & Mezuk, 2012). This study controlled for

depression and the MESA cohort adjusted for both depressive symptoms and antidepressant medication usage. The findings were unchanged after these adjustments. This suggests that the pathways linking depression and T2D might not be implicated in anxiety or that anxiety alone is not as important a psychological factor as depression in predicting T2D.

Another explanation for equivocal findings might be sex differences. An analysis of three large US population cohort studies found that phobic anxiety was associated with subsequent diabetes onset (Farvid et al., 2014). However, this association was only robust to adjustment for lifestyle factors in two out of the three cohort studies assessed. A discussion of the issues surrounding the inclusion of lifestyle factors as covariates will be presented in section 1.5.4. Interestingly, in the cohorts where an association was detected the samples were completely female and the cohort with no association was entirely male. In another recent study of participants from the National Health and Nutrition Examination Survey (NHANES), there was no association between anxiety and incident T2D in overall sample (Demmer et al., 2015). However, when dividing the groups by sex, anxiety symptoms were significantly associated with increased T2D risk in women (Risk ratio = 2.19; 95% CI 1.17 – 4.09), but not in men (Risk ratio = 0.85; 95% CI 0.56 – 1.28).

In sum, the literature looking at anxiety and new onset diabetes is mixed. There are several possible explanations for these equivocal findings. It may be that the symptoms of depression and anxiety overlap or that there is no independent association between anxiety and T2D. Differences in measurements across studies or sex differences in the association might also account for the results.

Psychological distress encompasses a range of co-morbid psychological factors, such as depressive and anxiety symptoms, general stress as well as sleep disturbance.

Distress has been investigated in relation to diabetes development, but associations have not been consistent. In a UK study of 9514 people, psychological distress at baseline was associated with incident T2D over 18 years follow-up adjusting for age, sex, education and income (HR 1.33; 95% CI 1.10 – 1.61). However, the relationship did not remain significant additional adjustment for health-related factors (Mommersteeg, Herr, Zijlstra, Schneider, & Pouwer, 2012). In the UK Whitehall II cohort, psychological distress did not predict T2D in the overall sample, but in a subsample of participants at high risk of T2D (pre-diabetes at baseline and >40 on the Framingham diabetes risk score), distress was associated with a 40.9% increased risk of T2D independent of age, sex, SES, anti-depressant usage, smoking and physical activity (Virtanen et al., 2014). No sex interaction effects were found in the UK studies, but an earlier Swedish study of 5227 individuals who were normoglycaemic at baseline reported an association between distress and subsequent T2D in male but not in female participants (Eriksson et al., 2008). The reasons for the mixed findings in the studies are unclear. It may be that initial health moderates the relationship and psychological distress accelerates progression to T2D only in high risk individuals. Another issue is that distress in of itself may be too broad a measure and that only particular aspects of it are related to future T2D.

1.5.2.2. *Exposure to life stress*

Chronic exposure to external stressors has also been implicated in T2D onset. To date the majority of research has explored the relationship between work stress and incident diabetes and several meta-analyses concerning various work stress constructs have been carried out (Cosgrove, Sargeant, Caleyachetty, & Griffin, 2012; Kivimäki et al., 2015;

Nyberg et al., 2013, 2014). Job strain, which is the combination of high job demands and low control at work, is a widely studied work stress construct (Karasek & Theorell, 1990). A meta-analysis investigating the link between job strain and T2D development pooled results from 13 prospective European cohort studies. Over a mean follow up of 10.3 years job strain was associated with a 1.15-fold (95% CI 1.06 – 1.25) increased risk of incident T2D (Nyberg et al., 2014). This association was robust to adjustment for both demographic and lifestyle-related covariates and extends previous pooled cross-sectional associations (Nyberg et al., 2013). A substantial number of studies of the relationship between long work hours and diabetes have also been carried out (Kivimäki et al., 2015). Meta-analytic results suggest that working 55 hours or more a week also increases the risk of developing T2D, but only in low socio-economic status (SES) groups (RR 1.29, 95% CI 1.06 - 1.57). The mechanisms underlying this finding are unknown, but it is possible that working long hours could reduce the time for health-protective behaviours. The reported association was independent of smoking, alcohol consumption, physical activity and obesity, but it is plausible that other unmeasured health-related factors such as sleep could mediate this relationship. Another possibility is that the reasoning for working long hours could differ between low and high SES groups. Low SES workers might work longer hours due to issues such as low-pay and lack of control over working hours. In contrast, individuals from higher SES groups often voluntarily work longer hours to move up the corporate ladder and to achieve goals rather than through financial necessity.

Perceived stress is a broader conceptualisation of psychosocial stress exposure that has been implicated in T2D development. Novak et al., (2013) investigated the relationship between perceived permanent stress (self-reported stress related to work or home life that was ongoing for a year or more) and incident T2D in a sample of 7251

men. Over the 35 year follow period men with permanent stress had a greatly increased risk of T2D (HR 1.52; 95% CI 1.26 – 1.82) compared with those reporting no or periodic stress. This relationship was not accounted for by conventional T2D risk factors (Novak et al., 2013). Similarly, Japanese men with high levels of “stress in daily life” have been found to have a greater risk of incident diabetes over a 3 year follow-up period (Toshihiro et al., 2008).

Findings from other prospective studies with both male and female participants have been equivocal. In the Copenhagen City Heart Study involving 7066 participants, men who reported daily emotional stress were two times more likely to develop T2D than those with low levels of stress over a 10 year follow-up period (OR= 2.36; 95% CI 1.22 - 4.59), but no associations were found for women (Rod, Grønbaek, Schnohr, Prescott, & Kristensen, 2009). Conversely, a study of 3759 Australian men and women found no relationship between perceived stress and the development of abnormal glucose tolerance in men over 5 years of follow-up, but detected an association in women (Williams, Magliano, Tapp, Oldenburg, & Shaw, 2013). It is unclear why some studies have found sex differences, but sample size might offer one possible explanation. The largest study to date investigated the relationship between perceived stress and subsequent diabetes development in 55,826 Japanese men and women over a 10-year follow-up period (Kato et al., 2009). In this analysis high levels of perceived stress were found to increase the risk of T2D onset in both men and women, but effects were stronger among male (OR 1.39; 95% CI 1.15 - 1.65) than female participants (OR 1.25; 95% CI 1.01 - 1.56) after adjustment for known T2D risk factors.

Another study assessed the association between perceived stress and T2D in 22,567 participants (71% men) from a French workforce cohort. Over 5.3 years follow-up, no association between perceived stress and future T2D was observed in the full

sample (Wiernik et al., 2016). However, perceived stress was significantly associated with new onset T2D in participants of low occupational status (OR 1.39; 95% CI: 1.02 – 1.90). The authors suggest that low occupational status might reflect greater work stress. If this is the case, these results would map onto previous meta-analytic findings indicating that long working hours are only associated with incident diabetes in those of low SES (Kivimäki et al., 2015).

Considering the current evidence as a whole, there appears to be an association between perceived stress and increased risk of diabetes in initially healthy populations. However, sex or SES (as measured by occupational status) may moderate this association. As in the case of depression, these longitudinal observational studies do not prove causality.

1.5.2.3. *Early life adversity*

Early life adversity has not been widely investigated as a risk factor for future diabetes onset, though it appears to be a significant issue for health-related processes such as telomere length and inflammation in adult life (Danese et al., 2011; Price, Kao, Burgers, Carpenter, & Tyrka, 2013). A review published in 2015 summarised the existing evidence on diabetes (Huang et al., 2015), analysing 7 prospective and cross-sectional studies with data on 87,251 participants. People who reported an adverse childhood experience had a 32% increased odds of diabetes (95% CI 1.16 - 1.51). Looking at different types of childhood stress, the strongest association was found for neglect (OR 1.92; 95% CI 1.43 - 2.57), followed by sexual abuse (OR 1.39; 95% CI 1.28 - 1.52) and physical abuse (OR 1.30; 95% CI 1.19 - 1.42). A limitation of this meta-analysis is that some of the studies included involved retrospective accounts of childhood adversity

which may not be accurate, whereas other studies were analyses of life course data. The studies varied in diabetes measurement, with both self-reported and objective measures being used. There are unanswered questions in this area such as whether there is a critical period in exposure to early life stress or whether there is a dose-response relationship between the frequency or duration of the stress and diabetes risk.

Pre-natal stress has also been suggested to play a role in the development of T2D. In a large Danish cohort study, participants who were exposed to prenatal stress (n= 45,302 out of nearly 1.9 million) because their mothers experienced bereavement in their prenatal life were found to have an elevated risk of future T2D (Incidence rate ratio 1.31; 95% CI 1.01 – 1.69). This association was independent of parental diabetes and other conventional T2D risk factors (Virk et al., 2012). The mechanisms underlying this association are likely to be complex and are currently poorly understood. One possibility is that the severe stress of bereavement might cause a rise in the mother's cortisol level, thus exposing the foetus to glucocorticoid excess (Holmes et al., 2006) which has been implicated in the pathogenesis of diabetes (Clare & Thurby-Hay, 2009; Newell-Price, Bertagna, Grossman, & Nieman, 2006). Another possibility is that maternal changes in cortisol could impact blood glucose concentrations, as cortisol trigger hepatic gluconeogenesis (Di Dalmazi, Pagotto, Pasquali, & Vicennati, 2012). In this way, stress-related increases in maternal glucose concentrations could enter foetal circulation predisposing the child to future issues with glucose metabolism.

1.5.2.4. *Personality traits*

Personality factors are not well researched in relation to T2D. Hostility is a trait that is typically conceptualized as a negative cynical attitude toward others, with a propensity

for anger or aggression (Cook & Medley, 1954). This trait has been associated prospectively with raised fasting glucose (Shen, Countryman, Spiro, & Niaura, 2008) and cross-sectionally with insulin resistance (Suarez, 2006; Zhang et al., 2006), HbA1c (Kawakami et al., 1995; Zhang et al., 2006) and prevalent T2D (Williams, Steptoe, Chambers, & Kooner, 2011). Additionally, angry temperament has been investigated in relation to T2D development. In a cohort study of 11,615 individuals who were disease-free at baseline, individuals with an angry temperament had a 1.34 increased hazard (95% CI 1.10 - 1.62) of incident diabetes over the 6 year follow-up period (Golden et al., 2006).

This association has also been investigated in the MESA cohort (Abraham et al., 2015), with a sample size of 5598 participants but a longer follow-up of 11.4 years. Participants reporting high levels of trait anger at baseline had a 48% greater risk of developing T2D than those with low anger (HR 1.48; 95% CI 1.04 - 2.12) independent of demographic factors, exercise, diet, alcohol use and smoking. However, the association was attenuated following adjustment for waist circumference. Another measure of high anger reactivity was also associated with future T2D in this sample (HR = 1.07; 95% CI 1.03 - 1.11) and this remained significant after adjustment for a range T2D risk factors including waist circumference. A discussion of the issues surrounding the inclusion of lifestyle factors as covariates will be presented in section 1.5.4.

Overall, although there have not been many studies investigating anger and hostility, the current evidence suggests that these characteristics may also be associated with an increased risk of T2D in later life. Due to this dearth of literature hostility offers an interesting factor to investigate further in people with T2D. The impact of trait hostility on biological responses to acute stress will be discussed in Chapter 4.

1.5.2.5. *Potentially protective psychosocial factors*

The vast majority of work has investigated relationships between negative psychosocial stress factors and future diabetes. To our knowledge only two studies have investigated associations with potentially protective positive factors. Crump et al., studied the relationship between resilience to stress in adolescence and T2D in later life (Crump, Sundquist, Winkleby, & Sundquist, 2016). The study used an impressive sample of over 1.5 million Swedish military conscripts who were assessed for stress resilience during a semi-structured interview at 18 years. The participants were followed up for an average of 25.7 years using national medical records. Participants with the lowest stress resilience had a 51% increased hazard of future diabetes compared to those with high levels of resilience (HR, 1.51; 95% CI 1.46- 1.57) independent of a range of T2D risk factors. These findings suggest that personal resilience to stress might be an important factor in the development of T2D. The other study looked at life satisfaction, emotional vitality and optimism in 7800 individuals from the Whitehall II cohort (Boehm, Trudel-Fitzgerald, Kivimaki, & Kubzansky, 2015). Over 13 years follow-up these wellbeing factors were not associated with incident T2D as assessed by self-reported T2D and glucose tolerance testing. The authors performed sub-analyses to assess whether associations varied by type of T2D diagnosis. Individuals with high life satisfaction (OR=0.85; 95% CI=0.76 - 0.95) and emotional vitality (OR=0.86; 95% CI=0.77 - 0.97) were less likely to report doctor diagnosed diabetes. No associations were found for optimism and none of the wellbeing indicators were associated with T2D as detected at the Whitehall clinical assessment. It is uncertain why associations

differed for doctor-diagnosed and screen-detected diabetes. They suggest that tests for screen detected diabetes could have lower specificity than tests conducted by a physician. However, this was not directly tested. This study adds to the literature as these factors previously had not been looked at in relation to T2D risk. However, these finding should be interpreted cautiously considering that associations were based on sub-analyses in 288 individuals with doctor diagnosed T2D.

1.5.3. Psychosocial factors and outcomes in T2D

1.5.3.1. *Depression in T2D*

In addition to increasing T2D risk over time in initially healthy populations, psychosocial factors are thought to contribute to disease progression in T2D patients. Indeed, the importance of psychosocial factors in people with diabetes has been recognized in UK and international diabetes care guidelines (Barnard, Peyrot, & Holt, 2012). Depression is common in T2D, with evidence suggesting that the prevalence of depression is significantly higher in individuals with diabetes compared with those without the condition (Ali, Stone, Peters, Davies, & Khunti, 2006; Anderson, Freedland, Clouse, & Lustman, 2001; Mommersteeg, Herr, Pouwer, Holt, & Loerbroks, 2013; Roy & Lloyd, 2012). A meta-analysis of 10 studies with 51,331 individuals estimated the prevalence of depression to be almost doubled in people with T2D compared to those without the condition (17.6% vs. 9.8%, OR = 1.6, 95%, CI 1.2 – 2.0) (Ali et al., 2006). The vast majority of the studies have been conducted in Western countries, but a study of 213,797 people in 47 countries from around the world has shown that people with diabetes have a twofold greater prevalence of depressive symptoms than those without diabetes (OR 2.36; 95% CI 1.91 – 2.92).

As well as increased prevalence in people with T2D, longitudinal studies indicate that T2D diagnosis is a risk factor for incident depression (Mezuk et al., 2008; Nouwen et al., 2010; Rotella & Mannucci, 2013). An analysis pooling data from 16 longitudinal studies involving 497,223 participants with an average follow-up of 5.8 years, indicated that people with T2D have a 25% increased risk of developing depression compared with controls without diabetes (Rotella & Mannucci, 2013). More recent longitudinal evidence has confirmed this relationship (Demakakos et al., 2014; Mezuk et al., 2015). For example, an analysis of 4238 people from English Longitudinal Study of Ageing (ELSA) found that diabetes was associated with a doubling of depressive symptoms over 4-years of follow-up (OR= 2.17; 95% CI 1.33 - 3.56), while a larger study of 37,043 Swedish twins T2D showed a 33% increased risk of major depression (HR 1.33; 95% CI = 1.02 – 1.72) (Demakakos et al., 2014; Mezuk et al., 2015). However, in both of these studies the association was only found in younger participants with T2D (≤ 55 or 64 years) and not in older individuals. The reasons for this are not known. It might be that middle-aged people with diabetes perceive their condition as a disease of old age that should not have happened to them and become more despondent following diagnosis. Alternatively, it could be that younger patients have more competing priorities (e.g. employment, raising children and financial commitments such as mortgages) and find diabetes care a greater burden to manage in comparison with older patients who are likely to have a grown up family and be retired. There is some evidence from smaller cross-sectional studies that T2D management might be more challenging for younger than older adults (Browne, Nefs, Pouwer, & Speight, 2015; O'Neil et al., 2014) but stronger prospective evidence from larger samples is required to explore these possibilities further.

It should be pointed out that the link between depression and diabetes is not unique to this condition. Similar associations have been observed for other common diseases such as osteoarthritis, CHD, chronic lung diseases and stroke (Huang, Dong, Lu, Yue, & Liu, 2010; Wikman, Wardle, & Steptoe, 2011). Nevertheless, co-morbid depression is a threat to quality of life in people with T2D (Ali et al., 2006; Schram, Baan, & Pouwer, 2009) and is of clinical importance. Lustman et al., conducted a meta-analysis of 24 cross-sectional studies investigating the association between depression and glycaemic control (Lustman et al., 2000). Depression was significantly associated with suboptimal glycaemic control in people with diabetes in this analysis, with stronger effects observed for interview-diagnosed depression compared with self-reported depression. More recent evidence has found an association between depression and poor glycaemic control (Rush, Whitebird, Rush, Solberg, & O'Connor, 2008) but results have not been consistent (Aikens, 2012). Depression has also been associated with non-adherence to diabetes treatment regimens (Gonzalez et al., 2008). Longitudinal evidence published since this meta-analysis has also found a relationship between depression and treatment non-adherence. For example, a study of 2759 individuals with diabetes found that participants with persistent or increasing depression symptoms had significantly poorer adherence to dietary and exercise regimens than their counterparts without depression over a 5-year follow-up period (Katon et al., 2010).

1.5.3.2. *Depression and outcomes in T2D*

An increasing body of literature indicates that depression exacerbates the risk of microvascular and macrovascular complications in people with T2D. According to an early meta-analysis of cross-sectional studies, depressed individuals with diabetes are

more likely to suffer from retinopathy, nephropathy and neuropathy, as well as the macrovascular complications of diabetes (de Groot, Anderson, Freedland, Clouse, & Lustman, 2001). However, due to limited evidence at the time type 1 diabetes and T2D samples could not be assessed separately.

Prospective studies have also found a relationship between comorbid depression and diabetes complications. For instance, in the Pathways Epidemiological Follow-up Study, a longitudinal cohort of over 4000 participants with T2D, comorbid depression has been associated with an increased risk of retinopathy (Sieu et al., 2011) and diabetic foot-ulcers (Williams et al., 2010), as well as more advanced microvascular complications such as blindness, amputations, end-stage renal disease and death from renal failure over a 5-year follow-up period (Lin et al., 2010). The associations reported in these studies were independent of prior complications, socio-demographic characteristics, health behaviours and diabetes self-care variables. Additionally, prospective evidence has detected an association between comorbid depression in diabetes and CHD (Black, Markides, & Ray, 2003; Lin et al., 2010). In the Pathways Study depressed individuals with T2D had a 24% increased risk (HR 1.24; 95% CI 1.0 - 1.54) of adverse macrovascular complications including MI, stroke, cardiovascular procedures and cardiac death over 5-years of follow-up (Lin et al., 2010).

Another study followed a cohort of 345,949 individuals who were free of CVD at baseline over a 7 year follow-up period. Results of the study showed that participants with T2D alone and those with major depression alone had a 30% increased risk of MI, whereas those with a double diagnosis (both T2D and major depression) had a 82% excess risk (HR 1.82, 95% CI 1.69 – 1.97) of subsequent MI compared to participants without either condition (Scherrer et al., 2011).

The largest study to date used national 'drug registers' and linked medical records to explore MI risk in people with treated with anti-depressant and anti-diabetic medications (Rådholm, Wiréhn, Chalmers, & Östgren, 2016). In this study medication usage was used as a marker of disease. Due to a sample size of almost 4 million people, the study was powered to explore MI risk stratified by sex. Over 3 years follow-up, the combined use of anti-diabetic and anti-depressant medication was associated with a significantly greater risk of MI compared with use of either medication alone. Women taking both drugs had a 7.4 increased hazard of MI (95% CI 6.3 – 8.6) than women without depression or diabetes. In men the corresponding hazard was 3.1 (95% CI 2.8 – 3.6). Despite the impressive sample size this study should be considered in light of some limitations. The authors did not have information on key confounders of this relationship such as BMI, smoking status or lipids. There is also the possibility that some of the participants were taking these medications for conditions other than T2D and depression.

Another study published in 2016 examined the association between depressive symptoms or perceived stress at baseline and risk of CVD in 22,003 US adults (Cummings et al., 2016). Over almost 6 years of follow-up, people with T2D who reported elevated depressive symptoms or perceived stress had a significantly increased incidence of stroke (HR 1.57; 95% CI 1.05 - 2.33) and acute CVD (HR 1.57; 95% CI 1.02 - 2.40). These associations were independent of demographics factors, but were attenuated when controlling for lifestyle factors. A limitation of this analysis is that the results for depressive symptoms alone and perceived stress alone were not presented. However, the authors assert that including both stress and depressive symptoms together was associated with greater CVD risk than either factor alone.

The majority of studies have focused on white samples in Western countries. To

address this issue, Ting et al. conducted a 7.4 year follow-up study in a sample of 7835 Chinese patients with T2D who were free of CVD at baseline (Ting et al., 2013). In this sample, diagnosed depression predicted CVD (HR=2.18; 95% CI=1.45 - 3.27) and the majority of the risk appeared to be driven by stroke (HR=3.55; 95% CI=2.15 - 5.84). The reported associations remained significant after adjustment for a wide range of conventional CVD risk factors

As might be expected from these findings, people with both depression and diabetes have greater CVD mortality rates. An analysis of 16 prospective studies (Van Dooren et al., 2013) indicated that comorbid depression in diabetes is associated with a 39% increased risk of cardiovascular death (HR 1.39, 95% CI 1.11 – 1.73) and 46% higher risk of all-cause mortality (HR 1.46, 95% CI 1.29 – 1.66). Other meta-analyses have reported similar associations between comorbid depression in T2D and future all-cause mortality (Hofmann, Köhler, Leichsenring, & Kruse, 2013; Park, Katon, & Wolf, 2013).

Given the evidence linking depression with poorer outcomes in people with diabetes it is not surprising that comorbid depression is a burden to healthcare systems. Evidence from review studies indicate that T2D patients with depression utilise more healthcare resources, seek more treatments and spend more time in hospital than their counterparts without depression, and as a result are more costly to treat (Egede, Bishu, Walker, & Dismuke, 2016; Molosankwe, Patel, Gagliardino, Knapp, & McDaid, 2012). However, it should be noted that most of the economic impact studies have been conducted in Western countries and that few of these studies assessed impact beyond one year follow-up.

1.5.3.3. *Anxiety in T2D*

In comparison with the research on comorbid depression, there is relatively little research on other psychosocial factors and their relationship to CVD risk in people with diabetes. However, there is some evidence that the prevalence of other psychosocial disorders is elevated in people with T2D in comparison with the general population. Overviews of this research suggest that diabetes is associated with 20% increased odds (OR 1.20; 95% CI 1.10 – 1.3) of having an anxiety disorder and 48% increased odds (OR 1.48; 95% CI, 1.02 – 1.93) of having elevated anxiety symptoms (Smith et al., 2013). These findings are in keeping with an earlier systematic review by Grigsby et al. which estimated that 14% of individuals with diabetes meet the criteria for a generalised anxiety disorder, while 40% have elevated anxiety symptoms (Grigsby, Anderson, Freedland, Clouse, & Lustman, 2002).

Co-morbid anxiety is of clinical relevance to people with diabetes. An association between anxiety and glycaemic control has been reported in a number of studies (Anderson et al., 2002; Collins, Corcoran, & Perry, 2009). Additionally, anxiety in T2D has been linked with functional disability. In a study of almost 2000 people with T2D, comorbid anxiety was associated with a 3-fold increase in the odds of functional disability (OR 3.01; 95% CI 1.38 - 6.57) independent of conventional risk factors (Smith & Schmitz, 2014).

Little research has investigated the association between anxiety and the development of complications. There is evidence that poor glycaemic control in T2D is associated with CVD, with event rates linked to the degree of hyperglycaemia (Emerging Risk Factors Collaboration et al., 2010; Goff et al., 2007). Epidemiological evidence suggests that every 1 mmol/L increase in fasting glucose predicts a 17% increase in future CVD risk (Anand et al., 2012). While, meta-analytic evidence

indicates that a 1% increase in HbA1c increases the risk of CVD by 18% (Selvin et al., 2004). Considering that anxiety is associated with poor glycaemic control, it is plausible that anxiety could also contribute to CVD complications. To date only one study has investigated the prospective relationship between anxiety and diabetes complications such as retinopathy, neuropathy or CVD, and no associations were found (Edwards & Mezuk, 2012). However, the number of cases of diabetes and anxiety was limited in this study, so it may have been underpowered to detect such effects.

1.5.3.4. *Psychological distress and diabetes-specific distress*

Psychological distress has been linked with CVD morbidity and increased mortality rates in people with diabetes. In a study of 1533 individuals with diagnosed T2D, distressed participants were found to have a 1.7 fold increased risk of a CVD event (HR: 1.69, 95% CI 1.05 - 2.70) and a 1.8-fold greater mortality rate (HR 1.76, 95% CI 1.23 - 2.53) compared to individuals without psychological distress over an average follow-up period of 5.4 years (Dalsgaard et al., 2014). This association was independent of CVD risk factors, and excluding participants who were receiving anti-depressive treatment did not impact the results. It would be interesting in future studies to tease out what particular aspects of psychological distress are most strongly linked to CVD.

As well as depression, anxiety and general psychological distress, diabetes-related emotional distress, a stress condition specifically resulting from concerns and worries about diabetes and its management, is common in people with diabetes (Fisher et al., 2007). According to estimates from a study of 8596 adults with diabetes from 17 countries, 44.6% of patients suffer from significant diabetes-related distress (Nicolucci et al., 2013). Diabetes-related distress is distinct from general psychological distress and

depression (Fisher et al., 2007, 2010). Diabetes-specific distress goes beyond depression-related low mood and emotional distress and refers to the unique burden of living with and managing this chronic condition. It encompasses distress related to self-management, regimen adherence and diabetes complications (Fisher et al., 2010; Fisher, Hessler, Polonsky, & Mullan, 2012). Theory-driven and factor-analysed standardised scales such as the 17-item Diabetes Distress Scale have been developed to measure this construct (Fisher, Glasgow, Mullan, Skaff, & Polonsky, 2008; Polonsky et al., 2005).

Diabetes-related distress is of clinical significance since several longitudinal studies suggest that it is associated with poor glycaemic control and that it has a greater impact on glycaemic management than depression (Aikens, 2012; Fisher et al., 2007, 2010). Additionally, findings from a recent study indicate that diabetes-related distress predicts future medication adherence in people with T2D (Aikens, 2012). No studies to date have investigated the association between diabetes distress and the macrovascular complications of diabetes. Prospective findings linking diabetes distress and glycaemic control offer the possibility that diabetes distress could affect CVD risk through reduced self-care behaviours and poorer glycaemic control.

1.5.3.1. *Potentially protective psychosocial factors*

Few studies have investigated potentially protective psychosocial factors in people with T2D and the majority of the research has been cross-sectional so it is not possible to assess the directionality of the relationship or to infer causality. Positive psychological factors have been correlated with glycaemic control in people with diabetes in two cross-sectional studies (Does et al., 1996; Papanas et al., 2010) but only one study has looked at the relationship between positive wellbeing and glycaemic control

prospectively (Tsenkova, Love, Singer, & Ryff, 2007). In this study of 97 older women positive affect at baseline was a significant predictor of lower HbA1c levels at 2 year follow-up adjusting for age, income, marital status, waist-hip ratio and statin usage. A limitation of this analysis was that the study sample did not have overt diabetes, with baseline HbA1c ranging from 4 - 6.6%, which reduces the generalisability of this study to diabetes patient groups.

Resilience has also been assessed as a potentially beneficial factor in people with T2D. In a cross-sectional study of 71 African-American women with T2D resilience was associated with reduced HbA1c. Other than the low sample size this study was limited by the use of a convenience sample and the lack of inclusion of control variables. The longitudinal relationship between resilience and glycaemic control was assessed in a study of 111 individuals with either type 1 or T2D (Yi, Vitaliano, Smith, Yi, & Weinger, 2008). Low resilience (as derived from a factor score of self-esteem, self-efficacy, self-mastery and optimism) was associated with worsening HbA1c over the 1-year follow-up period. However, the statistical analysis used in this study was questionable as no adjusted model was presented as the authors claimed that covariates such as age, sex and type of diabetes were not significantly related to either the outcome or exposure.

When conducting the literature review for this chapter no studies assessing the relationship between positive psychosocial factors and macrovascular complications in people with T2D were found. One study assessed the relationship between positive affect and future mortality in people with T2D (Moskowitz, Epel, & Acree, 2008). In this analysis of 715 participants and 2673 controls positive affect was significantly associated with a reduced risk of mortality in people with T2D (HR = 0.87, 95% CI 0.76 – 0.99) over 10-years of follow-up. No association was detected in the comparison

group. However, when covariates were added to the model the association was no longer significant. Looking at the 4 individual components (self-esteem, hopeful, happy, life enjoyment) of the positive affect scale, enjoyment of life remained a significant predictor of reduced mortality in people with T2D controlling for age, ethnicity, self-reported health status, and physical activity (HR = 0.89, CI 0.79 – 0.99). No associations were found for the other components.

1.5.4. Limitations of the research on psychosocial factors

Considering the literature as a whole, various negative psychosocial factors have (for the most part) been shown to increase the risk of diabetes in initially healthy populations independent of conventional risk factors. Meta-analyses of prospective studies have investigated depression and work stress as risk factors for new onset diabetes and there is growing evidence from large cohort studies that other psychosocial factors influence diabetes risk. There is less research on the involvement of psychosocial factors in CVD risk in people with existing diabetes. To date, most of the research in this area has been on depression, with evidence that a double diagnosis of diabetes and depression increases the risk of CVD in this population.

There are limitations to the research carried out in this field. Firstly, the healthy cohort studies involved bigger samples and longer follow-up periods than the studies of people with existing diabetes. A lot of the research on diagnosed diabetes has been cross-sectional and has focused on the prevalence of negative emotional disorders. This makes the possibility that the condition itself influenced reports of psychosocial stress difficult to rule out. Additionally, the studies of initially healthy individuals have

investigated a much wider range of psychosocial factors than the studies of people with existing diabetes.

The prospective studies of people with diagnosed diabetes tended to have shorter follow-up periods than the healthy cohort studies. The duration of follow-up is of importance as it has been suggested that psychosocial stress following diabetes diagnosis could be short-lived. Studies which have assessed the temporal association between anti-depressant prescriptions and diabetes suggest that there is an increase in anti-depressant use shortly after diabetes diagnosis, but this does not continue long-term (Kivimäki et al., 2010; Knol, Geerlings, Grobbee, Egberts, & Heerdink, 2009). Additionally, a systematic review of 24 studies examining adjustment to diabetes in recently diagnosed patients found diagnosis to have limited long-term emotional impact (Thoolen, De Ridder, Bensing, Gorter, & Rutten, 2008), a finding which has been replicated in subsequent research (Paddison et al., 2011). Taken together, this suggests that reports of psychosocial stress in people with diabetes might be temporary and could be a product of the initial emotional response to diagnosis of a serious chronic condition, rather than an ongoing issue.

Nevertheless, longer-term adaptation as indexed by a reduction in self-reported stress and anti-depressant usage does not necessarily indicate a successful adjustment to living with diabetes. In this line, there is evidence that complex treatment regimens at the more advanced stages of diabetes might also be associated with depression (Thoolen, de Ridder, Bensing, Gorter, & Rutten, 2006). Cross-sectional and prospective studies indicate that depression is significantly greater in individuals receiving insulin therapy than those receiving oral anti-diabetic medications (Katon et al., 2004; Pan et al., 2010; Sun et al., 2015). Future research with longer follow-up periods is required to shed further light on these issues.

Another limitation of the literature is that psychosocial factors have mostly been assessed using self-report questionnaire. Therefore, issues of information bias might be present. Most of the studies only assessed psychosocial factors at one time point, so it is unknown how possible changes in these factors over time could be linked to T2D. There is also heterogeneity between studies in the measures used to assess psychosocial factors. Furthermore, it has been suggested that psychosocial stress factors such as depression, anxiety and distress overlap and that a general disposition toward negative affectivity may be more important for disease risk and subsequent outcomes than any specific psychosocial factor alone (Engum, 2007; Suls & Bunde, 2005). It is also possible that the psychosocial factors described in this chapter do not work in isolation and might cluster. For example, perceived stress in one's working life could lead to depressive symptoms outside of work.

The measurement of diabetes varied from study to study with both objective and self-reported indicators used. It should also be noted that negative emotional disorders are common in many chronic conditions and are not exclusive to diabetes. Several meta-analyses have assessed the association between depression and chronic disease and have found prevalence rates to be similar across conditions such as diabetes, cardiac diseases, lung diseases, arthritis, hypertension and functional impairments (Egede, 2007; Huang et al., 2010; Moussavi et al., 2007). This suggests that there is no specific association between diabetes and depression, distinct from the association between depression and other chronic conditions.

Another issue is causality. The majority of the evidence in both initially healthy cohorts and in studies of those with diagnosed diabetes has been observational, so it is difficult to draw causal conclusions. Researchers attempt to rule out the notion that the association between psychosocial factors and diabetes is

non-causal by adjusting for potentially confounding factors. However, demonstrating there is an association between psychosocial stress and diabetes independent of shared risk factors does not excluded the possibility that unmeasured or poorly measured factors account for the relationship (Tabák et al., 2014). Additionally, different analyses include different covariates and there is no standardised approach to their inclusion.

Many of the studies reviewed included lifestyle factors such as smoking and physical activity as covariates in the analysis. Different studies included different covariates and of course measurement of these factors differs between studies and this issue is of importance. For example, people are likely to over-estimate their physical activity levels using self-report measures, whereas an objective activity measure could provide more realistic data (Strath et al., 2013). Lifestyle factors offer a possible indirect pathway linking psychosocial factors with future health risk and could also moderate or mediate the relationship between psychosocial factors and outcomes in people with an existing health condition. For a more detailed discussion of this pathway please see section 1.6 below. The majority of the studies reviewed above detected an association between psychosocial factors and diabetes independent of lifestyle factors. However, some did not (e.g. Abraham et al., 2015; Farvid et al., 2014). Finding an association that is robust to adjustment for lifestyle factors does not necessarily indicate that these factors are unimportant. It is well known that psychosocial factors affect health behaviours, for example both obesity and physical inactivity increase the risk of developing diabetes and both of these factors are known to moderate depressive symptoms (Cooney et al., 2013; Fabricatore et al., 2011). In turn in people with overt diabetes, depression adversely effects adherence to recommended diet and exercise regimens (Katon et al., 2004; Katon et al., 2010).

Furthermore, both psychosocial and lifestyle factors impact biological systems that are implicated in disease. Therefore, merely controlling for these factors does not tease out the complexities of the relationships between these processes.

As with all research the issue of publication bias might play a role, since studies that report null findings are less likely to be published than those that report statistically significant associations (Song et al., 2010). This possibility could lead to an overestimation of the relationship between psychosocial factors and diabetes.

Reverse causality is unlikely to have accounted for the associations in healthy cohort studies that had extended follow-up periods and excluded people with prevalent diabetes at baseline. However, this does not entirely exclude the possibility of reverse causation. In studies that relied on self-report measures of diabetes, it is possible that some of the participants included could have had pre-diabetes at baseline. Furthermore, it has been argued that the increased risk of depression in people with diabetes might not necessarily reflect new onset depression as most studies did not exclude participants with a lifetime history of depression (Rotella & Mannucci, 2013). Therefore some of the increased risk of depression in people with diabetes could be accounted for by recurrent depression in individuals with history of the disorder (Brown, Majumdar, Newman, & Johnson, 2006).

The case for causality in the relationship would be strengthened by evidence of plausible mechanisms through which psychosocial factors could influence disease processes. The following sections will review the potential pathways through which psychosocial factors and diabetes could be linked.

1.6. Psychobiological pathways linking stress with T2D and CVD

The precise mechanisms linking psychosocial stress to CVD and diabetes remain to be elucidated. However, several potentially interrelated pathways that plausibly account for the link have been proposed. One possibility is that the adverse relationship between psychosocial factors and T2D may be mediated via behavioural pathways. Behavioural mechanisms include poor diet, physical inactivity, excess alcohol consumption and smoking all of which are modifiable risk factors for CVD development (WHO, 2011; Yusuf et al., 2004). Reduced adherence to self-care behaviours and cardio-protective medications such as BP and lipid lowering drugs could also play a role.

Several lines of research support this mechanism. Depression, anxiety and diabetes-related distress are associated with suboptimal glycaemic control (Anderson et al., 2002; Collins et al., 2009; Fisher et al., 2010; Lustman et al., 2000; Rush et al., 2008) and there is evidence that the risk of CVD increases in line with the degree of hyperglycaemia (Emerging Risk Factors Collaboration et al., 2010; Goff et al., 2007). Depression in diabetes adversely affects adherence to recommended diet and exercise regimens (Gonzalez et al., 2008; Katon et al., 2010). According to a meta-analysis of 47 studies, comorbid depression in diabetes increases non-adherence to a range of behaviours including diet, medication usage and exercise (Gonzalez et al., 2008). Longitudinal evidence published since this meta-analysis has also found a relationship between depression and treatment non-adherence. For example, a study of 2759 individuals with diabetes, found that participants with persistent or increasing depression symptoms had significantly poorer adherence to dietary and exercise regimens than their counterparts without depression over a 5-year follow-up period (Katon et al., 2010). A cross-sectional study with a similar sample size of 2646

individuals with T2D, reported that physical inactivity doubled in the presence of depressive symptoms (Koopmans et al., 2009). Considerably less research has investigated the behavioural pathways linking other psychosocial factors and T2D. The Copenhagen City Heart Study of 7066 adults found that perceived stress was associated with physical inactivity and unsuccessful smoking cessation or alcohol reduction attempts over 10 year follow-up as well as the development of overt diabetes among men (Rod et al., 2009).

Negative psychosocial factors that are common in T2D may also decrease motivation for healthy lifestyle choices and in turn these may impact CVD risk. However, the association between T2D and CVD is not fully explained by behavioural risk factors (Emerging Risk Factors Collaboration, 2011; Emerging Risk Factors Collaboration et al., 2010) and results from RCTs suggest that the modification of behavioural risk factors do not significantly lower CVD outcomes in people with T2D despite the fact that lifestyle change has a marked effect on diabetes incidence (Fox et al., 2015). This offers the possibility that a direct biological pathway could link psychosocial factors with CVD risk in people with T2D. The following section will provide a brief overview of the stress response system and the main techniques used to investigate psychobiological mechanisms.

1.6.1. Allostasis and the stress response system

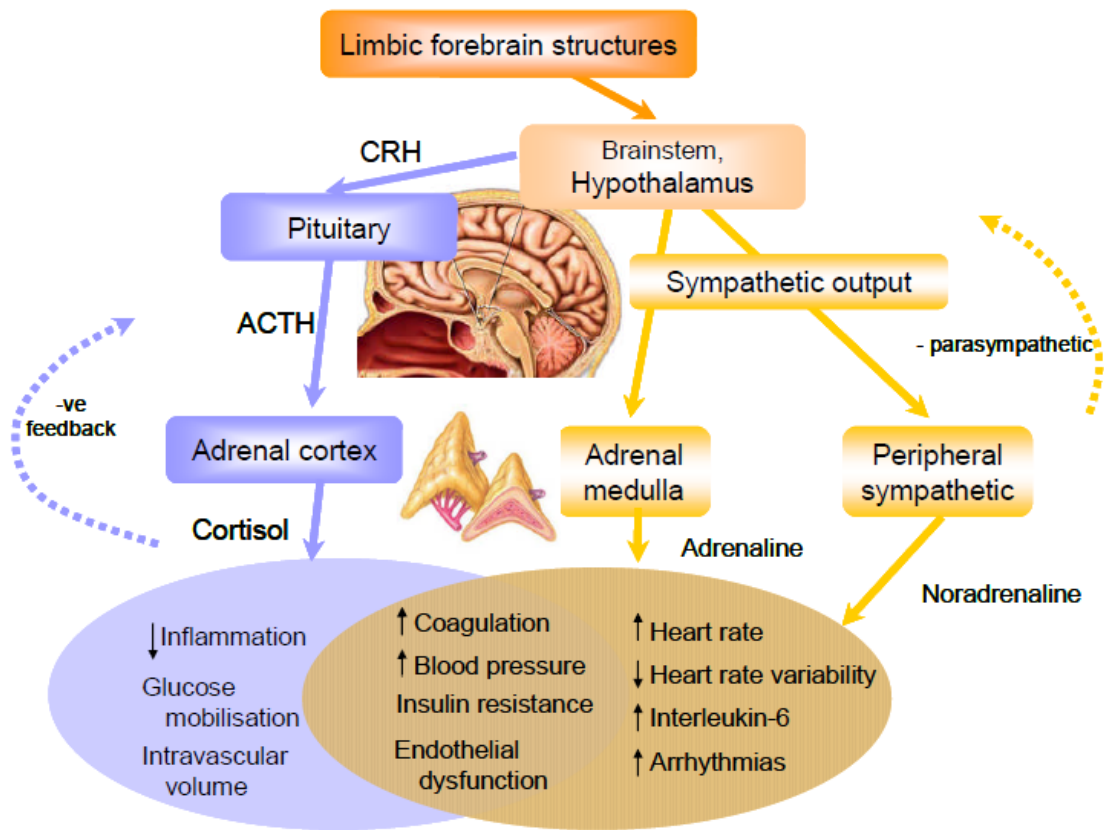
All behavioural responses to environmental events are underpinned by complex multisystem biological responses that sustain the actions taken by the organism. The concept of allostasis describes the process by which organisms respond to changes in the environment through adjustments in multiple biological systems to maintain biological stability or homeostasis (McEwen, 1998). Perceived threats to allostasis

initiate an acute stress response. The primary biological systems activated during the physiological stress response are the hypothalamic–pituitary–adrenocortical (HPA) axis and sympathetic adrenomedullary (SAM) axis (Brotman, Golden, & Wittstein, 2007). An illustration of this system can be seen in Figure 1.3.

The HPA axis is activated by corticotropin-releasing hormone from the hypothalamus, which stimulates corticotropin release from the pituitary, which in turn prompts the release of the glucocorticoid hormone cortisol from the adrenal gland. Cortisol is the end product of the HPA axis and has vital physiological functions including the mobilisation of energy stores (through the breakdown of carbohydrates), the suppression of the inflammatory response and the stimulation of the cardiovascular system, which through collaboration with sympathetic system serves to increase BP.

The sympathetic nervous system innervates tissues throughout the body including the heart, the vasculature and the adrenal medulla. The adrenal medulla releases epinephrine (also known as adrenalin) and the peripheral sympathetic nerves that line the vasculature release norepinephrine (also known as noradrenalin). This sympathetic activation causes increases in heart rate and BP, decreases in heart rate variability and induces energy mobilisation and pro-inflammatory cytokine release.

Figure 1.3 The acute stress response

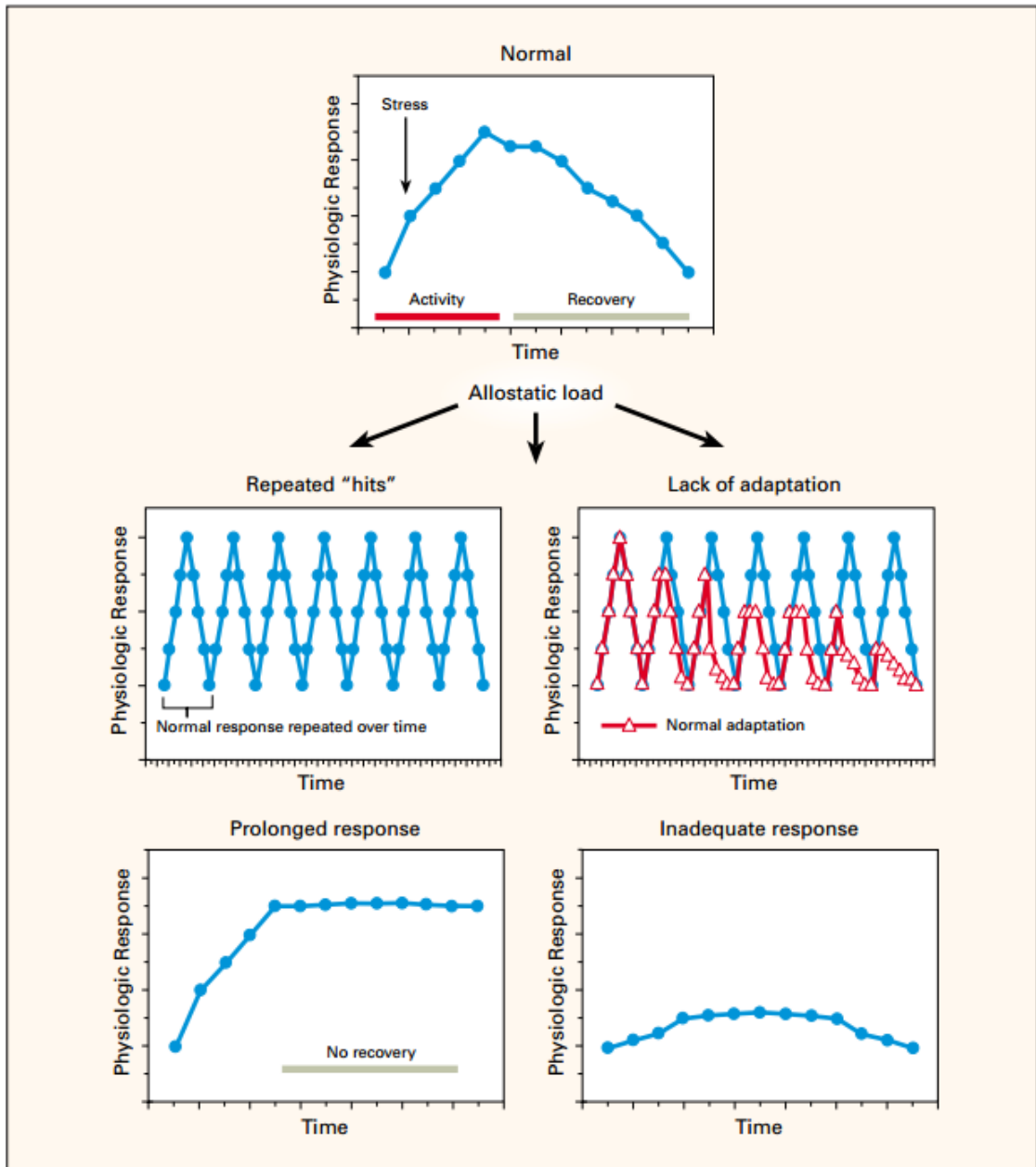


The activation of the HPA axis is shown in purple and activation of the SAM axis is shown in yellow.

Source: Adapted from Brotman et al., (2007).

Allostasis is an adaptive process that is essential for maintaining homeostasis. However, repeated or sustained stimulation of the stress system is thought to lead to ‘wear and tear’ known as allostatic load. In this way, repeated stress exposure can promote dysregulated physiological reactivity, resulting in heightened, prolonged, or diminished responses to stress, thus increasing vulnerability to disease and contributing to negative health outcomes over time (McEwen & Wingfield, 2003). Figure 1.4 illustrates the different response patterns in which allostatic states can deviate from healthy response profiles (McEwen, 1998).

Figure 1.4 Types of allostatic load



Source: McEwen (1998)

The top graph represents the normal response to a stressful experience. The following row depicts two circumstances which begin as healthy responses but over time can become maladaptive. The 'repeated hits' scenario is due to frequent exposure to multiple stressors and the 'lack of adaptation' scenario reflects a failure to habituate to stress. The bottom panel highlights two other circumstances which can reflect an

allostatic state. The ‘prolonged response’ is an exaggerated response to stress with an individual failing to return to baseline levels following stress exposure. The ‘inadequate response’ represents a significant deviation from the norm, by which there is an insufficient reaction to stress.

Allostatic load is often quantified by assessing a range of biomarkers (e.g., BP, cortisol, catecholamines, HbA1c, waist circumference, cholesterol, inflammatory markers), and allocating individuals scores based on the number of variables on which values are elevated compared with the sample distribution (Gruenewald, Seeman, Ryff, Karlamangla, & Singer, 2006; Seeman, McEwen, Rowe, & Singer, 2001). Considering the multiple biological effects of the HPA and the SAM axes, it is clear that the cardiovascular, neuroendocrine and immune systems are all inter-related. In this way, a dysregulated response in one system can impact one of the other systems. For example, it is known that the neuroendocrine and inflammatory systems are strongly linked and that cortisol has a regulatory effect on inflammation (Miller, Chen, & Zhou, 2007).

High allostatic load has been associated with a range of adverse health outcomes (Juster, McEwen, & Lupien, 2010). Early validation of the concept of allostatic load came from the MacArthur cohort of older American adults (aged 70-79 years). At 3 year follow-up high allostatic load was associated with an increased risk of CVD (Seeman et al., 2001) and after a 7 year follow-up allostatic load was associated with all-cause mortality independent of socio-demographic factors and baseline health status. Subsequent prospective research has also found an association between high allostatic load and future all-cause mortality (Crimmins, Kim, & Seeman, 2009; Karlamangla, Singer, & Seeman, 2006). For example, in a study of 12,000 older individuals from the NHANES cohort, those with a high allostatic load were estimated to have a 6 year shorter life expectancy than sex and income matched controls with low allostatic load

(Crimmins et al., 2009). Higher allostatic load has been associated prospectively with disability and functional limitations (Gruenewald, Seeman, Karlamangla, & Sarkisian, 2009; Karlamangla, Singer, McEwen, Rowe, & Seeman, 2002; Read & Grundy, 2014) as well as cognitive impairment in older adults (Karlamangla et al., 2014).

Interestingly, this allostatic index of dysregulation across multiple biological systems has tended to have greater predictive value for health outcomes than any single biological predictor alone (Juster et al., 2010; Karlamangla et al., 2014; Seeman et al., 2001). However, it should be noted that the concept of allostatic load has been criticised as different studies use different biomarkers to measure of allostatic load and as such there is no standardised approach as to what factors to include (Beckie, 2012).

Few studies have investigated allostatic load in relation to diabetes and the available evidence is mixed. One study of over a 1000 middle-aged Puerto Ricans living in Boston found that high allostatic load was associated with increased risk of diabetes and other chronic conditions (Mattei, Demissie, Falcon, Ordovas, & Tucker, 2010), but another investigation that focused specifically on diabetes failed to find that the components of allostatic load clustered reliably in a sample of 53 individuals (Carlsson, Nixon Andreasson, & Wändell, 2011).

These studies assessed allostatic load using biological measures that were taken at rest. Another aspect of allostasis is that it is manifest in modifications of dynamic responses to challenge, not only in basal measures (McEwen, 1998). Adaptive biological responses to stress involve brisk increases in activation (stress reactivity) as the person mobilizes for vigorous activity, followed by prompt recovery back to baseline levels. High allostatic load disrupts these dynamic biological responses, resulting in changes in the morphology of responses (see Figure 1.4). Stress reactivity will be discussed in greater detail later on in this chapter.

It is also important to note that biological reactivity to stress differs between individuals. The magnitude of the stress response and the ability to recover effectively are believed to be determined by multiple factors including genetics, personal perception of the specific stressor and coping resources (Juster et al., 2010; Lovallo & Gerin, 2003). Therefore, whether or not psychosocial stress promotes incident T2D or cardiovascular complications in T2D for a particular individual will depend on both their intrinsic stress responsivity and stress exposure in daily life, in addition to other conventional health risk factors (Steptoe & Kivimäki, 2012, 2013).

1.6.2. Methods for investigating psychobiological pathways

The association between psychosocial factors and biological correlates of the human stress system can be investigated using a number of different research strategies. Psychophysiological stress testing involves the measurement of biological responses to acute challenges in a laboratory setting. The advantage of this method is that it allows detailed dynamic responses to be studied under controlled conditions, reducing the impact of other factors that may confound associations. Several different types of laboratory stress task have been developed including socially evaluative, information processing, psychomotor and physical stimuli tasks (Steptoe & Poole, 2010). The choice of stress task can influence the reactions which are elicited. For example, socially evaluative tasks such as a public speaking task in which performance is rated by the researcher are thought to elicit stronger cortisol responses to stress than more novel tasks such as the mirror tracing task (Dickerson & Kemeny, 2004). However, there are trade-offs to consider when choosing the stress tasks. Taking the example of public speaking, this task is considered more ecological valid than a novel task that an

individual would possibly never encounter in daily life, but participants who are experienced public speakers might not find such a task stressful.

As discussed in sections 1.5.1-1.5.3 observational studies have associated psychosocial stress factors with subsequent CVD and T2D development in initially healthy populations as well as with poorer outcomes in existing patient groups. Another interesting aspect of laboratory stress research is the ability to look psychosocial differences in relation to physical responses to acute mental stress. A large body of research has been conducted in this field. For example, a 2008 meta-analysis investigating the relationship between psychosocial factors and acute physiological responses to laboratory stress included over 700 studies covering a range of psychosocial factors as well as multiple biological systems (Chida & Hamer, 2008). The findings of this analysis suggest that different psychosocial traits have different associations with physiological stress responses but taking the studies together there appears to be an integrated biological stress response pattern consisting of either hyper-reactivity or hypo-reactivity to stress. This patterning of physiology stress reactivity that is associated with chronic psychosocial factors outside of the laboratory environment maps onto the concept of allostatic load, with deviations from normal stress reactivity being indexed by heightened responses to stress as well as blunted reactions to stress.

The studies described in Chapters 3 and 4 use psychophysiological stress testing to assess biological stress responsivity in people with diabetes. The methods used for these studies will be presented in Chapter 2. The limitations of using psychophysiological tasks to study biological stress processes must be acknowledged. Firstly, as the stimuli are brief, only acute biological responses can be recorded which limits this method. Additionally, the stress tasks are often arbitrary and may bear little resemblance to everyday life. To address some of the limitations of this method,

psychophysiological testing is often complemented by naturalistic and ambulatory monitoring methods.

Naturalistic studies involve the sampling of biological variables during everyday life and offer the ability to assess biological activity under natural conditions, thus circumventing some of the problems with laboratory studies concerning ecological validity (Steptoe & Poole, 2010). Ambulatory studies typically involve continuous or repeated measures of biological activity such repeated measures of cortisol from saliva samples taken over the course of day or ambulatory BP monitoring where a device can be pre-set to take measurements at different points during the day. Naturalistic studies can be used to assess average levels of activation over the day and also patterns of activation. For example, cortisol is known to have a marked diurnal patterning with distinct components (Adam & Kumari, 2009). This patterning will be discussed in more detail below in Section 1.7.3. The different features of daily cortisol secretion, as well as average cortisol output over the day can be investigated in naturalistic studies in a way that would not be possible in a brief laboratory stress testing period. Naturalistic measures such as salivary cortisol can be incorporated into large-scale epidemiological studies which allow the assessment of biological measures in larger numbers than is feasible with laboratory testing (Adam & Kumari, 2009). The studies described in Chapters 6 and 7 use naturalistic monitoring data from the Whitehall II cohort study to look at aspects of diurnal cortisol secretion in relation to diabetes. The methods used for these studies will be described in Chapter 5.

Naturalistic monitoring studies are also not without limitations. Firstly, participants in these studies are generally aware that they are being monitored and may alter their behaviour accordingly. For example, in weekly activity monitoring studies the first and last days of wear time are usually discarded as participants often deviate

from their normal activity pattern in response to wearing a device (Hamer et al., 2014; Hamer, Venuraju, Lahiri, Rossi, & Steptoe, 2012). Additionally, some devices used in naturalistic studies such as ambulatory BP monitors can be intrusive and therefore participants might not adhere to the study protocol regarding their use. There are also limits to the number of biological processes which can be measured using naturalistic monitoring due to technical restrictions. For example, markers which can only be accurately measured through blood such as inflammatory cytokines do not lend themselves to this method. Unlike laboratory studies there is no control over potentially confounding variables which are of relevance to psychobiological processes such as smoking and food intake. Naturalistic studies generally require participants to keep a diary during the monitoring period to try and account for these effects.

1.7. Overview of pathways investigated in this thesis

Allostatic load is frequently quantified by measuring a range of biomarkers. The following sections focus on reviewing the evidence linking diabetes with markers of cardiovascular function, metabolic function, neuroendocrine activation and inflammation since these systems will be investigated further in later chapters.

1.7.1. Cardiovascular function and T2D

1.7.1.1. *Markers of cardiovascular function and CVD*

Sympathetic activation of the autonomic nervous system can be indexed by BP, heart rate and cardiac output, whereas parasympathetic activation is indexed by heart rate variability (HRV). Naturally these measures of cardiovascular function have clinical relevance for assessing CVD risk. BP is the physical pressure that the blood mass exerts

on the walls of the arteries and it is reported using two figures; systolic blood pressure (SBP) which is the pressure of the blood when it is leaving the heart and diastolic blood pressure (DBP) which is the pressure of the blood when the heart rests between beats (Townsend et al., 2015). BP is one of the most important risk factors for CVD (Campbell et al., 2014; James et al., 2014), as increased BP can cause damage to the endothelium (the lining of the blood vessels) and contribute to the atherosclerotic process. Heart rate is the number of heart beats per minute. Elevated resting heart rate values have been associated with atherosclerosis (Fox et al., 2007) as well as an increased risk of cardiac mortality (Jouven et al., 2005; Williams & Merhige, 2012). Cardiac output is the volume of blood pumped by the heart per minute and it is an indicator of how efficiently the heart can meet the demands of the body. Cardiac output is derived from heart rate and stroke volume and reduced cardiac output has been associated with heart failure (Vincent, 2008). HRV refers to beat to beat variation in heart rate. Healthy individuals have a high degree of heart rate beat to beat variation. This variation is controlled by the sino-atrial node of the heart which is innervated by both sympathetic and parasympathetic neurons. Therefore, HRV is a measure of sympathetic/parasympathetic balance or sympathovagal balance. For a detailed overview of the different aspects of HRV please see Thayer et al. (Thayer, Hansen, & Johnson, 2010). Autonomic imbalance is generally characterised by hyperactive sympathetic nervous system activity and hypoactive parasympathetic activity. Reduced HRV, which indicates an imbalance between sympathetic and parasympathetic modulation of the heart, has been implicated in the long-term development of CVD and is associated with CVD morbidity and mortality (Thayer & Lane, 2007).

1.7.1.2. *Markers of cardiovascular function and T2D*

High BP is a risk factor for new onset diabetes (International Diabetes Federation, 2015). However, the relationship between BP and incident diabetes in initially healthy populations has not been as widely researched as other T2D risk factors such as obesity. A recent study investigated the link between objectively measured BP and risk of diabetes using linked electronic health records from a UK primary care population (Emdin, Anderson, Woodward, & Rahimi, 2015). In this cohort of 4.1 million adults who were free of diabetes and CVD at baseline, both SBP and DBP were found to have a positively graded association with diabetes risk independent of age, sex, BMI, smoking, anti-hypertensive and lipid-lowering medication. Specifically, a 20 mmHg higher SBP and a 10 mmHg higher DBP were associated with a 58% (HR: 1.58; 95% CI: 1.56 - 1.59) and 52% increased (HR: 1.52; 95% CI: 1.51 - 1.54) risk of new onset diabetes over an average follow-up of 6.8 years. This paper also included a meta-analysis of 30 previous prospective studies. The pooled results across studies indicated that for a 20 mmHg increase in SBP the relative risk of diabetes was 1.77 (95% CI 1.53 - 2.05). Taken together, it is clear that increased BP is a significant risk factor for T2D but it is unclear whether the association is causal. Proposed pathways through which BP may increase the risk of T2D include insulin resistance (Knowles & Reaven, 2016) and inflammation (Emdin et al., 2015).

In people with diagnosed diabetes BP control is a key clinical target (American Diabetes Association, 2015), as CVD risk in people with T2D increases in a linear fashion as SBP rises (Adler et al., 2000). According to the 2015 UK Diabetes audit an estimated 68.7% of people with T2D are achieving the recommended BP level of less than 140/90 mmHg (HSCIC, 2015). However, evidence from prospective intervention studies such as the LookAHEAD trial (Look AHEAD Research Group et al., 2013) and

the ACCORD trial (ACCORD Study Group et al., 2010) suggests that intensive modification of BP (target 130/80 mmHg) does not significantly lower the risk of adverse CVD outcomes in people with T2D (American Diabetes Association, 2015; Arguedas, Leiva, & Wright, 2013; McBrien et al, 2012). Results from a recent Cochrane review encourage BP lowering from 140/90 mmHg but not more intensive lowering due to a possible risk of adverse outcomes and limited evidence of effectiveness (Arguedas et al., 2013).

Resting heart rate has also been investigated as a risk factor for new onset diabetes. A 2015 meta-analysis of 10 cohort studies with almost 120,000 participants found a dose response relationship between resting heart rate and incident T2D, with a 19% increased risk of diabetes for every 10 beats per minute (bpm) increment in heart rate (95% CI: 1.07 - 1.34) (Aune, ó Hartaigh, & Vatten, 2015). Resting heart rate is a marker of autonomic regulation (Lahiri, Kannankeril, & Goldberger, 2008) and it is plausible that sympathetic/parasympathetic imbalance might contribute to the association between increased heart rate and T2D. Autonomic regulation has been associated with central fat deposition, inflammation and components of the metabolic syndrome (Licht et al., 2010; Mancia et al., 2007; Sajadieh et al., 2004; Ziegler et al., 2006) which are all risk factors for T2D.

Lowered HRV is an early indicator of cardiovascular autonomic neuropathy, one of the most serious complications of diabetes (Pop-Busui, 2010, 2012; Vinik & Ziegler, 2007). The condition consists of damage to the autonomic nerve fibres of the heart and blood vessels leading to abnormal heart rate control and vascular dynamics (Vinik & Ziegler, 2007). Abnormalities in HRV can cause rapid heart rate (known as tachycardia) with resting values of greater than 100 bpm and occasional increments up to 130 bpm (Pop-Busui, 2012). In the advanced stages of neuropathy heart rate can become fixed

and unresponsive to exercise, stress or sleep which indicates almost total cardiac denervation and severe cardiac neuropathy (American Diabetes Association, 2015; Vinik & Ziegler, 2007). Autonomic dysfunction can also cause blunting of SBP, DBP and cardiac output responsivity (Sacre et al., 2010; Vinik & Ziegler, 2007).

Cross-sectional evidence indicates that sympathetic/parasympathetic balance could be altered in even before overt diabetes onset. For example, in the Framingham Heart Study of 1919 people, HRV was observed to be reduced in individuals with pre-diabetes, as well as those with overt diabetes compared with their normoglycaemic counterparts (Singh et al., 2000). A subsequent study with 1440 participants also found impaired HRV in those with IGT and diabetes independent of demographic and clinical covariates (Wu et al., 2007).

In people with existing diabetes, cardiovascular autonomic neuropathy has been associated with silent myocardial ischaemia, which is ischaemia where no or little pain is felt (Cohn, Fox, Of, & Daly, 2003). Vinik & Ziegler conducted a meta-analysis of 12 cross-sectional studies and found the pooled prevalence rate ratio of silent myocardial ischaemia to be 1.96 (95% CI 1.53 - 2.51) in people with T2D with cardiovascular autonomic neuropathy compared with their counterparts without the complication (Vinik & Ziegler, 2007). A more recent study (Young et al., 2009) of 1123 people with T2D found that cardiac autonomic dysfunction was a strong predictor of future silent ischaemia as well as other cardiovascular events (HR 4.33; 95% CI 2.14 - 8.75). Cardiovascular autonomic neuropathy might also be linked with stroke. A study of 1458 individuals with T2D found that decreased HRV was a strong predictor of stroke (HR 2.7, 95% CI 1.3 - 5.5) over 7 years of follow-up (Ko et al., 2008). An earlier study also showed a similar association (Töyry, Niskanen, Länsimies, Partanen, & Uusitupa, 1996).

As well as cardiovascular morbidity, cardiovascular autonomic neuropathy has also been associated with cardiovascular and all-cause mortality. A meta-analysis of 15 prospective studies including almost 3000 participants with T2D, found that future mortality was significantly higher in patients with cardiovascular autonomic neuropathy than in those who had no abnormalities at baseline (RR 3.65, 95% CI, 2.66 - 4.47). This association was independent of conventional CVD risk factors (Maser, Mitchell, Vinik, & Freeman, 2003). A subsequent study of over 8000 participants with T2D found that cardiovascular autonomic neuropathy predicted all-cause (HR 2.14; 95 % CI, 1.37 – 3.37) and CVD mortality (HR 2.62; 95 % CI, 1.4 – 4.91) independently of baseline CVD, diabetes duration as well as other conventional CVD risk factors (Pop-Busui et al., 2010). Taking the evidence together, it appears that sympathetic/parasympathetic balance can influence outcomes in T2D. However, it should be noted that the studies differed in how cardiovascular autonomic neuropathy was assessed as there is no widely accepted single approach for its diagnosis. Most studies used HRV to assess cardiovascular autonomic neuropathy, as this is the most commonly used approach clinically (Pop-Busui, 2010).

1.7.1.3. *Cardiovascular stress responsivity*

The research above concerned the assessment of cardiovascular function in observational studies and clinical settings. Cardiovascular function can also be measured dynamically in response to acute standardised stress. Cardiovascular reactivity in the laboratory setting has been related to future health status in everyday life. Chida & Steptoe, (2010) conducted a meta-analysis of 36 studies that assessed

cardiovascular responses to laboratory stress in healthy individuals and subsequently tracked their health over time. The results of this analysis suggest that exaggerated cardiovascular responses to acute stress and poorer stress recovery are associated with an increased risk of incident hypertension, subclinical atherosclerosis as well as CVD events independently of traditional risk factors such as smoking and lipids. The most consistent associations were reported for SBP and DBP. As discussed in section 1.6.1 and illustrated in Figure 1.4 heightened biological reactivity to stress and the poorer recovery from stress are indicative of the ‘prolonged response’ aspect of high allostatic load (McEwen, 1998). This meta-analysis demonstrates that a high allostatic profile in cardiovascular stress reactivity the laboratory environment is linked with future health risk in everyday life.

Considering that physiological responses to laboratory stress have been associated with future CVD risk status it is surprising that stress reactivity has not been more widely investigated in people with T2D. One small study of 30 men with diabetes and 30 controls found that SBP responses to a mental arithmetic task were elevated and HRV responses blunted in the participants with diabetes compared with the controls (Deepak, Nallulwar, & Khode, 2014). No differences for DBP or heart rate were detected. The elevated SBP responses to stress in this study could be indicative of the ‘prolonged response’ component of allostatic load whereas the blunting in HRV could reflect of the ‘inadequate response’ aspect of allostatic load (McEwen, 1998). However, the results of this study should be interpreted with caution for several reasons. Firstly, the control group was weak. The controls were age and sex matched with the participants with diabetes (albeit across a diverse age range of 30-65 years) but the group was unusual due to the exclusion of controls who took regular exercise, consumed alcohol or smoked. Secondly, no covariates were included in the statistical

analysis which increases the possibility of confounding. Additionally, BP was not measured continuously over the course of the laboratory session, with only two measurements taken at rest and immediately post-task, which means the pattern of reactivity and post-stress recovery could not be reliably assessed.

1.7.1.4. *Summary and cardiovascular function hypotheses*

BP, heart rate, cardiac output and HRV have been widely studied as CVD risk markers. Meta-analytic evidence suggests that elevated BP and heart rate increase the risk of diabetes in initially healthy populations. Research has linked reduced HRV with cardiovascular autonomic neuropathy one of the most serious diabetes complications and small number of cross-sectional studies suggest that HRV might be dysregulated in people before overt diabetes onset.

Cardiovascular stress reactivity has been researched in healthy individuals and pooled evidence suggests that heightened cardiovascular responses to laboratory stress (which are reflective of high allostatic load) are associated with future cardiovascular risk status. There is a dearth of research on psychophysiological stress reactivity in people with diabetes and whether their reactivity differs from healthy controls. It is also unclear whether the high levels psychosocial stress experienced by people with T2D in everyday life could influence stress reactivity in this population. Therefore, this PhD will investigate the following hypotheses in relation to cardiovascular function:

1. Do cardiovascular responses to acute laboratory stress differ in people with T2D compared to healthy controls? (Study 1)

2. Do psychosocial stress factors influence cardiovascular responses to acute laboratory stress in people with T2D? (Study 2)

1.7.2. Metabolic function and T2D

Metabolic markers of increased cardiovascular risk include BMI, HbA1c, and total cholesterol (Abraham, Brunner, Eriksson, & Robertson, 2007). Obesity is a major risk factor for T2D (Guh et al., 2009) and rising obesity rates worldwide (Ng et al., 2014) are thought to be a major contributor to the increasing prevalence of diabetes (Danaei et al., 2011; International Diabetes Federation, 2015). For the purposes of this PhD, BMI will be included as a covariate in all analyses to ascertain whether associations between diabetes and biological pathways are independent of overweight/obesity. HbA1c and other markers of glucose dysregulation will be used to ascertain diabetes status. Therefore, cholesterol offers an interesting marker to assess metabolic function in people with T2D.

Cholesterol plays a key role in CVD. For example, a meta-analysis of 61 prospective cohort studies with almost 900,000 participants found a positive association between total cholesterol and future CVD mortality with no apparent threshold (Prospective Studies Collaboration et al., 2007). Previously it was thought that the passive accumulation of lipids and cholesterol in the arterial wall was solely responsible for atherosclerosis. However, it is now understood that atherosclerosis is an inflammatory disorder that involves an interaction between lipid accumulation, vascular injury and inflammatory responses (Anogeianaki et al., 2011; Libby et al., 2011; Ross, 1999). The Jupiter RCT of 17,802 men and women from 26 countries was set up to assess whether statin treatment could prevent CVD events among individuals who were at vascular risk due to elevated concentrations of the inflammatory marker C-reactive

protein (CRP) rather than heightened cholesterol levels (Ridker, 2009). In this trial of people with no history of CVD but raised CRP levels statin therapy was found to reduce the risk of adverse cardiac events and CVD mortality (Ridker et al., 2008). This trial highlights the interaction between cholesterol and inflammation in CVD processes.

High cholesterol is not considered to be a major risk factor for T2D (American Diabetes Association, 2015; International Diabetes Federation, 2015), but there is evidence that statin use is associated with a slightly increased risk of incident diabetes (Rajpathak et al., 2009; Sattar et al., 2010). The benefit of CVD reduction is suggested to outweigh the increased diabetes risk as a pooled-analysis of 13 RCTs with over 90,000 individuals found that treatment of 255 people with statins for 4 years resulted in one extra case of diabetes, while simultaneously preventing 5.4 vascular events among those 255 patients (Sattar et al., 2010).

In people with diagnosed diabetes cholesterol control is a key clinical target (American Diabetes Association, 2015), due to the strong association between cholesterol and the macrovascular complications of diabetes. According to the 2015 UK Diabetes audit an estimated 40.5% of people with T2D are achieving the recommended total cholesterol level of less 4mmol/L (HSCIC, 2015). Statin therapy is recommended as a cardioprotective treatment for most individuals with T2D (American Diabetes Association, 2015). Considering the different types of cholesterol, raised low density lipoprotein (LDL) cholesterol is known to increase CVD risk, whereas raised high density lipoprotein (HDL) cholesterol is thought to have a protective effect (American Diabetes Association, 2015). A meta-analysis of 14 randomised trials of statin therapy suggest that for every 1 mmol/L reduction in LDL cholesterol, participants with diabetes have a 9% reduced risk of all-cause mortality and a 13% reduced risk of vascular mortality (Cholesterol Treatment Trialists' (CTT) Collaborators et al., 2008).

The authors also found a 21% reduction in vascular events in people with diabetes per mmol/L reduction in LDL cholesterol. However, not all trials and cholesterol treatment combinations have been shown to reduce the risk of CVD in this population (Ginsberg, 2011; The AIM-HIGH Investigators, 2011). Treatment to raise low HDL cholesterol is not recommended due to the lack of proven efficacy on CVD mortality and the possible increase in risk of ischemic stroke in those taking a combination of LDL and HDL targeting therapies (The AIM-HIGH Investigators, 2011).

Circulating lipids including cholesterol are known to increase in response to acute stress and emotional arousal (Bachen, Muldoon, Matthews, & Manuck, 2002; Dimsdale & Herd, 1982). The association between lipid stress responsivity and lipid profiles in everyday life has also been investigated longitudinally. In a study of almost 200 participants LDL, HDL and total cholesterol were found to increase in response to a standardised stress protocol. At three years follow-up those in the highest cholesterol stress response tertile had significantly greater odds of developing clinically elevated cholesterol compared with those in the lowest stress response tertile (Steptoe & Brydon, 2005). This association was independent of baseline cholesterol levels and a range of demographic and clinical covariates. Considering this finding it is plausible that acute cholesterol stress responsivity can contribute to the development of elevated cholesterol levels in everyday life. Furthermore, heightened stress reactivity could be reflective of high allostatic load.

1.7.2.1. *Summary and cardiovascular function hypotheses*

Cholesterol plays a major role in the development of CVD and therefore it is a key clinical target in people with diabetes. Little is known about physiological stress

reactivity in people with diabetes. No previous studies have assessed whether cholesterol stress reactivity differs in people with diabetes compared with healthy individuals. To address this gap in the literature, this PhD will investigate the following hypothesis in relation to cholesterol stress reactivity:

1. Do cholesterol responses to acute laboratory stress differ in people with T2D compared to healthy controls? (Study 1)

1.7.3. Neuroendocrine function and T2D

1.7.3.1. *Cortisol and T2D*

Cortisol secretion is another potential biological mechanism that could link stress with health. Cortisol plays a pivotal role in many physiological processes relevant to diabetes. It directly reduces insulin sensitivity and decreases insulin secretion by acting through glucocorticoid receptors, which are expressed on pancreatic β -cells. It triggers hepatic gluconeogenesis, promotes lipolysis and the release of fatty free acids into the circulation and the accumulation of triglycerides in adipose tissue (Di Dalmazi et al., 2012). Increased fatty acid levels and triglyceride build up in adipose tissue are associated with an increased risk of diabetes, but it is unclear whether these associations are causal (Pankow et al., 2004; Silva et al., 2011).

Chronic over-activation of the HPA axis can lead to dysregulated cortisol output (McEwen, 1998). As cortisol is essential for life any deviation from the optimal range is thought to have deleterious effects and neuroendocrine dysfunction has been implicated in diabetes. Pathological (Clayton, Raskauskiene, Reulen, & Jones, 2011) and experimental (Connell et al., 1987) exposure to excessive cortisol is related to metabolic disturbances such as hypertension, abnormal glucose metabolism and central obesity, all

of which are risk factors for T2D (Anagnostis, Athyros, Tziomalos, Karagiannis, & Mikhailidis, 2009). Elevated cortisol concentrations, assessed from single plasma samples (Phillips et al., 1998) and 24 hour urinary free samples (Misra et al., 2008) have been associated with raised plasma glucose (Phillips et al., 1998) and insulin resistance (Misra et al., 2008; Phillips et al., 1998) in healthy participants. Long-term cortisol excess as seen in Cushing's syndrome (Lacroix, Feelders, Stratakis, & Nieman, 2015; Newell-Price et al., 2006) and in glucocorticoid-treated patients (Clare & Thurby-Hay, 2009) increases susceptibility for hyperglycaemia and manifest T2D. In keeping with this Cushing's syndrome is recognised to be more prevalent in people with T2D than in the general population (Gungunes et al., 2014).

Previous research has investigated the link between diabetes and cortisol using various single time point cortisol measurements. Raised plasma cortisol levels have been associated with elevated fasting glucose in people with diabetes (Reynolds et al., 2010). Higher plasma cortisol levels after dexamethasone suppression test (Bruehl et al., 2007; Chiodini et al., 2005), higher 24 hour urinary free cortisol samples (Chiodini et al., 2005) and greater adrenal gland volume have also been reported people with T2D (Godoy-Matos et al., 2006), which taken together suggests subclinical hypercortisolism is prevalent in this population. However, not all studies report altered cortisol secretion in T2D (Asfeldt, 1972) or only demonstrate increased HPA axis activity in a subset of people with diabetes (Chiodini et al., 2007). The reason for the mixed evidence in this area is probably due to a lack of consideration of the diurnal patterning of cortisol, as well as studies with a predominately small number of participants who were selected from convenience samples.

1.7.3.2. *Cortisol and stress responsivity*

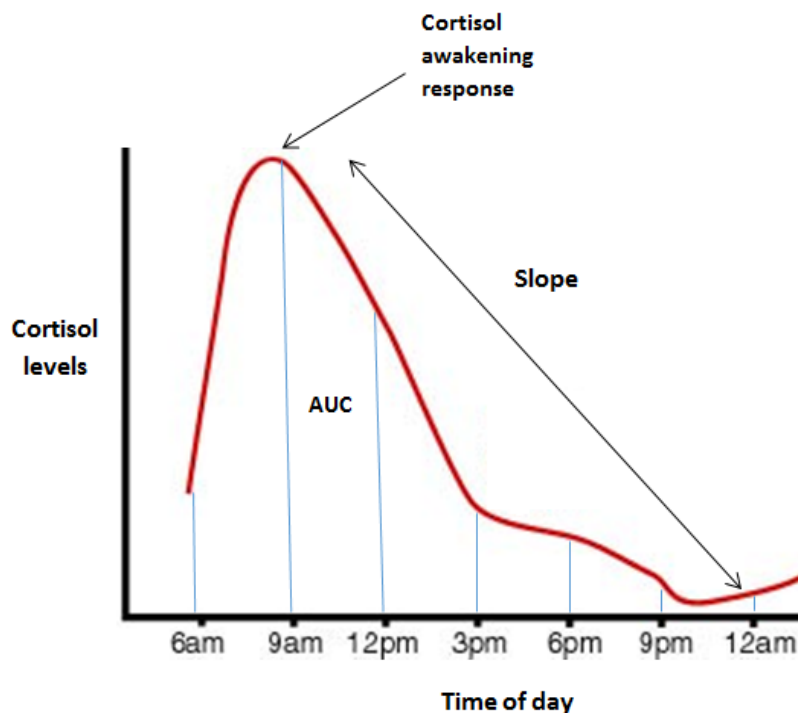
Cortisol levels are elevated by exposure to stress (Chida & Steptoe, 2009a; Miller et al., 2007) and heightened cortisol responses to stress have been linked to future CVD risk status (Hamer, Endrighi, Venuraju, Lahiri, & Steptoe, 2012; Hamer & Steptoe, 2012). In a study of almost 500 healthy individuals from the Whitehall II cohort, heightened cortisol responses to stress were shown to predict incident hypertension 3 years later. This association was independent of conventional demographic and clinical risk factors including BP at the time of the stress laboratory assessment (Hamer & Steptoe, 2012). In the same cohort heightened cortisol stress reactivity was associated with the progression of objectively measured coronary artery calcification over the same follow-up period (Hamer et al., 2012). Again, this association was robust to adjustment for numerous covariates. The results of these studies suggest that heightened cortisol stress reactivity could be an indicator of heightened allostatic load and that HPA function is a potential mechanism through which psychosocial stress may influence the risk of CVD. Despite the high risk of CVD and the potential role of neuroendocrine dysfunction in people with T2D, cortisol stress responsivity is under researched in this population. While reviewing the literature for this chapter no study that assessed cortisol responsivity to acute stress in people with T2D was found. This gap in the literature will be addressed with the studies presented in Chapters 3 and 4.

1.7.3.3. *Daily cortisol secretion in and CVD and T2D*

As well as acute cortisol stress responsivity, daily cortisol secretion and in particular the marked diurnal patterning in the release of cortisol has been the focus of large-scale HPA axis research (Adam & Kumari, 2009). The distinctive profile of daily cortisol

release is illustrated in Figure 1.5. It is clear from this illustration that cortisol levels vary considerably over the day. The pattern is typically characterized by high cortisol levels on waking, followed by an increase that reaches a peak 30 minutes after waking (termed the cortisol awakening response (CAR)) and subsequent decline across day (Adam & Kumari, 2009). Another parameter of interest is total cortisol output over the day. This can be calculated using area under the curve (AUC) (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

Figure 1.5 Circadian cortisol release



HPA axis dysregulation is thought to cause reduction in the amplitude of the diurnal cortisol pattern, or a flatter slope in cortisol across the day (Adam & Kumari, 2009). Flattening of the diurnal cortisol slope can be driven by low cortisol output on waking and/or higher evening cortisol concentrations. Dysregulation in cortisol output can also cause changes to the profound increase in cortisol concentrations after waking (Fries, Dettenborn, & Kirschbaum, 2009). The peak in cortisol levels or the CAR is calculated

by subtracting cortisol concentrations on waking from the concentration 30 minutes after waking. The CAR and the slope are thought to reflect different neurobiological control systems, therefore the 30 minute peak after waking sample is traditionally not included when calculating the cortisol slope (Adam & Kumari, 2009).

The relationship between these cortisol parameters and CVD outcomes has been assessed in both community and clinical samples. In an analysis of 4047 community-dwelling people from the Whitehall II cohort, a flatter daily cortisol slope and raised evening cortisol levels predicted cardiovascular mortality over 6 years of follow-up (Kumari, Shipley, Stafford, & Kivimaki, 2011). No significant associations for morning cortisol or the CAR were detected. In another study of community-dwelling older individuals urinary cortisol output over 24 hours was associated with CVD death over 6 years follow-up in the InCHIANTI study (Vogelzangs et al., 2010). In clinical populations associations between daily cortisol output and future outcomes have also been assessed. In a study of 250 participants who underwent coronary artery bypass graft surgery, pre-surgical flattening of the daily cortisol was associated with an increased risk of future adverse cardiac events and all-cause mortality (Ronaldson et al., 2015). Both morning and evening cortisol concentrations were found to account for changes in the daily cortisol slope in this study. No associations were detected for AUC in this study.

Dysregulation of daily cortisol secretion has been associated with subclinical atherosclerosis. In the Coronary Artery Risk Development in Young Adults study of 718 black and white adults those with the flattest cortisol slopes had an increased presence of coronary artery calcification. While, in the Rotterdam Study of 1886 people, total cortisol output over the day assessed by AUC was associated with atherosclerosis of the carotid arteries (Dekker et al., 2008).

The reported cross-sectional associations between diurnal cortisol patterns and T2D are mixed. In the MESA cohort, participants with T2D were found to have a significantly lower CAR than those without T2D (Champaneri et al., 2012). There was a sex difference in associations with the AUC; women with T2D had a significantly higher AUC than controls, but no associations were seen in men. Bruehl et al., observed a blunted CAR in participants with T2D, but found no association of T2D with slope in cortisol across the day or the AUC (Bruehl, Wolf, & Convit, 2009). In contrast, Lederbogen et al., observed a flattened slope in diurnal cortisol secretion and raised evening cortisol among those with diabetes compared with healthy controls in 979 individuals from a community cohort, but detected no association with the AUC (Lederbogen et al., 2011). Whereas, Vreeburg et al., observed no association between diabetes status and the CAR or diurnal cortisol slope or the AUC in a sample of 491 men and women (Vreeburg et al., 2009).

The reasons for these divergent findings are unclear. It is possible that differences in participant characteristics or in the number and timing of cortisol samples between studies may be involved. For example, the study by Bruehl *et al.*, was limited by low participant numbers and a lack of adjustment for potential confounding factors (Bruehl et al., 2009). Whereas, Champaneri *et al.*, used a much larger cohort of over a 1000 individuals (Champaneri et al., 2012) but the sample was ethnically diverse and over 60% of the participants were of Hispanic origin. With regards to sample timing in the NESDA cohort, participants provided four saliva samples, two within an hour of waking and two late-evening samples (Vreeburg et al., 2009). Whereas the study of the MESA cohort used up to 18 salivary cortisol samples collected over 3 days (Champaneri et al., 2012). It is possible that the lack of information on late morning and afternoon cortisol levels in the NESDA cohort study (Vreeburg et al., 2009) reduced the

authors' ability to examine the curvilinear nature of the decline in cortisol across the day (Adam & Kumari, 2009).

Prospective evidence relating neuroendocrine dysfunction with incident T2D is sparse. In the Longitudinal Ageing Study of Amsterdam (LASA) morning and evening salivary cortisol were measured in 998 initially healthy people. Raised evening cortisol was associated with future T2D in female participants (OR = 1.33), but no associations were found for men in the study (Schoorlemmer, Peeters, van Schoor, & Lips, 2009). In the MESA cohort changes over time in cortisol parameters were assessed in people with prevalent diabetes rather than looking at whether changes in cortisol predict new onset diabetes (Spanakis et al., 2016). To date no study has examined the relationship between the complete diurnal cortisol profile and incident T2D in an initially healthy population.

1.7.3.4. *Summary and neuroendocrine function hypotheses*

In summary it has been suggested that cortisol plays a role in the pathogenesis of diabetes. Heightened cortisol reactivity in the laboratory has been associated with the progression of coronary artery calcification and alterations in diurnal cortisol have been associated with CVD mortality. Stress plays a role in both CVD and T2D and cortisol levels are elevated by exposure to stress. Therefore, it is plausible that stress-induced cortisol release may influence the increased risk of CVD in T2D. As research in the area is lacking this PhD will address the following research questions:

1. Do cortisol responses to acute laboratory stress differ in people with T2D compared to healthy controls? (Study 1)

2. Do psychosocial stress factors influence cortisol responses to acute laboratory stress in people with T2D? (Study 2)
3. Is diurnal cortisol secretion altered in people with T2D compared to individuals without the condition? (Study 3)
4. Is altered daily cortisol secretion associated with new onset T2D in an initially healthy sample? (Study 4)

1.7.4. Inflammation and T2D

Another potential mechanism by which stress may influence T2D and CVD is through immune system activation. Diabetes has been characterised as a chronic low-grade inflammatory state involving multiple inflammatory mechanisms and metabolic pathways (Akash, Rehman, & Chen, 2013; Donath & Shoelson, 2011; Fève & Bastard, 2009).

Obesity is common in T2D (Guh et al., 2009) and visceral adipose tissue is major source of inflammatory factors collectively termed ‘adipokines’ (Galic, Oakhill, & Steinberg, 2010). These include cytokines such as interleukin (IL)-6, IL-1 β , and tumour necrosis factor- α (TNF- α), as well as hormone-like factors such as leptin, adiponectin, resistin, chemokines and acute phase proteins (Galic et al., 2010; Trayhurn & Wood, 2005). These factors derived from adipose tissue induce inflammation in the corresponding tissue, as well as in the β -cells of the pancreas, which in turn effects insulin sensitivity (Ehse, Ellingsgaard, Böni-Schnetzler, & Donath, 2009; Tilg & Moschen, 2008; Zhao, Feng, & Chen, 2006). Circulating adipokines levels have been shown to effect insulin sensitivity in vivo (Stuart & Baune, 2012). Heightened concentrations of pro-inflammatory adipokines such as IL-1 β , IL-6 and TNF- α and resistin promote insulin resistance by inhibiting enzymes involved in fatty-acid

oxidation, and down-regulating gene transcription of proteins involved in insulin-stimulated glucose transport and lipid uptake in adipose tissue. Conversely, adiponectin and leptin increase insulin sensitivity by stimulating these mechanisms (Pickup, 2004; Tilg & Moschen, 2008).

Circulating pro-inflammatory adipokine concentrations are elevated in T2D (Pickup, 2004). Recent evidence from a study of over 15,000 individuals in Germany suggests that there is a dose-response relationship between glucose status and inflammation (Grossmann et al., 2015). In this analysis CRP, fibrinogen, IL-1 receptor antagonist (IL-1RA) and IL-18 concentrations were found to increase in line with glucose dysregulation, with the lowest values being found in normoglycaemic individuals and increasing values found in people with pre-diabetes followed by the highest concentrations in those with diagnosed T2D. Overt tissue inflammation has also been detected in people with T2D (Akash, Rehman, & Chen, 2013).

Heightened inflammatory cytokine concentrations are also predictive of T2D development in initially healthy populations. Wang *et al.*, conducted a meta-analysis of prospective studies investigating the association between IL-6 and CRP concentrations and subsequent T2D (Wang et al., 2013). Results of the study indicate that heightened concentrations of both IL-6 (RR 1.31; 95% CI 1.17 - 1.46) and CRP (RR 1.26; 95% CI 1.16 - 1.37) significantly increase the risk of future T2D. Studies published since this meta-analysis have also detected a longitudinal relationship between CRP and incident increases in glucose parameters (Ahmadi-Abhari, Kaptoge, Luben, Wareham, & Khaw, 2015; Oda, 2015; Parrinello et al., 2015).

Similar prospective associations have been found for other markers of inflammation such as TNF- α , IL-1RA and IL-18 (Carstensen et al., 2010; de Rekeneire et al., 2006; Herder et al., 2009; Hivert et al., 2009; Luotola et al., 2011; Barbara

Thorand et al., 2005) though the magnitude of the association differs between studies and might vary depending on sample characteristics (Negi et al., 2012). It should also be noted that although IL1-RA is the antagonist to IL-1, it is often used as a proxy for IL-1 β activity and is considered to reflect an attempt by the body to counteract increased IL-1 β activity (Donath & Shoelson, 2011).

Taken together, the observational evidence supports a link between T2D and inflammation. However, observational studies are unable to reliably determine causality due to potential issues of confounding and reverse causality. Another type of study known as Mendelian randomisation exploits the properties of common genetic variation (random allocation of alleles at the time of conception) to estimate the causal contribution of a biological factor of choice with disease outcomes (Davey Smith & Hemani, 2014). Several Mendelian randomisation studies have sought to investigate the relationship between genes coding for inflammatory biomarkers and diabetes. One large study reported a near significant effect of a functional variant causing impaired signalling at the IL-6 receptor with reduced T2D risk (Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium, 2012) However, in a meta-analysis the same functional variant was not related to T2D risk (IL6R Genetics Consortium Emerging Risk Factors Collaboration, 2012). Studies investigating CRP and IL-1 genetic variants have not detected significant associations with diabetes (Swerdlow, 2016). Considering the evidence from Mendelian studies as a whole, thus far there is no evidence of a causal link between inflammatory related genetic variants and T2D. However, this area has not been widely researched and it is plausible that future studies may detect an association (Swerdlow, 2016).

Aside from questions of causality raised in the Mendelian randomisation studies, observational evidence has supported an association between heightened inflammation

and T2D and conversely, elevated anti-inflammatory adipokine concentrations have been reported to have anti-diabetic effects. For example, raised adiponectin concentrations have been associated with better glycaemic control in people with T2D (Mantzoros, Li, Manson, Meigs, & Hu, 2005) and meta-analytic results indicate that higher adiponectin levels lower the risk of T2D onset in a dose-response fashion (Li, Shin, Ding, & van Dam, 2009). Subsequent research published since this meta-analysis has also shown that low adiponectin levels are predictive of new onset diabetes (Marques-Vidal et al., 2012; Thorand et al., 2010). Additionally, treatment with anti-inflammatory medication may offer protection against T2D onset in certain populations (Solomon et al., 2011) and treatment with anti-inflammatory drugs is being investigated as a potential therapy in people with existing T2D (Akash, Rehman, Sun, & Chen, 2013; Akash, Shen, Rehman, & Chen, 2012; Donath & Shoelson, 2011). Some anti-diabetic medications such as glitazones or insulin have been shown to lower inflammation, independently of the glucose lowering effects (Goldfine, Fonseca, & Shoelson, 2011; Kahn et al., 2010). Statins have also been shown to reduce inflammation (Balk et al., 2003; Goldfine et al., 2011). Considering the link between inflammation and obesity, it is not surprising that weight reduction either through lifestyle change interventions or bariatric surgery has also been shown to decrease inflammatory markers in people with diabetes (The Diabetes Prevention Program Research Group, 2005; Viardot, Lord, & Samaras, 2010).

Aside from the anti-inflammatory effects of commonly prescribed diabetes treatments, selective blockade of IL-1 receptor and the inhibition of the NF- κ B pathway (the protein complex which controls cytokine production) are the most promising treatments to date. Both strategies appear to increase β -cell secretory function and insulin sensitivity, as well as reducing blood glucose (Fleischman, Shoelson, Bernier, &

Goldfine, 2008; Goldfine et al., 2013; Larsen et al., 2009; Sloan-Lancaster et al., 2013). As well as supporting the targeting of inflammation as a treatment for T2D, these studies add credence to the idea that inflammation is integral to the pathogenesis of T2D. However, research into anti-inflammatory treatments for diabetes is at an early stage and questions remain over the precise mechanism of action of these drugs, how durable their effects are and whether they have any impact on T2D complications.

1.7.4.1. *Inflammation and CVD in people with diabetes*

CVD has been characterised as an inflammatory condition (Hansson, 2005) and the progression of atherosclerosis is considered to be a multi-stage inflammatory processes (Galkina & Ley, 2009). Heightened inflammatory adipokines have been associated with CVD development and poorer outcomes in CHD patients (Danesh et al., 2008; Libby et al., 2011; The Emerging Risk Factors Collaboration, 2010). Evidence from Mendelian randomisation studies suggests that IL-6 plays a causal role in CHD (IL6R Genetics Consortium Emerging Risk Factors Collaboration, 2012; Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium, 2012). CRP has been associated with CVD in observational studies, but based on available evidence from Mendelian Randomisation studies a causal role for CRP in the pathogenesis of CVD is less certain (C. Reactive Protein Coronary Heart Disease Genetics Collaboration, 2011; Elliott et al, 2009)

There is evidence that inflammation might play a role in linking T2D with increased incidence of CHD. At the cellular level, the atherosclerotic plaques of people with diabetes have a higher expression of inflammatory receptors and proteins and greater inflammatory cell infiltration compared to people without T2D (Burke et al.,

2004; Marfella et al., 2006; Moreno et al., 2000). Results from population studies indicate that CRP and fibrinogen (an inflammatory clotting factor) are associated with sub-clinical atherosclerosis (Ray et al., 2009). For example, in the Atherosclerosis Risk in Communities (ARIC) cohort involving 921 patients with T2D and 11,964 controls fibrinogen was significantly correlated with carotid intima-media thickness, with stronger associations detected in those with T2D (Metcalf, Folsom, Davis, Wu, & Heiss, 2000). Similarly, in a study of 3534 participants, CRP concentrations were positively associated with intima-media thickness in individuals with diabetes. No association was detected in participants without T2D (Sander et al., 2006).

Heightened concentrations of inflammatory markers have also been prospectively related to cardiac events in people with T2D. The association between IL-6, CRP and fibrinogen and the risk of macrovascular events and mortality was assessed in 3865 people with T2D from the ADVANCE trial (Lowe et al., 2014). All 3 markers were associated with an increased risk of macrovascular complications, as well as all-cause mortality controlling for age, sex and ADVANCE trial treatment group. However, only IL-6 was found to be a significant predictor of macrovascular events (HR per SD increase 1.37, 95% CI 1.24 - 1.51) and mortality (HR 1.35, 95% CI 1.23 - 1.49) with additional adjustment for other risk factors as well as fibrinogen and CRP concentrations.

Other studies have also reported prospective associations between raised CRP and future macrovascular events and CVD mortality in people with T2D (Graziella Bruno et al., 2009; Matsumoto et al., 2003; Schulze et al., 2004; Soinio, Marniemi, Laakso, Lehto, & Rönnekaa, 2006). However, none of these studies of CRP included a control group of people without diabetes, so it is unclear whether the predictive value of CRP differs between people with diabetes and those free of the condition. One pooled

analysis of 25,979 adults (4.9% of whom had diabetes) who took part in the British and Scottish health surveys directly addressed this issue (Kengne, Batty, Hamer, Stamatakis, & Czernichow, 2012). Over an average of 7 years of follow-up raised CRP levels were associated with increased CVD mortality and all-mortality, but the association did not differ depending on diabetes status. Similar findings were reported in sub-group analysis as part of a large meta-analytic study (The Emerging Risk Factors Collaboration, 2010).

Some other studies prior to the ADVANCE trial analysis (Lowe et al., 2014) assessed the relationship between fibrinogen and CVD outcomes in people with T2D (Bruno et al., 2005; Wattanakit et al., 2005). Wattanakit *et al.*, studied 1651 individuals with T2D from the ARIC cohort over a 10 year period and found that participants who were in the highest quartile of fibrinogen had a substantially increased risk of peripheral arterial disease compared with those in the lowest quartile (Wattanakit et al., 2005). Another study by Bruno *et al.*, assessed 1565 people over 11 year follow-up and found that raised fibrinogen was a significant predictor of CVD mortality in people with diabetes (Bruno et al., 2005). These studies did not compare the predictive value of fibrinogen for CVD events in people with and without diabetes. Another large analysis of data from the British and Scottish health survey addressed this gap in the literature and observed that the association of fibrinogen with CVD mortality and all-cause mortality was similar in people with and without diabetes (Kengne, Czernichow, Stamatakis, Hamer, & Batty, 2013).

There was only one other prospective study prior to the ADVANCE trial analysis (Lowe et al., 2014) that assessed IL-6 and complications in T2D (Schöttker et al., 2013). In this Austrian study of just over 1000 people with T2D, 161 subjects experienced a primary cardiovascular event over the 8 year follow-up period. However,

IL-6 was associated only with an increased risk of CVD events in participants with renal dysfunction. The ADVANCE trial study (Lowe et al., 2014) had a larger sample and better defined covariates which included other inflammatory markers. This may account for the ability to detect stronger effects in this study. When reviewing the literature for this chapter no study assessing whether the predictive value of IL-6 differed by diabetes status was found. However, the ADVANCE trial analysis suggests that IL-6 adds significantly to the prediction of CVD events in people with diabetes beyond conventional risk factors. The authors suggest that improving prognostic assessment with novel biomarkers is difficult to assess as the conventional risk factors already discriminate risk well.

1.7.4.2. *Acute laboratory stress*

Inflammatory markers can be assessed in laboratory stress studies, although responses are delayed in comparison other biological markers (Brydon, Edwards, Mohamed-Ali, & Steptoe, 2004; Steptoe, Hamer, & Chida, 2007). Elevated adipokine concentrations are observed in response to acute laboratory stress (Steptoe et al., 2007) and in people reporting high levels of psychosocial stress (Hänsel, Hong, Cámara, & von Känel, 2010; Hemingway et al., 2003; Kiecolt-Glaser et al., 2003). There is some evidence that heightened inflammatory responses to stress are linked to future CVD risk status. In a study of 153 healthy individuals from the Whitehall II cohort, heightened IL-6 and fibrinogen responses to stress were shown to predict incident increases in BP 3 years later (Brydon & Steptoe, 2005). This association was independent of conventional demographic and clinical risk factors including BP at the time of the stress laboratory assessment. In another study of this cohort, individuals with heightened fibrinogen and

TNF- α responses to stress were found to have greater carotid arterial stiffness 3 years later (Ellins et al., 2008). No associations were found for IL-6. Taken together, these studies suggest that heightened inflammatory responses to stress might be a marker of high allostatic load and that this heightened reactivity could contribute to future CVD risk. Despite the heightened risk of CVD in people with diabetes, while reviewing the literature for this chapter no study that assessed inflammatory responses to acute stress in people with T2D was found.

1.7.4.3. *Summary and inflammatory hypotheses*

Both inflammation and psychosocial stress appear to play an important role in the development and progression of T2D and CVD. Given this evidence, it is plausible that stress may affect processes that are central to the pathophysiology of both conditions and that stress responses may be altered in people with T2D. Therefore, the role of stress-related inflammatory processes in people with T2D warrants further investigation.

1. Do inflammatory responses to acute laboratory stress differ in people with T2D compared to healthy controls? (Study 1)
2. Do psychosocial stress factors influence inflammatory responses to acute laboratory stress in people with T2D? (Study 2)

1.8. Summary

T2D is chronic disorder with increasing prevalence that strongly contributes to the risk of CVD. Observational evidence indicates that psychosocial stress plays a role in the

development and progression of both T2D and CVD. A variety of negative psychosocial factors have (for the most part) been shown to increase the risk of diabetes in initially healthy populations. There is less research on the involvement of psychosocial factors in CVD risk in people with existing diabetes. To date, most of the research in this area has been on depression with evidence that a double diagnosis of diabetes and depression increases the risk of CVD in this population. Most of the studies are observational, so causal conclusions are difficult to draw.

The mechanisms through which psychosocial stress factors increase diabetes risk and affect outcomes in people with existing diabetes are yet to be fully understood. It is likely that both behavioural and biological pathways are involved. Considering that the association between T2D and CVD is not fully explained by behavioural risk factors and results from RCTs suggest that the modification of behavioural risk factors do not significantly lower CVD outcomes in people with T2D despite the fact that lifestyle change has a marked effect on diabetes incidence, this thesis will focus on the pathophysiological effects of stress in relation to T2D using both psychophysiological stress testing, as well as naturalistic monitoring.

2. The Diabetes Study: Introduction and methods

2.1. Introduction

This chapter will provide an overview of the methods employed in what we have called in Andrew Steptoe's group 'the Diabetes Study'. The data from this trial is used in the studies presented in Chapters 3 and 4. The main aim of the Diabetes Study was to assess physiological responses to acute mental stress across multiple biological systems in people with T2D and to compare the responses to those of healthy controls. A secondary aim of the study was to assess the impact of psychosocial factors on biological responses to acute laboratory stress in people with T2D.

The Diabetes Study had four major components. The first part of the study involved participants completing a questionnaire booklet containing scales on lifestyle behaviours, affective factors and psychosocial measures among others. The second part of the study concerned psychophysiological stress testing over a single laboratory session. The third and fourth parts of the study involved naturalistic monitoring, with participants completing a week of objective physical activity assessment and a single day of at home salivary cortisol monitoring. Further detail on the different aspects of the study will be provided throughout this chapter.

2.2. Participant recruitment

The sample consisted of 140 people aged 50-75 years with doctor diagnosed T2D who were recruited from diabetes outpatient and primary care clinics in the London area. Finer details on the recruitment strategy can be found in section 2.6.1. Enrolment was restricted to patients without a history of CHD, inflammatory diseases, allergies or mood disorders. These exclusion criteria were selected to reduce potential interference with psychophysiological stress responses and to exclude individuals with diabetes who

had existing CHD. The study response rate was difficult to assess as primary care clinics differed in their requirements surrounding the confidentiality of patient data and the sending of recruitment letters. This meant that in some practices it was unknown how many letters were sent out to potentially eligible individuals. This limitation will be discussed in greater detail in Section 8.3.1 of Chapter 8. In the seven days prior to testing, all participants were prohibited from taking anti-inflammatory or anti-histamine medication. On the day of testing, participants were rescheduled if they reported colds or other infections. Participants were instructed to avoid caffeinated beverages and smoking for at least 2 hours before the session and to avoid vigorous exercise and alcohol from the previous evening. All participants gave full informed consent to take part in the study and ethical approval was granted by the National Research Ethics Service. The study was powered to detect small to medium effect sizes ($\delta = 0.32$, $p < 0.05$).

2.3. Questionnaire and naturalistic measures

The Diabetes Study was a large study of physiological response to stress in people with diabetes. For simplicity only measures that are used for the purposes of this PhD will be discussed here. Copies of the questionnaire measures mentioned in this section can be found in the Appendices at the end of this thesis.

2.3.1. Demographic measures

Personal details were collected from all participants including age, sex and marital status. Self-reported household income was used as an indicator of SES, as household income is thought to be a better measure of SES than personal income or education in older age groups (Banks, Karlsen, & Oldfield, 2003). Participants were categorised into

two income groups ($< \text{£}40,000$ and $\geq \text{£}40,000$). Education was also assessed in the study and was categorised into 3 groups, less than high school, high school or equivalent and college or higher.

2.3.2. Clinical information

Participants were telephone screened for any co-morbidity that would have excluded them from taking part in the study. In the questionnaire booklet that was completed before the laboratory session the participants self-reported any co-morbidities and current medication usage. To objectively confirm what medications the participants were taking and also for safety during the laboratory session participants were required to bring their current medication with them. Medication was allocated to six categories: oral diabetic medication (metformin, etc.), insulin and other injected diabetic medication, aspirin, beta-blockers, other hypertensive medication (ACE inhibitors, calcium channel blockers, etc.), and statins. Height and weight were objectively assessed at the start of the laboratory session with the participants wearing light clothing. Height was measured to the nearest 0.1 cm using a stadiometer and weight was assessed using a Tanita scale (Tanita Corporation of America, Inc., Arlington Heights, IL) BMI was computed as body mass in kilograms divided by the square of the height in meters (kg/m^2). HbA1c was assessed from the baseline blood sample taken at the laboratory stress session.

2.3.3. Health behaviour measures

2.3.3.1. *Sleep*

Sleep problems were assessed using an adapted 5 item version of the Jenkins sleep problems questionnaire (Jenkins, Stanton, Niemcryk, & Rose, 1988). The questionnaire assesses sleep issues in the past month with items such as “how often did you have trouble falling asleep” and “how often did you have trouble staying asleep”. In addition to the original 4 items, a fifth item was included “how often in the past month did you have disturbed or restless sleep?” (Kumari, Badrick, Ferrie, et al., 2009). The response options are rated on scale from 1 = ‘not at all’ to 6 = ‘22-31 days’. Scores were totalled and averaged with higher scores indicating greater sleep disturbance. This scale has been previously used in clinical samples (Jenkins, Stanton, & Jono, 1994) as well as in large epidemiological cohorts (Kumari, Badrick, Ferrie, et al., 2009; Lallukka et al., 2012). The internal consistency (Cronbach α) of the scale was .86 in this sample.

2.3.3.2. *Physical Activity*

Objective physical activity was assessed using an accelerometer (Actigraph GT3X, Pensacola, Florida, USA) that records movement vertically and horizontally. The accelerometer records the intensity, frequency and duration of physical activity as well as general activity levels. Participants wore the actigraphs at hip level during waking hours for 7 consecutive days. To be included in the physical activity analysis all participants needed recorded wear time for a minimum of 10 hours a day for 5 days. The first and last days of wear were excluded. A time interval of at least 60 minutes of zero activity counts was defined as non-wear time. The raw data were processed using specialist software (MAHUffe, Cambridge, UK). The data derived from this device was

classified using previously defined cut-points (Harris, Owen, Victor, Adams, & Cook, 2009) calculating daily times in each intensity band: sedentary, light, moderate and vigorous activity. Two papers have been published using actigraph data from the this study and further details on the activity measures can be found there (Hamer et al., 2014; Hamer, Bostock, Hackett, & Steptoe, 2013).

2.3.3.3. *Alcohol Consumption*

Alcohol consumption was assessed using a seven-day recall questionnaire, in which the type and quantity of alcoholic drink was recorded. Five different categories of alcoholic drink were assessed (beer, lager, cider; wine; martini, sherry, port; spirits; other) and the quantity of alcohol was recorded as a pint, glass or measure depending on the type of alcohol. If no alcohol was consumed the participants recorded their consumption as zero. When processing this data the overall quantity of alcohol was totalled, one quantity of any alcohol was counted as '1 unit'. The data were then split into three categories no alcohol consumption, below and above recommended levels. The recommended level for women was classified as 14 units or less a week and for men 21 units or less a week. These cut-points were based on the 1995 UK guidelines as the most recent guidelines do not recognise sex difference in the relationship between alcohol consumption and health risk and were not designed to be used for research purposes (Department of Health, 2015).

2.3.3.4. *Smoking*

Self-reported current smoking status was assessed using one item and a binary response of yes/no was recorded.

2.3.4. Psychosocial measures

2.3.4.1. *Hostility*

Cynical hostility was measured using the 10 item Cook Medley Cynical Hostility Scale (Cook & Medley, 1954). The Cynical Hostility scale is a widely used self-report measure of hostility, assessing cynical and mistrustful attitudes towards others and has previously been related to physiological stress responses (Brydon et al., 2010; Chida & Hamer, 2008). The items (e.g. “I think most people would lie to get ahead” and “It is safer to trust no one”) were scored using a binary (true/false) format. Total scores ranged from 0-10 with higher scores indicating greater hostility. The internal consistency (Cronbach’s alpha) of the scale was 0.80 in this sample.

2.3.4.2. *Depression*

Depression was measured using the 20 item revised version of the CES-D which is a standard measure of depressive symptomatology (Radloff, 1977). The CES-D is widely used in population-based research (e.g. Brunner et al., 2014; Demakakos, Zaninotto, & Nouwen, 2014). Participants were asked to rate statements based upon the feelings they experienced over the past week. Items included statements such as ‘I felt that everything I did was an effort’ and ‘I felt that I could not shake off the blues’. Items were rated on a 4 point scale from 1 ‘rarely or none of the time’ to 4 ‘all or most of all of the time’. The

total score is calculated as sum of the responses to all 20 questions. Items were assessed using the continuous range of scores rather than using cut-points, with higher scores indicating greater depression. The Cronbach's alpha of the scale was 0.86 in this sample.

2.3.4.3. *Optimism*

Optimism was measured using the 10-item Life Orientation Test-Revised (LOT-R), a widely used measure of optimism that evaluates generalised positive or negative expectancies in life (Scheier, Carver & Bridges, 1994). This scale has previously been used in acute laboratory stress trials (Brydon, Walker, Wawrzyniak, Chart, & Steptoe, 2009; Nes, Segerstrom, & Sephton, 2005). Participants were asked to indicate the extent of their agreement with each item (e.g. "I'm always optimistic about my future") from 0 (strongly disagree) to 4 (strongly agree). Six items were used to derive the optimism score, so ratings can range from 0 to 24, with higher scores indicating greater optimism. The remaining four questions on the LOT-R are filler items. The Cronbach α was .83 in this sample.

2.3.4.4. *Loneliness*

Loneliness was assessed with the revised UCLA loneliness scale (Russell, Peplau, & Cutrona, 1980). This scale has previously been related to physiological stress responses (Hackett, Hamer, Endrighi, Brydon, & Steptoe, 2012). This questionnaire consists of 20 items which are rated on a four point scale from 1 = never to 4 = often. Items include 'I feel isolated from others' and 'I feel I am no longer close to anyone'. Total loneliness

scores were calculated by summing the responses for all items. Total scores ranged from 20 to 80, with higher scores indicating greater loneliness. The Cronbach's alpha of the scale was 0.94 in this sample.

2.3.4.5. *Financial strain*

Financial strain was assessed with an 8-item adaptation of the economic strain measure of Pearlin *et al.* (Pearlin, Menaghan, Lieberman, & Mullan, 1981). The questionnaire asks about the financial ease of spending on certain items (e.g. 'Are you able to afford furniture or household equipment that needs to be replaced?' and 'Do you have problems paying your bills?'). Responses to the items were rated on a 3 point scale from 1 'no difficulty' to 3 'very great difficulty'. The items were totalled with a range of 8-24, with higher values indicating greater financial strain. The Cronbach's alpha of the scale was 0.95 in this sample. For ease of interpretation this was collapsed into a binary variable with values = 8 representing no financial strain and values >8 meaning some financial strain.

2.3.4.6. *Subjective stress*

Subjective stress was measured over the course of the laboratory session using a 7-point rating scale with higher values indicating greater stress.

2.3.5. **Laboratory mental stress tasks**

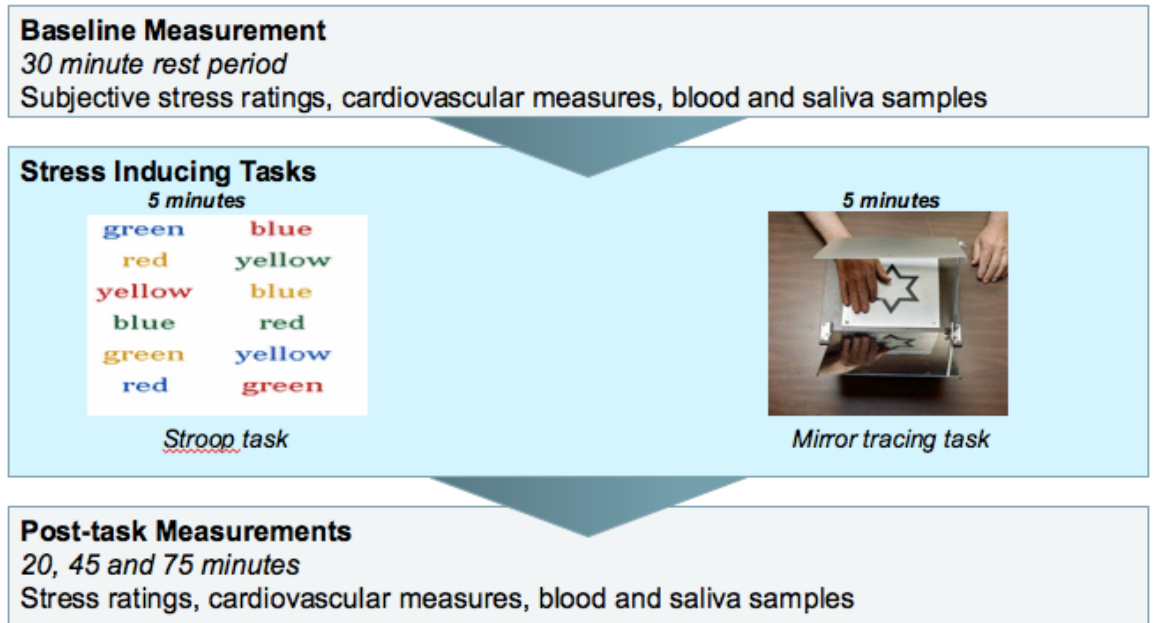
Mental stress was induced in the laboratory with two 5-minute behavioural tasks administered in random order. The first was a computerised version of the Stroop

colour-word interference task which involved successive presentation of target colour words (e.g. green, blue) printed in an incongruous colour. The second task was mirror tracing, which involved tracing a star that could only be seen in mirror image using a mental stylus. When the stylus came off the star a mistake was registered and a loud beep was emitted by the device (Lafayette Instruments Corp., Lafayette, IN, USA). Participants were told that the average person could complete five circuits of the star in the allocated time. These tasks were selected because they have previously been shown to stimulate similar appraisals of involvement and engagement from participants across the social gradient and have been used in a number of previous studies in the Psychobiology Group at University College London (Steptoe et al., 2002).

2.3.5.1. *Mental stress testing procedure*

We tested participants individually in a light- and temperature- controlled laboratory. Sessions were held either in the morning or in the afternoon. An overview of the timing of the psychophysiology stress testing period can be seen in Figure 2.1.

Figure 2.1 Overview of psychophysiology stress testing session



At the beginning of the session, anthropometric measures were obtained using standardised techniques and BMI was computed. Participants were fitted with a finger cuff so that SBP, DBP and heart rate could be continuously monitored using a Finometer device (TNO-TPD Biomedical Instrumentation, Amsterdam, Holland). The Finometer detects the full profile of cardiovascular responses by providing beat by beat data and shows good reproducibility and accuracy for cardiovascular monitoring in a range of settings (Castiglioni et al., 1999; Imholz et al., 1993). Cardiac output was determined from the device by Model flow 2.1 software using the aortic flow waveform method (Wesseling, Jansen, Settels, & Schreuder, 1993). HRV was also monitored from the device using the standard deviation of heart rate inter-beat-intervals. A venous cannula was inserted for the collection of blood samples. The participant rested for 30 minutes and the last 5 minutes of data were averaged to constitute baseline cardiovascular values. At this time, a baseline blood sample was drawn, saliva was collected for the analysis of cortisol and a subjective stress rating was obtained. We then

administered the two 5-minute behavioural tasks. Five-minute recordings of SBP, DBP and heart rate, cardiac output and HRV were made during each of the tasks, and subjective stress ratings, blood and saliva samples were taken immediately after the tasks. Monitoring of post-task recovery continued for 75 minutes. Further subjective stress ratings, cardiovascular measurements and blood samples were obtained at 45 and 75 minutes post-tasks. Additional saliva samples were obtained at 20, 45 and 75 minutes after the tasks.

As well as laboratory cortisol, saliva samples were collected over a normal day in order to measure components of daily cortisol secretion. Participants collected five samples at waking, 30 minutes later, and then within three 30-minute time windows in the morning (10:00–10:30), afternoon (16:00–16:30) and evening (20:00–20:30). The participants were instructed not to eat, consume caffeine or smoke in the 30 minutes before sample collection. Violations of this protocol and sample timing were recorded in a log. As diurnal cortisol is assessed in the much larger Whitehall II cohort sample in Chapters 6 and 7, daily cortisol secretion in participants of the Diabetes Study will not be included in this thesis.

2.3.5.2. *Processing of biological measures*

Blood samples were collected in EDTA tubes and centrifuged immediately at 2500 rpm for 10 min at room temperature. Plasma was removed from the tube and aliquoted into 0.5 ml portions and stored at -80°C until analysis. Plasma IL-6 was assayed using a Quantikine high sensitivity two-site enzyme-linked immunosorbent assay (ELISA) from R&D Systems (Oxford, UK). The sensitivity of the assay ranged from 0.016 - 0.110 pg/ml and the intra and inter assay coefficient of variations of 7.3% and 7.7%

respectively. Plasma IL-6 was assayed from all four blood samples. Total cholesterol was measured in a centrifugal analyser by enzymatic colorimetric methods and was assessed from the baseline, task, and 45 min post-task blood samples. Cortisol was assessed from saliva samples using a time resolved immunoassay with fluorescence detection, at the University of Dresden. The intra- and inter-assay coefficients of variation were less than 8%.

2.4. Data storage

Data were collected and stored in line with ethical guidelines and the project was registered with the UCL Data Protection Office. Considering the sensitivity nature of information collected, the data were treated as strictly confidential. Participant consent forms and contact details were stored separately from the questionnaire data. All participant questionnaires were labelled using a unique anonymised participant ID. All hard copies of the data were stored in locked filing cabinets. The data that was entered into the computer from hard copy was anonymised used the assigned participant IDs. Personal identification information was kept separately from questionnaire and biological data. All electronic data were kept on password protected computers with access only available to authorised study researchers.

2.5. Harmonising the Diabetes Study and Heart Scan Study datasets

The main aim of the analysis presented in Chapter 3 was to assess physiological responses to acute laboratory stress in people with diabetes compared with the responses of healthy controls. The controls for this analysis came from the Heart Scan Study, an earlier study which was carried out at the Psychobiology Group. The advantage of selecting these participants as controls was that the same activity measures

and questionnaire measures were used in this study as in the Diabetes Study. The participants of the Heart Scan Study also underwent an identical stress task protocol as the participants in the Diabetes Study.

The Heart Scan Study participants were recruited from the Whitehall II epidemiological cohort between 2006 and 2008 and the aim of this study was to investigate socioeconomic and psychosocial factors, physiological stress responsivity, and subclinical CAD (Hamer et al., 2012). All participants of the Heart Scan Study gave full informed consent, and ethical approval was obtained from the National Research Ethics Service. A total sample of 543 participants of white European origin with no history or objective signs of CHD, no previous diagnosis or treatment for hypertension, diabetes, inflammatory diseases, or allergies was recruited.

The absence of diabetes in participants from the Heart Scan Study was confirmed by low HbA1c levels ($\leq 6.5\%$, or 48 mmol/mol) and negative OGTT over the previous 20 years as objectively measured at Whitehall clinical assessments. Every person with diabetes was matched with two healthy controls as closely as possible by age, sex, and income category. In case-control analyses more than one control per case is used to increase statistical efficiency and power (Rose & van der Laan, 2009). In this study a ratio of two controls to one case was used due to a physical limit on the number of suitable controls available in the Heart Scan Study. As can be seen from Table 2.1 the matching procedure was successful; there was no difference between the groups in sex and income (p 's = 1) and the groups did not significantly differ in age ($p = .65$). The harmonised dataset had final sample size of 140 diabetes participants and 280 matched non-diabetic individuals.

Table 2.1 Characteristics of the diabetes and control groups

Values presented as numbers (%) and means (SD)

	Diabetes (n = 140)	No Diabetes (n = 280)	Group difference (p value)
Men / Women	88 / 52	176 / 104	1.00
Age - mean \pm SD	64.0 \pm 6.3	63.7 \pm 7.0	0.65
Household income – N (%)			
<£40,000	194 (71.6%)	95 (71.4%)	1.00
≥£40,000	77 (28.4%)	38 (28.6%)	

2.6. My involvement and contribution

The Diabetes Study was one of four major studies that formed part of a Psychobiology Group 5-year British Heart Foundation programme grant (2010-2015). The overarching aim of this programme grant was to investigate the links between psychosocial factors, biological processes and CHD. The design and aims of the Diabetes Study were set out by my supervisors Professors Steptoe and Hamer. While the Diabetes Study was part of a larger programme of work I was involved with all stages of the project from gaining ethical approval, to participant recruitment, data collection, data analysis and manuscript write-up. I have benefitted from contributing to the larger project by co-authoring papers using this data that do not form part of thesis (e.g. Carvalho et al., 2015; Hamer et al., 2014; Hamer, Bostock, Hackett, & Steptoe, 2013). The paragraphs below provide more detail on my role during different stages of the project.

2.6.1. Ethical approval and participant recruitment

When I started working on the Diabetes Study ethical approval had been granted as a substantial ethics amendment to the first Psychobiology Study that was set by Professor

Steptoe in 1997 (REC number 97/0356). My first role was to start participant recruitment. The recruitment strategy was to identify potential participants who met the study criteria based on the hospital records of individuals who were attending diabetes outpatient clinics at University College Hospital, London. However, it became clear over time that majority of people with diabetes attending outpatient clinics had many co-morbidities and therefore did not meet the stringent inclusion criteria. At this point I helped write an ethics amendment for permission to recruit participants from primary care clinics in Camden, London. I also helped apply for 'site specific approval' for the individual clinics we recruited from. From this point I was involved in all aspects of the recruitment process from liaising with practice managers at the individual clinics, to sending out recruitment letters and consent forms, telephone screening potentially eligible participants, sending out questionnaire booklets and actigraphs to the participants and arranging appointments at the laboratory. Copies of the study consent form and information sheet can be found in the Appendices.

2.6.2. Data collection

I ran all the psychophysiological stress testing sessions for the Diabetes Study working with a team of two research nurses and one medical doctor. The stress testing sessions required me to use equipment for cardiovascular measurement and blood glucose monitoring and to run the stress tasks. I also developed competencies in blood processing and learned how to perform biochemical assays.

2.6.3. Data cleaning and statistical analysis

I set up the datasets for the study and did the majority of data entry with the help of one of the research nurses. I helped clean the Diabetes Study data working with Professor

Step toe. I harmonised the Diabetes Study and Heart Scan Study datasets and performed the matching of the diabetes participants with appropriate healthy controls from the Heart Scan Study working under the tutelage of Professor Step toe. I conducted the statistical analysis for this thesis under the guidance of my supervisors. The research questions that form the basis of Chapters 3 and 4 were mutually established through discussion with my primary supervisor Professor Andrew Step toe.

3. Study 1: Comparing physiological responses to stress in people with and without diabetes

3.1. Overview

This chapter concerns the findings from the first analysis of the Diabetes Study. As discussed in Chapter 1 psychological stress-related processes are thought to contribute to the pathogenesis of T2D, but the biological mechanisms involved are poorly understood. In this study the notion that people with T2D experience chronic allostatic load, manifest as dynamic disturbances in reactivity to and recovery from stress across multiple biological systems, coupled with heightened experience of chronic life stress was tested. An experimental comparison of acute laboratory stress responses in 140 people with diabetes and 280 controls was carried out. Details on the methods can be found in Chapter 2. A brief overview of the study background is presented followed by the results of this study and a discussion of the findings.

Note: Some of the results presented in this chapter have been published in Steptoe et al., (2014). For the purpose of this PhD additional analyses were conducted assessing cardiac index and HRV stress responsivity, as well as group differences in health behaviours and psychological measures.

3.2. Introduction

Stress has been suggested to be involved in the pathogenesis of T2D (Pouwer, 2009; Pouwer, Kupper, & Adriaanse, 2010). Psychosocial factors from emotional disorders (e.g. depression), to personality traits (e.g. anger/hostility) and external stressors (e.g. work stress) have prospectively been related to new onset diabetes in initially healthy

populations (Abraham et al., 2015; Mezuk et al., 2008; Novak et al., 2013; Nyberg et al., 2014). In people with an existing diabetes diagnosis, psychosocial factors have been related to poorer self-management and worse glycaemic control (Collins et al., 2009; Gonzalez et al., 2008; Katon et al., 2010; Rush et al., 2008). There is also emerging evidence that psychosocial stress factors might increase the risk of complications in people with the condition (Lin et al., 2010; Rådholm et al., 2016; Sieu et al., 2011). A detailed discussion of the involvement of psychosocial factors in T2D can be found in sections 1.5 and 1.6 of Chapter 1.

In brief, the diverse associations between stress-related processes and diabetes reported in these studies are only partly accounted for by lifestyle factors such as physical inactivity, alcohol consumption, or adiposity, suggesting that direct psychobiological pathways may be involved. A helpful concept in this regard is allostatic load. The concept of allostasis describes the process by which organisms respond to changes in the environment through adjustments in multiple biological systems to maintain biological stability or homeostasis (McEwen, 1998). Allostasis is an adaptive process that is essential for maintaining homeostasis. However, repeated or sustained stimulation of the stress system is thought to lead to ‘wear and tear’ known as allostatic load. In this way, repeated stress exposure can promote dysregulated physiological reactivity, resulting in heightened, prolonged, or diminished responses to stress, thus increasing vulnerability to disease and contributing to negative health outcomes over time (McEwen & Wingfield, 2003).

Further detail on allostasis and allostatic load can be found in section 1.6.1 of Chapter 1 but for the purposes of this chapter it is important to reiterate that high allostatic load has been associated with a range of adverse health outcomes (e.g. Crimmins, Kim, & Seeman, 2009; Gruenewald et al., 2006; Juster, McEwen, & Lupien,

2010 etc.) and there is evidence that dysregulation across multiple biological systems has greater predictive value for health outcomes than any single biological predictor alone (Juster et al., 2010; Karlamangla et al., 2014; Seeman et al., 2001).

Few studies have investigated allostatic load in relation to diabetes and the available evidence is mixed. One study of over a 1000 middle-aged Puerto Ricans living in Boston found that high allostatic load was associated with increased risk of diabetes and other chronic conditions (Mattei et al., 2010), but another investigation that focused specifically on diabetes failed to find that the components of allostatic load clustered reliably in a sample of 53 individuals (Carlsson et al., 2011).

These studies assessed allostatic load using biological measures that were taken at rest. Another aspect of allostasis is that it is manifest in modifications of dynamic responses to challenge, not only in basal measures (McEwen, 1998). Adaptive biological responses to stress involve brisk increases in activation (stress reactivity) as the person mobilizes for vigorous activity, followed by prompt recovery back to baseline levels. High allostatic load disrupts these dynamic biological responses, resulting in changes in the morphology of responses (see Figure 1.4 in Chapter 1).

In this study the notion that people with T2D experience high allostatic load, manifest as dynamic disturbances in reactivity to and recovery from stress across multiple biological systems, coupled with heightened experience of chronic life stress was tested. An experimental comparison of acute laboratory stress responses in 140 people with diabetes and 280 controls was carried out. As hypothesised in Chapter 1, we expected disturbances in the diabetes group compared with controls in cardiovascular (SBP, DBP, heart rate, cardiac index and HRV), metabolic (as indexed by cholesterol), neuroendocrine (as indexed by cortisol), and inflammatory (as indexed by IL-6) measures. An allostatic state can be reflected by the occurrence of ‘prolonged

responses' such that the individual fails to return to baseline levels after stress exposure (impaired post-stress recovery). It also can be demonstrated through 'inadequate responses' by which there is an insufficient reaction to stress (blunted stress reactivity) (McEwen, 1998). In keeping with this concept of allostatic load we hypothesized that in response to standardized mental stress, people with T2D would show impaired post-stress recovery and blunted stress reactivity in BP, heart rate, cardiac index, HRV and cortisol, and serum cholesterol. It should be noted that in healthy individuals HRV generally decreases in response to stress. Therefore, blunting of this measure would mean less of a decrease in response to stress. With regards to IL-6, we hypothesised that concentrations would be raised in the diabetes group as insufficient glucocorticoid signalling (reflected by blunted cortisol) may have a permission effect on inflammatory markers such as IL-6 (Miller et al., 2007; Raison & Miller, 2003). We also measured several psychological factors as detailed in Chapter 2, conjecturing that people with diabetes would report more emotional distress and greater stress in their lives compared with individuals without diabetes.

3.3. Statistical analysis

SBP, DBP, heart rate, cardiac output and HRV were averaged into 5-minute means for baseline, the two tasks, and the two recovery periods. Cardiac output was transformed into cardiac index by correcting for body surface area. We conducted repeated measures analysis of variance to assess patterns of change across the session, with group and sex as the between-person factors and trial as the within-person factor. Because this study was a matched case-control design, analysis of variance and general estimating equation models were not appropriate for comparisons between groups. Instead, we analysed differences between groups in stress reactivity and stress recovery by using conditional

logistic regression, which takes account of the matched case-control design (Elwood, 2007). Group membership (diabetes or control) was the outcome variable for all analyses. Difference scores between tasks and baseline (for stress reactivity) and differences between tasks and recovery measures (for recovery analyses) were computed and used as independent variables for the analyses, and these were entered along with covariates into the models. Results are presented as adjusted odds of being in the diabetes as opposed to the control group per unit change in the predictor variable, with 95% confidence intervals (95% CI). A value >1 indicates that larger values of the predictor are associated with increased odds of being in the diabetes group, while values <1 indicate that larger values are associated with reduced odds of being in the diabetes group. Stress responses in IL-6 are delayed in comparison with other measures, so we assessed reactivity as differences between values recorded at 45 min and 75 min compared with baseline. Post-stress recovery in cardiovascular measures, cortisol, and cholesterol was measured by using difference scores between task and recovery period means. We log transformed cortisol, IL-6 and HRV before analysis because of skews in the distribution. Raw values for IL-6 and cortisol are presented in the Results for interpretative ease. HRV results are presented as log values +5 for ease of interpretation.

There were 28 (20%) ethnic minority participants in the diabetes group. Removing these participants from the analysis did not change the direction of effects, but some analyses no longer reached significance most likely due to the reduction in participant numbers. Health behaviour differences between the groups are also presented. The health behaviour analyses were unadjusted except for the objective physical activity analysis where registered actigraph wear time was included as a covariate. Adding physical activity, sleep problems or alcohol consumption to the analyses did not affect the results so these factors were not included in the final models.

However, all analyses of physiological data were adjusted for BMI and smoking status, because these variables are known to influence stress responses. Time of day of stress testing was included as a covariate, because profiles of response to stress may vary across the day. We also adjusted for education because of differences between groups and because this factor might affect stress responsivity (Steptoe et al., 2002). We selected medication covariates by testing for associations between medication status and stress responses within the diabetes group. Thus, cardiovascular analyses were additionally adjusted for the use of beta blockers in addition to education, BMI, and smoking; cortisol analyses for the use of statins; and cholesterol responses for the use of statins and aspirin. Sensitivity analyses were conducted removing medication covariates from the cardiovascular and cholesterol analyses. Removing medication covariates from these analyses did not change the results. Psychological differences between groups were analysed by using conditional logistic regression and unadjusted values are presented. We also explored interrelationships between cardiovascular and other biological responses, by computing product–moment correlations. All analyses were conducted using SPSS version 22 (SPSS, Chicago, IL, USA).

3.4. Results

3.4.1. Participants

As discussed in Chapter 2, 140 people with T2D were matched by age, sex and income with 280 healthy individuals who underwent an identical stress testing procedure. The matching procedure was deemed successful as the groups did not significantly differ in age, sex or income. A group comparison of other demographic and clinical characteristics can be found in Table 3.1. The participants with diabetes were better educated than the healthy controls and were significantly more likely to smoke. As

expected, BMI, waist circumference and HbA1c were significantly greater in the diabetes than control group. When looking at medications, the majority of the diabetes participants were taking oral medications such as metformin, and hypertensive medications were also common. None of the healthy controls were taking any medications apart from a small proportion of prescribed statins (7.9%).

Table 3.1 Characteristics of the diabetes and healthy control groups

	Diabetes (n = 140)	No Diabetes (n = 280)	Group difference (p value)
Education			< 0.001
Less than high school	37 (26.8%)	94 (35.9%)	
High school	14 (10.0%)	77 (29.4%)	
College degree or higher	87 (63.0%)	91 (34.7%)	
Smoker (% yes)	20 (14.4%)	18 (6.4%)	= 0.011
BMI (kg/m ²)	30.8 ± 5.72	25.9 ± 3.82	< 0.001
Waist (cm)	105.5 ± 13.5	87.5 ± 12.6	< 0.001
HbA1c %	7.25 ± 1.42	5.47 ± 0.53	< 0.001
HbA1c mmol/mol	56 ± 15.5	36 ± 5.8	< 0.001
Medication			
Statins	106 (77.9%)	22 (7.9%)	< 0.001
Oral diabetic medication	109 (80.1%)	-	-
Insulin, other anti-diabetics	15 (11.0%)	-	-
Aspirin	48 (35.3%)	-	-
Beta-blockers	16 (11.8%)	-	-
Other anti-hypertensives	96 (70.6%)	-	-

Values are presented as n (%) and means ± standard deviations

3.4.2. Physiological responses to mental stress

As mentioned in Chapter 2, participants were tested individually in the laboratory either in the morning or in the afternoon and time of testing may impact biological responses.

The groups did not differ in the proportion of laboratory sessions taking place in either the morning or the afternoon ($p = 0.233$). The mental stress tasks elicited substantial subjective stress responses, with increases from $1.50 \pm (\text{SD}) 0.91$ to 4.51 ± 1.54 in the diabetes and 1.42 ± 0.83 to 4.08 ± 1.42 in the no diabetes group on the 7-point subjective stress scale. The mean values in the two groups for subjective stress and the other measures over the laboratory session can be found in Table 3.2.

Table 3.2 Mean (SD) values of the physiological and subjective stress measures in the two groups

	Group	Baseline	Task response	Post stress recovery 45 min	Post stress recovery 75 min
SBP ^a (mmHg)	Healthy	127.28 ± 14.53	159.29 ± 21.55	136.76 ± 17.63	138.53 ± 17.78
	Diabetes	126.29 ± 13.54	149.90 ± 20.48	134.53 ± 20.60	137.42 ± 17.16
DBP ^a (mmHg)	Healthy	75.25 ± 9.49	90.01 ± 12.50	80.52 ± 11.30	81.66 ± 11.71
	Diabetes	71.81 ± 10.22	84.41 ± 12.56	78.08 ± 15.11	79.59 ± 14.01
Heart rate ^a (bpm)	Healthy	65.98 ± 8.68	74.50 ± 10.28	63.85 ± 7.97	63.99 ± 8.16
	Diabetes	71.82 ± 12.43	76.40 ± 12.35	70.21 ± 12.33	70.34 ± 12.02
Cardiac index (L/min/m ²) ^a	Healthy	3.17 ± 0.67	3.50 ± 0.79	2.88 ± 0.65	2.85 ± 0.66
	Diabetes	4.11 ± 0.97	4.12 ± 0.97	3.62 ± 0.90	3.60 ± 0.89
HRV (s) log+5 ^a	Healthy	1.94 ± 0.54	1.86 ± 0.54	2.14 ± 0.55	2.15 ± 0.55
	Diabetes	1.71 ± 0.62	1.61 ± 0.65	1.88 ± 0.68	1.89 ± 0.62
Cortisol ^b (nmol/l)	Healthy	6.69 ± 4.31	6.57 ± 4.75*	5.60 ± 4.39	5.26 ± 3.74
	Diabetes	9.92 ± 5.40	7.70 ± 3.98	6.92 ± 4.08	7.20 ± 5.59
IL-6 (pg/ml) ^c	Healthy	1.36 ± 0.84	1.38 ± 0.85	1.64 ± 0.98	1.77 ± 1.12
	Diabetes	2.03 ± 1.13	2.04 ± 1.09	2.14 ± 1.20	2.27 ± 1.21
Cholesterol (mmol/l) ^d	Healthy	5.22 ± 0.94	5.47 ± 0.97	5.34 ± 0.94	
	Diabetes	4.23 ± 1.08	4.33 ± 1.09	4.30 ± 1.11	
Stress rating ^a	Healthy	1.42 ± 0.83	4.08 ± 1.42	1.41 ± 0.83	1.36 ± 0.76
	Diabetes	1.51 ± 0.91	4.51 ± 1.54	1.53 ± 0.97	1.43 ± 0.95

^a Adjusted for education, smoking, BMI, beta-blockers and time of testing

^b Adjusted for education, smoking, BMI, cholesterol medication and time of testing

^c Adjusted for education, smoking, BMI and time of testing

^d Adjusted for education, smoking, BMI, cholesterol medication, aspirin and time of testing

*For cortisol the task response was the 20-minute peak in cortisol minus post-task values

Table 3.3 shows the unadjusted results of the conditional logistic regression on group membership. Table 3.4 shows the adjusted results. As can be seen the increase in subjective stress during tasks did not significantly differ between the two groups. SBP, DBP and heart rate also increased during stress tasks, returning toward baseline over the post-stress recovery period (Table 3.2). The diabetes group showed a pattern of cardiovascular responses characteristic of high allostatic load (Figure 3.1, Figure 3.2 and Table 3.3 and 3.4). Notably, SBP stress reactivity was blunted in the diabetes compared with the control group (adjusted odds of being in the diabetes group per mmHg increase in reactivity = 0.97, 95% CI 0.94–0.99, $p = 0.003$), whereas recovery was reduced both at 40–45 min post-stress (OR 0.98, 95% CI 0.96–0.99, $p = 0.013$) and at 70–75 min post-stress (OR 0.97, 95% CI 0.94–0.99, $p = 0.002$). We recorded a similar profile for DBP (Figure 3.1) and heart rate (Figure 3.2); thus, for both variables, stress reactivity was lower in the diabetes group, and post-stress recovery was impaired (statistical details in Table 3.3 and 3.4). In addition, the diabetes group had lower DBP but higher heart rate than the healthy control group throughout the stress session ($p < 0.001$). Looking at cardiac index, participants with diabetes had greater values at baseline than the healthy control group (OR 3.54, 95% CI 2.03 – 6.19, $p < 0.001$). However, similar to the other cardiovascular analyses, stress reactivity was lower and post-stress recovery was impaired in the diabetes group compared with the healthy control group (Figure 3.2 and Table 3.3 and 3.4). No associations with HRV were detected (detail in Table 3.3 and 3.4) and no interactions by sex were detected in any of the cardiovascular analyses. Considering the results together, the pattern is consistent with the allostatic load model, with blunted stress responsivity and impaired recovery following stress in the diabetes group compared with the healthy control group

Table 3.3 Unadjusted stress response & recovery: conditional logistic regression on group membership

	Baseline		Task response		Post-stress recovery 45min		Post-stress recovery 75min	
	Odds of diabetes (95% CI)	P	Odds of diabetes (95% CI)	P	Odds of diabetes (95% CI)	P	Odds of diabetes (95% CI)	P
Systolic BP (mmHg)	0.99 (0.98 – 1.01)	0.562	0.96 (0.94 – 0.98)	<0.001	0.97 (0.96 – 0.99)	<0.001	0.97 (0.96 – 0.98)	<0.001
Diastolic BP (mmHg)	0.97 (0.95 - 0.99)	0.003	0.96 (0.93 – 0.99)	0.006	0.96 (0.94 – 0.98)	0.002	0.96 (0.93 – 0.98)	0.001
Heart rate (bpm)	1.05 (1.03 – 1.08)	<0.001	0.86 (0.81 – 0.91)	<0.001	0.86 (0.82 – 0.91)	<0.001	0.86 (0.82– 0.91)	<0.001
Cardiac Index (L/min/m²)	4.22 (2.78 – 6.41)	<0.001	0.25 (0.14- 0.42)	<0.001	0.69 (0.47 – 1.07)	0.100	0.69 (0.46 – 1.04)	0.076
HRV (s) log+5	0.55 (0.37 – 0.82)	0.002	0.93 (0.60 – 1.46)	0.764	0.92 (0.62 - 1.38)	0.699	0.93 (0.62 – 1.40)	0.716
Cortisol (nmol/l)	5.33 (3.22 – 8.84)	<0.001	0.09 (0.40 – 0.23)*	<0.001	0.86 (0.54 – 1.40)	0.555	0.76 (0.50 – 1.16)	0.197
IL-6 (pg/ml)	3.80 (2.42 – 5.95)	<0.001			0.13 (0.05 – 0.36)	<0.001	0.23 (0.10 - 0.52)	<0.001
Cholesterol (mmol/l)	0.29 (0.21 – 0.40)	<0.001	0.006 (0.00-.032)	<0.001	0.057 (0.01 – 0.24)	<0.001		
Stress rating	1.08 (0.86 – 1.36)	0.493	1.17 (1.02-1.33)	0.022	1.14 (1.00 – 1.30)	0.046	1.14 (0.99 – 1.30)	0.054

*For cortisol the task response was the 20-minute peak in cortisol minus post-task values

Table 3.4 Adjusted stress response & recovery: conditional logistic regression on group membership

	Baseline		Task response		Post-stress recovery 45min		Post-stress recovery 75min	
	Adjusted odds of diabetes (95% CI)	P	Adjusted odds of diabetes (95% CI)	P	Adjusted odds of diabetes (95% CI)	P	Adjusted odds of diabetes (95% CI)	P
Systolic BP^a (mmHg)	0.99 (0.96 – 1.01)	0.246	0.97 (0.94 – 0.99)	0.003	0.98 (0.96 – 0.99)	0.013	0.97 (0.94 – 0.99)	0.002
Diastolic BP^a (mmHg)	0.95 (0.91 - 0.98)	0.006	0.95 (0.90 – 0.99)	0.020	0.96 (0.92 – 0.99)	0.021	0.95 (0.91 – 0.98)	0.005
Heart rate^a (bpm)	1.05 (1.01 – 1.08)	0.006	0.86 (0.79 – 0.93)	<0.001	0.78 (0.70 – 0.87)	<0.001	0.78 (0.70– 0.87)	<0.001
Cardiac Index^a (L/min/m ²)	3.54 (2.03 – 6.19)	<0.001	0.23 (0.10- 0.50)	<0.001	0.52 (0.28 – 0.97)	0.037	0.51 (0.29 – 0.92)	0.025
HRV^a (s) log+5	0.64 (0.35 – 1.16)	0.139	0.62 (0.30 – 1.27)	0.191	1.17 (0.65 - 2.10)	0.616	1.17 (0.58 – 2.40)	0.660
Cortisol^b (nmol/l)	12.56 (2.88 – 54.83)	<0.001	0.01 (0.00 – 0.10)*	<0.001	1.09 (0.33 – 3.54)	0.887	0.77 (0.27 – 2.17)	0.621
IL-6^c (pg/ml)	2.23 (1.18 – 4.23)	0.014			0.20 (0.47 – 0.85)	0.029	0.33 (0.11 - 0.96)	0.042
Cholesterol^d (mmol/l)	0.29 (0.13 – 0.62)	0.002	0.001 (0.00-.008)	0.003	0.002 (0.00 – 0.16)	0.005		
Stress rating^a	1.08 (0.79 – 1.48)	0.638	1.19 (0.99-1.42)	0.052	1.15 (0.97 – 1.38)	0.104	1.15 (0.96 – 1.38)	0.129

^a Adjusted for education, smoking, BMI, beta-blockers and time of testing

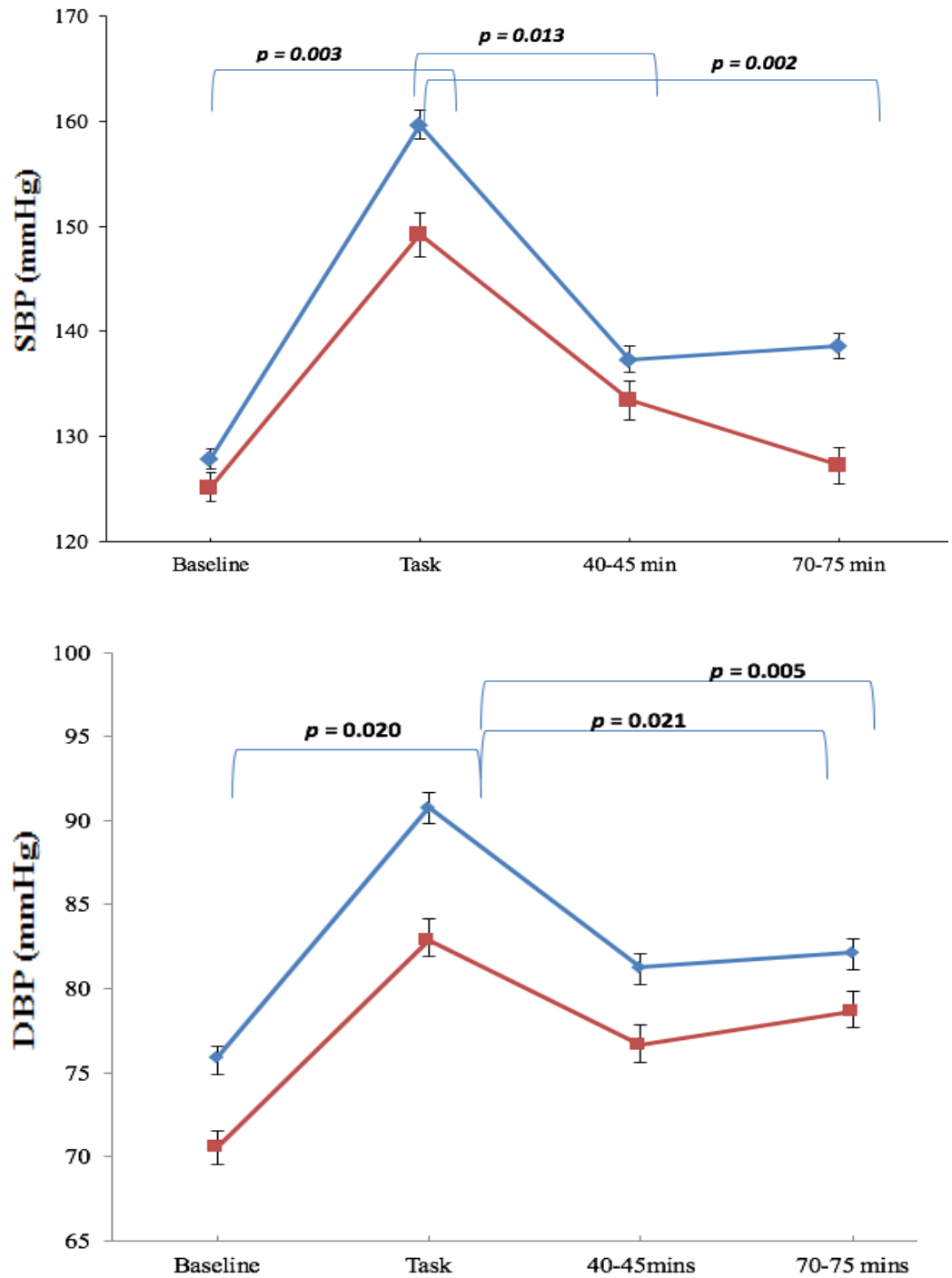
^b Adjusted for education, smoking, BMI, cholesterol medication and time of testing

^c Adjusted for education, smoking, BMI and time of testing

^d Adjusted for education, smoking, BMI, cholesterol medication, aspirin and time of testing

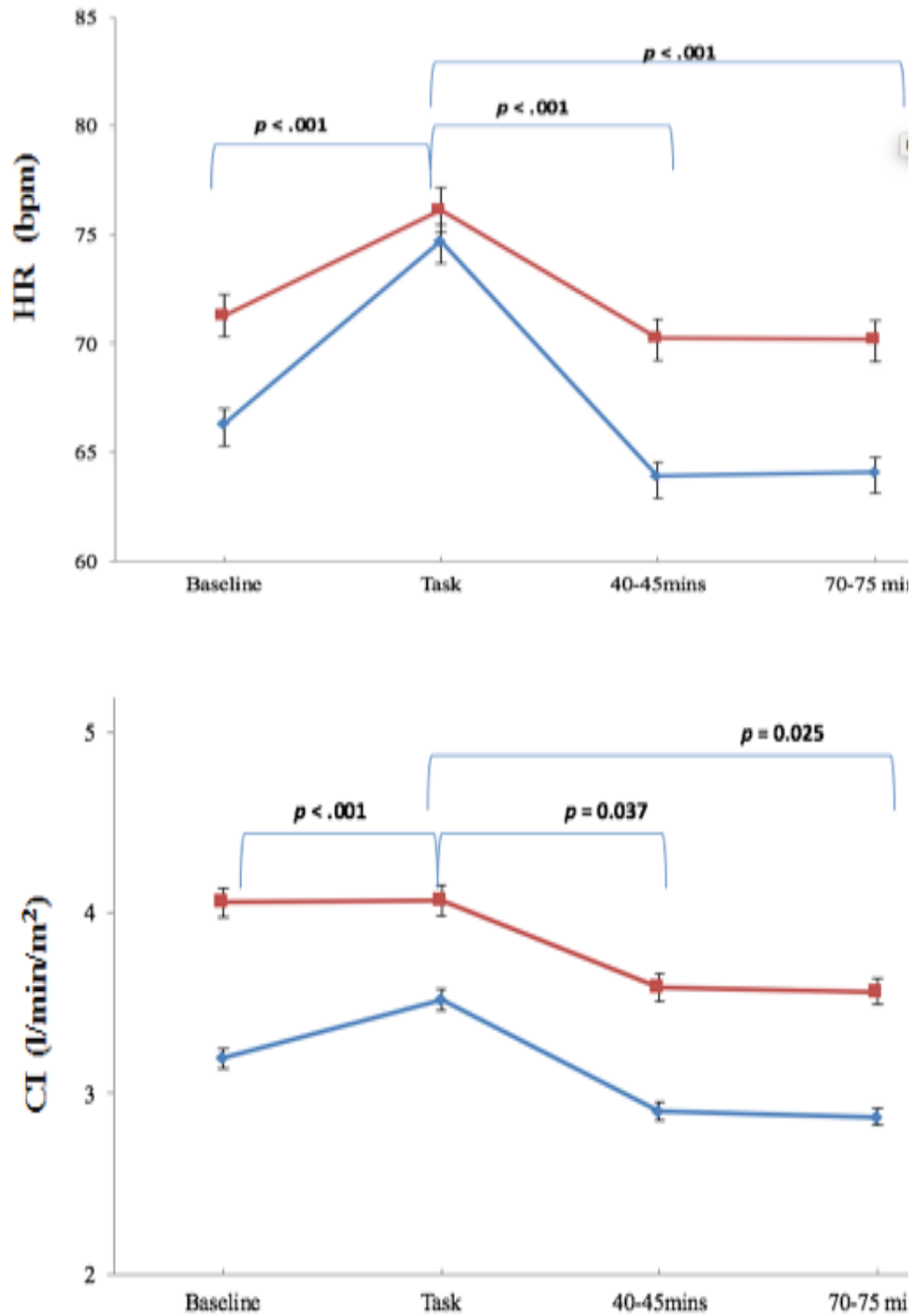
*For cortisol the task response was the 20-minute peak in cortisol minus post-task values

Figure 3.1 SBP and DBP responses across the laboratory session



Mean SBP and DBP values across the session in the diabetes (red line) and control (blue line) groups. All data adjusted for education, BMI, smoking, beta-blockers, and time of testing. P values indicate group differences in stress reactivity (baseline-task difference) and post-stress recovery (task-post-task differences) as detailed in Table 3.3 and 3.4. Error bars are SEM.

Figure 3.2 Heart rate and cardiac index responses across the laboratory session



Mean heart rate and cardiac index values across the session in the diabetes (red line) and control (blue line) groups. All data adjusted for education, BMI, smoking, beta-blockers, and time of testing. P values indicate group differences in stress reactivity (baseline-task difference) and post-stress recovery (task-post-task differences) as detailed in Table 3.3 and 3.4. Error bars are SEM.

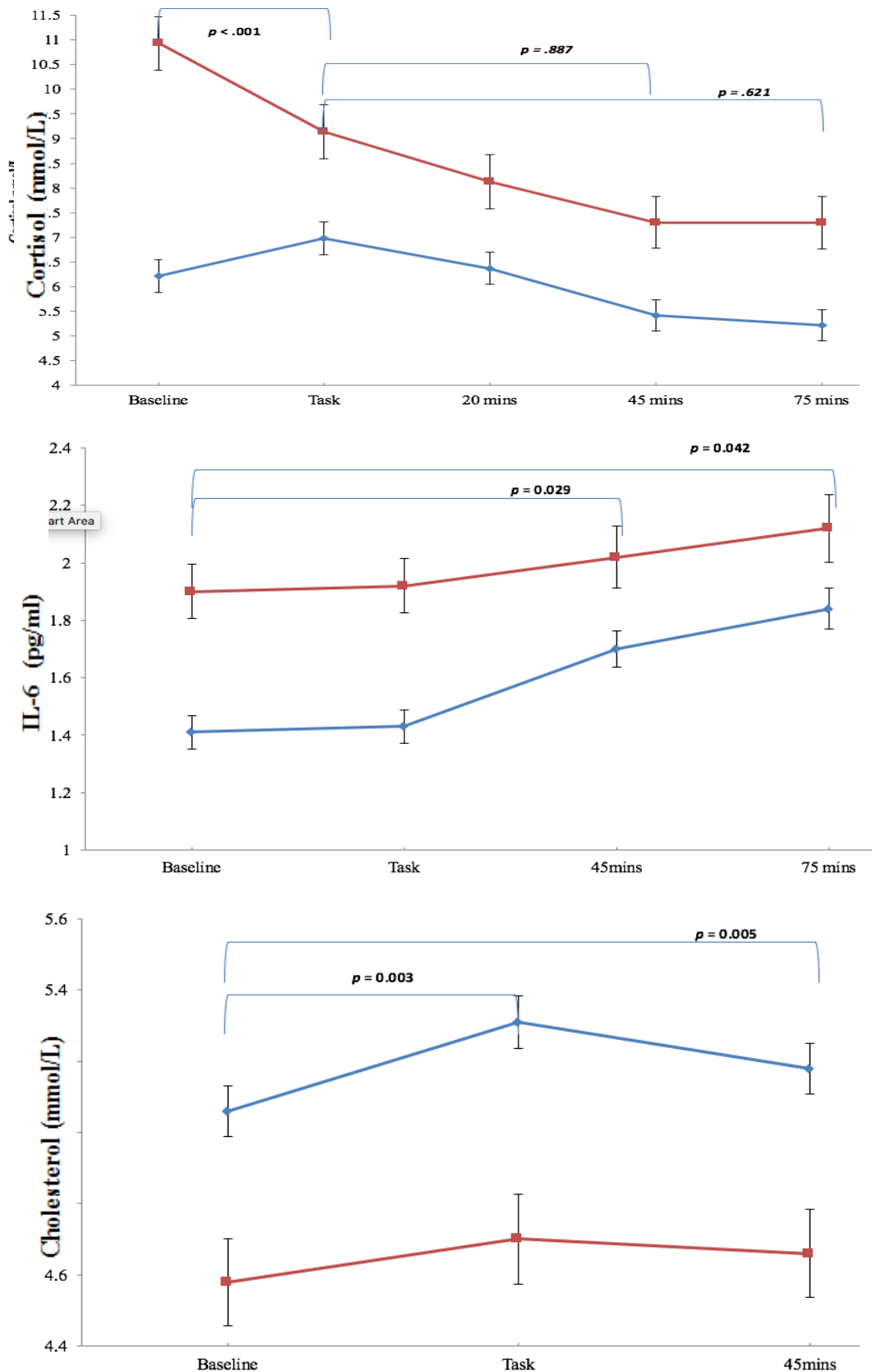
3.4.3. Cortisol, IL-6 and cholesterol responses to stress

We found that baseline cortisol concentrations were substantially greater in the participants with diabetes (OR 12.56, 95% CI 2.88–54.83, $p < 0.001$) and that cortisol concentrations subsequently fell across the task period (Figure 3.3 and Table 3.3 and 3.4). Consequently, the groups differed in cortisol responses to stress, with increases in the control group and decreases in the diabetes group (OR 0.01, 95% CI 0.00–0.10, $p < 0.001$). The groups converged over the post-task recovery period.

Plasma IL-6 concentration was higher in the diabetes group at baseline ($p = 0.014$). We recorded increases in IL-6 following stress in both groups, but the increase was blunted in the diabetes group both at 45 min (OR= 0.20, 95% CI 0.47 – 0.85, $p = 0.029$) and 75 min (OR 0.33, 95% CI 0.11 - 0.96, $p = 0.042$) after tasks. Nonetheless, the concentration of IL-6 remained higher in absolute terms in the diabetes than control groups.

Baseline cholesterol was significantly lower in the diabetes than the control group (adjusted odds of being in the diabetes group per unit mmol increase in cholesterol = 0.29; 95% CI 0.13 – 0.62, $p = 0.002$). The profile of total cholesterol stress responses was again consistent with high allostatic load (Figure 3.3), since the rise in cholesterol with stress was blunted in the diabetes group (OR = 0.001, 95% CI 0.000–0.008, $p = 0.003$), whereas the recovery following stress was reduced (OR 0.018, 95% CI 0.002–0.16, $p = 0.05$). Sex did not interact with diabetes status in any of these analyses.

Figure 3.3 Cortisol, IL-6 and cholesterol across the laboratory session



Mean cortisol (upper), plasma IL-6 (centre), and total cholesterol (lower) in the diabetes (red line) and control (blue line) groups. Cortisol values adjusted for education, BMI, smoking status, use of statins, and time of testing. IL-6 was adjusted for education, BMI, smoking, and time of testing. Total cholesterol was adjusted for education, BMI, smoking, aspirin, use of statins, and time of testing. P values indicate group differences in stress reactivity (baseline–task difference) and post-stress recovery (task–post-task difference) as detailed in Table 3.3 and 3.4. Error bars are SEM.

3.4.4. Stress-related psychological factors

We measured stress-related psychological factors using standardized questionnaires as detailed in Chapter 2. The participants with diabetes showed a higher stress profile than controls in terms of negative emotional responses and greater reported stress experience (Table 3.5). They reported significantly greater hostility, more depressive symptoms, less optimism and more financial strain than the healthy control group. There was a trend for greater loneliness in the diabetes group but this did not reach significance ($p = 0.053$). We did not observe any interactions between sex and diabetes status in these analyses.

Table 3.5 Psychosocial factors in the diabetes and control groups

	Diabetes (n = 140)	No diabetes (n = 280)	Odds of diabetes* (95% CI)	Group difference (p value)
Hostility	3.78 ± 2.8	2.72 ± 2.4	1.19 (1.09- 1.29)	< 0.001
Depression	11.86 ± 8.9	6.47 ± 6.6	1.12 (1.08- 1.16)	< 0.001
Optimism	14.40 ± 4.3	15.5 ± 3.8	0.93 (0.89- 0.98)	= 0.008
Loneliness	37.19 ± 12.0	35.03 ± 10.8	1.01 (1.00- 1.03)	= 0.053
Financial strain	84 (71.2%)	122 (43.7%)	3.56 (2.14- 5.92)	< 0.001

Values are presented as n (%) and means ± standard deviations

*Unadjusted odds of being in the diabetes group per unit change in the independent variable

3.4.5. Health Behaviours

Alcohol consumption and smoking were measured by self-report and sleep problems were assessed using a standardized questionnaire. Objective physical activity was assessed over a week using an actigraph. Further detail on these measures can be found in Chapter 2. A group comparison of the health behaviours can be found in Table 3.6. The participants with diabetes reported greater sleep difficulties over the previous month ($p = 0.001$) and higher levels of smoking than the control group ($p = 0.011$). Alcohol consumption was greater in the control group, significantly more controls reported consumption above recommended weekly amounts ($p < 0.001$) and more of the diabetes group reported no alcohol consumption (35.6% vs 17.9%, $p < 0.001$; detail not in Table). With regards to physical activity, individuals with diabetes engaged in less light activity and were more sedentary than the controls ($p < 0.001$). A sensitivity analysis was conducted to ascertain whether these health behaviours impacted the physiological stress response findings. The physiological stress reactivity or the post-stress recovery results across all of the biological systems were not attenuated by the inclusion of these individual health factors as covariates.

Table 3.6 Health behaviours in the diabetes and control groups

	Diabetes (n = 140)	No diabetes (n = 280)	Odds of diabetes* (95% CI)	Group difference (p value)
Sleep problems	2.85 ± 1.35	2.37 ± 1.10		< 0.001
Physical activity (min/day) †				
Moderate	31.90 ± 24.49	35.98 ± 25.27	0.99 (0.89-1.00)	=0.173
Light	184.11± 69.75	208.92 ± 63.42	0.99 (0.990-1.0)	< 0.001
Sedentary	659.83±108.0	637.96 ± 77.55	1.01 (1.00-1.01)	< 0.001
Alcohol > guideline	9 (6.7%)	33 (11.8%)	0.45 (0.29-0.68)	< 0.001
Smoking (% yes)	20 (14.4%)	18 (6.4%)		= 0.011

Values are presented as n (%) and means ± standard deviations

*Unadjusted odds of being in the diabetes group per unit change in the independent variable

† Physical activity adjusted for registered actigraph wear time, other analyses are unadjusted

3.5. Intercorrelation between responses in different systems

For the most part there were significant associations between responses in the different systems monitored in this study. SBP, DBP, heart rate and cardiac index were correlated positively with each other (Table 3.7). However, no consistent relationship was detected between HRV and the other cardiovascular measures. Heart rate and cardiac index were not related to HRV but SBP and DBP stress reactivity (baseline–task difference) and post-stress recovery (task–45 minute post-task difference) were positively associated with HRV post-stress recovery (Table 3.7).

All of the cardiovascular stress reactivity measures were negatively correlated with baseline IL-6 concentrations (Table 3.8) but no associations between the cardiovascular measures were found for IL-6 reactivity and recovery values. SBP, DBP, heart rate and cardiac index were positively related to cortisol responses to mental stress

(Table 3.8). No association between HRV and cortisol was detected. SBP, DBP, heart rate and cardiac index reactions to tasks were positively related to cholesterol responses to mental stress and post-stress recovery. HRV post stress recovery (task–75 minute post-task difference) was positively associated with cholesterol (Table 3.8).

Table 3.7 Intercorrelations between the different cardiovascular measures

	SBP task	SBP 45 mins	SBP 75 mins	DBP task	DBP 45 mins	DBP 75 mins	Heart rate task	Heart rate 45 min	Heart rate 75 min	CI task	CI 45 mins	CI 75 mins	HRV task	HRV 45 mins	HRV 75 mins
SBP task	1	.674**	.716**	.863**	.506**	.494**	.454**	.396**	.409**	.290**	.267**	.298**	-.027	.112*	.069
SBP 45 mins	.674**	1	.811**	.564**	.848**	.658**	.435**	.421**	.417**	.260**	.178**	.220**	.061	.177**	.027
SBP 75 mins	.716**	.811**	1	.595**	.696**	.804**	.456**	.428**	.432**	.280**	.194**	.225**	.053	.080	.016
DBP task	.863**	.564**	.595**	1	.510**	.500**	.385**	.344**	.353**	-.039	.125*	.178**	-.047	.138**	.087
DBP 45 mins	.506**	.848**	.696**	.510**	1	.813**	.345**	.381**	.353**	.118*	.117*	-.020	.091	.162**	.025
DBP 75 mins	.494**	.658**	.804**	.500**	.813**	1	.332**	.349**	.363**	.112*	-.076	.123*	.106*	.028	-.009
Heart rate task	.454**	.435**	.456**	.385**	.345**	.332**	1	.863**	.873**	.568**	.445**	.447**	-.055	-.004	-.004
Heart rate 45 mins	.396**	.421**	.428**	.344**	.381**	.349**	.863**	1	.909**	.481**	.473**	.437**	.014	.017	.013
Heart rate 75 mins	.409**	.417**	.432**	.353**	.353**	.363**	.873**	.909**	1	.477**	.456**	.442**	-.008	.021	.025
CI task	.290**	.260**	.280**	-.039	.118*	.112*	.568**	.481**	.477**	1	.575**	.541**	-.061	-.018	.010
CI 45 mins	.267**	.178**	.194**	.125*	.117*	-.076	.445**	.473**	.456**	.575**	1	.847**	-.086	-.003	.043
CI 75 mins	.298**	.220**	.225**	.178**	-.020	.123*	.447**	.437**	.442**	.541**	.847**	1	.117*	.075	.053
HRV task	-.027	.061	.053	-.047	.091	.106*	-.055	.014	-.008	-.061	-.086	.117*	1	.493**	.554**
HRV 45 mins	.112*	.177**	.080	.138**	.162**	.028	-.004	.017	.021	-.018	-.003	.075	.493**	1	.653**
HRV 75 mins	.069	.027	.016	.087	.025	-.009	-.004	.013	.025	.010	.043	.053	.554**	.653**	1

Values presented are differences in stress reactivity (baseline–task difference) and post-stress recovery (task–post-task differences)

Note: In this table for presentation purposes cardiac index is abbreviated to CI. CI continues to refer to confidence interval elsewhere in this thesis.

* Correlation is significant at the 0.05 level ** Correlation is significant at the 0.01 level

Table 3.8 Intercorrelations across different systems

	IL-6 baseline	IL-6 45 min	IL-6 45 min	Cortisol baseline	Cortisol 20 min	Cortisol 45 min	Cortisol 75 min	Cholesterol baseline	Cholesterol task	Cholesterol 45 min
SBP task	-.174**	-.034	-.042	-.039	.207**	.110*	.167**	.121*	.336**	.278**
SBP 45 min	-.174**	-.011	-.033	-.048	.220**	.075	.140**	.135**	.271**	.277**
SBP 45 min	-.174**	-.011	-.033	-.048	.220**	.075	.140**	.145**	.307**	.328**
SBP 75 min	-.182**	-.011	-.044	-.057	.236**	.084	.161**	.086	.269**	.234**
DBP task	-.125*	-.016	-.004	-.008	.132**	.104*	.135**	.143**	.194**	.215**
DBP 45 min	-.183**	.010	-.003	-.035	.174**	.062	.111*	.152**	.219**	.229**
DBP 75 min	-.172**	.013	-.023	-.055	.174**	.072	.121*	.144**	.283**	.308**
Heart rate task	-.159**	-.005	-.039	-.105*	.200**	.023	.042	.153**	.266**	.268**
Heart rate 45 min	-.153**	.046	.006	-.131*	.212**	.065	.100	.124*	.306**	.261**
Heart rate 75 min	-.140**	.039	-.014	-.093	.166**	.059	.107*	.127*	.216**	.250**
CI task	-.156**	-.068	-.020	-.109*	.201**	.001	.082	.018	.168**	.209**
CI 45 min	-.013	-.046	-.057	-.038	.100	.016	.027	.012	.190**	.203**
CI 75 min	-.023	-.045	-.062	-.009	.101	-.010	.014	.063	.058	.104
HRV task	.096	.046	.009	-.017	.026	.003	.012	.021	.045	-.014
HRV 45 min	-.102*	.017	.024	-.017	-.005	.033	.073	.000	-.056	-.078
HRV 75 min	-.153**	.017	.040	.003	-.045	.064	.055	.121*	.336**	.278**

Values presented are differences in stress reactivity (baseline–task difference) and post-stress recovery (task–post-task differences)

Note: In this table for presentation purposes cardiac index is abbreviated to CI. CI continues to refer to confidence interval elsewhere in this thesis.

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

3.6. Discussion

3.6.1. Results summary

This study explored the hypothesis that people with T2D experience chronic allostatic load, manifest in alterations in dynamic physiological responses to standardized mental stress and greater psychological distress and experience of chronic life stress, in comparison with age, sex, and income matched controls. We found that post-stress recovery was attenuated in the diabetes group in SBP and DBP, heart rate, cardiac index and total cholesterol, together with blunted stress reactivity in SBP, DBP heart rate, cardiac index, cortisol, and cholesterol concentration. These effects were independent of covariates including medication and were evident in both men and women. Additional sensitivity analyses adjusting for health behaviours did not alter the pattern of results. The acute increases in cholesterol concentration are likely to be due in part to reductions in blood volume following stress, leading to greater hemoconcentration. Plasma IL-6 concentration was higher in people with T2D, so that although the increases following mental stress were smaller than those of controls, absolute levels remained higher. The diabetes group had higher baseline cortisol than controls in the laboratory. Contrary to the hypothesis no associations were detected for HRV. With regards to psychosocial stress, we found that participants with diabetes had more depressive and hostile symptoms, and reported greater chronic stress in terms of financial strain.

3.6.2. Earlier studies of allostatic load and diabetes

The multisystem measures of allostatic load described in the literature typically involve factors taken under resting conditions and include several components of the metabolic syndrome such as elevated BP, triglycerides, and waist circumference (McCaffery, Marsland, Strohacker, Muldoon, & Manuck, 2012). The clustering of risk factors in the

metabolic syndrome has been associated with increased T2D risk in initially healthy populations (International Diabetes Federation, 2006; Stern, Williams, González-Villalpando, Hunt, & Haffner, 2004). Therefore, it is surprising that allostatic load as a broader, cumulative measure of multiple health risk factors has not been more widely researched in relation to T2D.

One cross-sectional study of middle-aged Puerto Ricans living in Boston showed that greater allostatic load was associated with increased risk of diabetes and other chronic conditions (Mattei et al., 2010). This study had over 1000 participants and included 10 parameters of biological functioning to calculate a cumulative measure of allostatic load. The analysis was robust to adjustment for a range of demographic and lifestyle factors including smoking, alcohol consumption and physical activity. However, the study was not without limitations. It did not have an exclusive focus on diabetes and less than 40% of the sample had glucose values in the diabetic range. The sample consisted of Puerto Ricans living in the US so the results may not be generalizable to other ethnic groups. Additionally, convenience sampling was used and it is possible that selection bias may have influenced the results as the study as recruitment was limited to areas known to have high concentrations of Hispanic residents. Additionally, the authors postulate that living as a migrant and a member of ethnic minority group in the US might be a chronic stressor. However, chronic life stress factors were not assessed in the study so this hypothesis could not be directly tested.

Another investigation focusing specifically on diabetes failed to find that the components of allostatic load clustered reliably in people with diabetes (Carlsson et al., 2011). This study was limited by a small sample size of 53 (of whom 45 were retained for the final analysis), as well as a convenience recruitment method by which the

participants were drawn from an RCT assessing the benefits of massage in people with T2D. The statistical analysis performed was also questionable as the authors had longitudinal data at 3 time-points but chose to analyse the data as cross-sectional snapshots rather than using appropriate repeated measures longitudinal statistics. Additionally, the analyses were poorly controlled as sex was the only covariate considered.

Both of these previous studies assessed allostatic load at rest and the results of the present study suggest that attention to the dynamic aspects of allostatic load may be fruitful. The review of the published literature conducted for Chapter 1 found no previous studies that have examined the dynamic responses to mental stress across multiple biological systems in T2D. One small study of 30 men with diabetes and 30 controls found that SBP responses to a mental arithmetic task were elevated and HRV responses blunted in participants with diabetes compared with the controls (Deepak et al., 2014). No differences for DBP or heart rate were detected. The elevated SBP responses to stress in this study could be indicative of the ‘prolonged response’ component of allostatic load whereas the blunting in HRV could reflect the ‘inadequate response’ aspect of allostatic load (McEwen, 1998). However, the results should be interpreted with caution due to numerous study limitations. These shortcomings were addressed in Chapter 1, but to reiterate in brief; the control group was weak due to the exclusion of individuals who took regular exercise, consumed alcohol or smoked. No covariates were included in the statistical analysis and BP was only assessed twice (once at rest and once immediately post-task) rather than continuously over the course of the laboratory session, meaning the pattern of responsivity and post-stress recovery could not be reliably assessed. Furthermore, this paper was published in a journal with a

doubtful reputation, and therefore the stringency of the peer-reviewing process is not certain.

3.6.3. Blunted cardiovascular stress reactivity

In the present study cardiovascular factors were continuously assessed with a Finometer which has been shown to have good reproducibility and accuracy for cardiovascular monitoring in a range of settings (Castiglioni et al., 1999; Imholz et al., 1993). Additionally, the statistical analysis performed was appropriate for a matched sample and our models were well-adjusted. Disturbances in stress reactivity and post-stress recovery reflected by blunted stress-reactivity and poorer recovery post-stress were detected for SBP, DBP, heart rate and cardiac index in the diabetes group. Although ‘inadequate responses’ are a recognised component of allostatic load (McEwen, 1998) much research has focused on heightened stress reactivity and its association with health risk (Phillips, Ginty, & Hughes, 2013). In turn, the natural corollary of this idea is the assumption that blunted cardiovascular stress reactivity may be beneficial to health (Phillips, 2011). Of particular relevance to the present sample is meta-analytic evidence that suggests exaggerated cardiovascular responses to stress are associated with increased risk of future CVD (Chida & Steptoe, 2010). Considering the greatly heightened risk CVD in people with diabetes (e.g. Emerging Risk Factors Collaboration et al., 2010; for greater detail see Chapter 1 section 1.4.2), the blunted responsiveness observed in the participants with diabetes may seem paradoxical.

Some studies published since the Chida & Steptoe, (2010) meta-analysis have reported associations between cardiovascular risk factors and blunted stress cardiovascular reactivity and a review of this area was conducted in 2013 (Phillips et al., 2013). To give a few examples, depression is a significant CVD risk factor (Nicholson

et al., 2006; Roest et al., 2010) and the association between depression and reactivity to an acute stress task was studied in the West of Scotland Twenty-07 cohort. In this analysis of 1647 participants depression was associated with reduced SBP and heart rate reactions to acute mental stress after adjusting for a range of covariates including BMI and anti-hypertensive medication. Furthermore, attenuated heart rate reactivity was prospectively related with increased depressive symptomatology 5 years later in the same cohort (Phillips, 2011). Similarly, in a study of 725 middle-aged adults from the Dutch Famine Birth cohort, depression and anxiety (both CVD risk factors) were associated with blunted SBP and heart rate stress reactivity (de Rooij, 2013). Other psychosocial stress factors such experiences of adversity (Lovallo, Farag, Sorocco, Cohoon, & Vincent, 2012) and the personality trait neuroticism (Bibbey, Carroll, Roseboom, Phillips, & de Rooij, 2013) have also been linked with attenuated cardiovascular reactivity. An earlier meta-analysis also associated anxiety, neuroticism and negative affectivity with reduced cardiovascular reactivity to stress and poorer post-stress recovery (Chida & Hamer, 2008).

The paragraph above should not be considered a thorough review of studies that have associated blunted cardiovascular stress reactivity with risk factors and the relevance of these studies to the present analysis is somewhat limited as we focused on a diseased sample with matched controls, rather than on cohorts of relatively healthy individuals. However, studies such as these offer an important demonstration that blunted cardiovascular reactivity to stress has previously been associated with health risk factors.

Studies with CVD patients have linked both heightened stress reactivity and blunted stress reactivity with poor cardiovascular prognosis. For example, a recent study in the Netherlands evaluated cardiovascular reactivity to stress in 100 individuals with

heart failure and assessed the potential relationship between laboratory stress reactivity and future mortality (Kupper, Denollet, Widdershoven, & Kop, 2015). Over 4 years of follow-up, mortality rates in the lowest DBP reactivity group were 2 times higher than in those in moderate DBP reactivity group (HR: 2.04, 95% CI 1.15 - 3.60) adjusting for age, baseline BP and implanted devices. A similar pattern emerged for SBP and future mortality but did not reach significance. Another study assessed cardiovascular responses to acute mental stress in 521 coronary artery by-pass patients 6 months after they underwent surgery (Herd et al., 2003). The authors found that that blunted SBP, DBP and heart rate responses to stress were associated with a 2-fold increase in clinical cardiovascular events during the 3 year follow-up period. The association was robust to adjustment for covariates including ejection fraction, age, sex, and prior MI. Contrary to these findings in a study of 79 patients with stable CAD, heightened DBP responsivity was linked with an increased incidence of future cardiac events over an average of 3.5 years follow-up (Krantz et al., 1999). No associations emerged for SBP and heart rate reactivity. It is important to consider studies that have associated exaggerated responsivity with health risk as well as studies that have similarly to the current study shown blunted stress reactivity in a diseased population. That both heightened and blunted responses have been observed adds credence to the theory of allostatic load which suggests that exaggerated responses, prolonged responses and diminished responses represent significant deviation from the physiological norm and therefore are potentially damaging to health (McEwen & Wingfield, 2003; McEwen, 1998).

In the present analysis, contrary to prediction no associations were found for HRV. Lowered HRV is an early indicator of cardiovascular autonomic neuropathy, one of the most serious complications of diabetes (Pop-Busui, 2010, 2012; Vinik & Ziegler, 2007). Baseline HRV values were lower in the diabetes group than in the controls but

the difference was not statistically significant. The reason why we detected blunting in the diabetes participants for the other cardiovascular measures but not for HRV is unclear. One possibility is that our measure of HRV was weak. We assessed HRV by looking at the standard deviation of heart rate inter-beat-intervals on the Finometer. Using this equipment we were unable to assess the numerous components of the time and frequency domains of HRV and specific aspects of these domains such as the respiratory sinus arrhythmia of the time domain have been shown to be relevant to diabetes (Masi, Hawkey, Rickett, & Cacioppo, 2007). Future research should be conducted using electrocardiogram monitors which are designed to measure these components.

3.6.4. Cortisol and IL-6 responses to stress

We found that cortisol stress reactivity was blunted in the diabetes group in comparison to the controls and that IL-6 was heightened in the participants with diabetes throughout the laboratory session. When conducting the literature review for Chapter 1 no previous studies assessing cortisol and IL-6 responses to laboratory stress in people with T2D were found. Blunted cortisol responses to stress have been associated with health risk factors in relatively healthy samples (Phillips et al., 2013). Considering the present study sample, research on individuals with CVD might have more weight than research in healthy individuals. To date, two studies assessing cortisol responses to stress have been conducted with CVD patients. The most recent of these assessed cortisol responses to the Trier Social Stress test in 91 participants, 46 of whom had CAD (Waller et al., 2016). The results of the study suggested that the participants with CAD had blunted cortisol stress reactivity compared with controls, after adjustment for age, sex, BMI and medications. In the other study both cortisol and inflammatory responses to stress were

assessed in 30 CAD patients and patterns of responsivity were compared with those of healthy age and sex matched controls (Nijm, Kristenson, Olsson, & Jonasson, 2007). Stress response were elicited using an acute physical stressor (exercise test), as well as two psychological stress tasks (anger-recall and mental arithmetic). The CAD patients were found to have significantly blunted cortisol reactivity in comparison to the controls during both the physical and psychological stress tasks. This association was robust to adjustment for potential confounders such as smoking, beta-blocker usage and statin therapy. Additionally, the patients experienced a stress-related increase in CRP 24 hours after stress testing which was not observed in the control group.

The combination of blunted cortisol and heightened inflammation was also found in the present study. The regulatory role of cortisol on inflammation could account for these findings. Typically cortisol has an inhibitory effect on pro-inflammatory cytokine production (Miller et al., 2007). However, long-term heightened cortisol concentrations may result in dysregulation of this system manifested through insufficient glucocorticoid signalling (Miller et al., 2007; Raison & Miller, 2003). In keeping with this idea, a sub-analysis of 37 participants with diabetes and 37 controls from this study found that glucocorticoid sensitivity was reduced in response to stress in individuals with diabetes (Carvalho et al., 2015). This blunting of cortisol may have a permission effect on inflammatory markers such as IL-6, allowing concentrations to rise (Miller et al., 2007; Raison & Miller, 2003). The high IL-6 concentration is likely related to the role of inflammation in T2D, promoting insulin resistance and dyslipidaemia by inhibiting enzymes involved in fatty acid oxidation, down-regulating the expression of genes involved in insulin-stimulated glucose transport and lipid uptake in adipocytes (Pickup, 2004; Tilg & Moschen, 2008). Greater detail on the role of

inflammation in T2D can be found in section 1.7.4 of Chapter 1. Further attention to the potential role of cortisol in diabetes will be given in later chapters.

3.6.5. Alternative explanations

Apart from heightened dynamic allostatic load, one alternative explanation for our results is that people with diabetes were less stressed by the behavioural challenges, so they produced smaller biological responses; however, the subjective ratings indicate that the diabetes and control groups were stressed to the same extent by the tasks. Lack of task engagement by the diabetes group is another possible explanation for the observed low stress reactivity. Low engagement might indicate that people who are low reactors intentionally hold back from fully engaging with the tasks to avoid a stressful experience. One relevant theory in this vein concerns central motivational dysregulation (Carroll, Lovallo, & Phillips, 2009; Lovallo, 2011; Phillips et al., 2013). It has been suggested that attenuated stress reactivity may be a peripheral marker of disengagement in the neural systems that support motivation. Areas within the greater amygdala system of the brain are implicated in the both regulation of stress responses and behaviour motivation. Neuroimaging studies investigating brain activity in relation to cardiovascular stress responsivity have found that subjects who display attenuated cardiovascular reactions have corresponding blunted neural reactions in the greater amygdala system (Gianaros et al., 2008; Gianaros, May, Siegle, & Jennings, 2005). This suggests that blunted stress reactivity is associated with compromised activity in the brain areas that support motivation. However, in the current study the groups did not differ in their ratings of task involvement (data not shown in results $p = 0.185$). Indeed, other studies that have reported blunted biological reactions to stress have also demonstrated that their findings were independent of participant ratings of task stress

and task engagement, as well as objective task performance scores (Phillips et al., 2013). Perceptions of task difficulty has also been put forward as explanations for blunted reactivity (Phillips et al., 2013). However, the groups did not differ in their assessment of the difficulty of the tasks in the present study (data not shown in results $p = 0.374$). Taken together, although our groups did not differ in their assessment of task difficulty, task engagement or perceived stress, it is important to collect this information to limit this sort of confounding.

Another possibility is that effects were influenced by the multiple medications used to control diabetes. These medications may have contributed to the low baseline levels of DBP and total cholesterol observed in the diabetes group, as anti-hypertensive medications are known to affect sympathetic reactivity to stress, though drug classes differ in effect (Lefrandt et al., 2001; Nazzaro, Merlo, Manzari, Cicco, & Pirrelli, 1993). A number of previous studies have found no effect of β -blockers on cardiovascular responses to stress (see Mills & Dimsdale, 1991 for a review). Similar to some other stress reactivity research studies (Bibbey et al., 2013; Carroll, Phillips, & Der, 2008; de Rooij, 2013; Nijm et al., 2007; Phillips, 2011; Phillips, Roseboom, Carroll, & de Rooij, 2012; Waller et al., 2016) we took account statistically of medications to limit confounding from these factors. Nevertheless, an effect of medication of stress reactivity cannot be excluded.

Finally, it is conceivable that the differences in the cardiovascular measures were early manifestations of neuropathy in people with diabetes, as the autonomic dysfunction of cardiovascular autonomic neuropathy can cause blunting of SBP, DBP and cardiac output responsivity (Sacre et al., 2010; Vinik & Ziegler, 2007). It could be that chronic life stress experienced by the participants with T2D leads to altered stress reactivity which might predispose them to an increased risk of diabetes complications.

3.6.6. Psychosocial differences between the groups

Moving on from the disturbances in biological stress reactivity, we also observed differences between the diabetes and control groups in self-reported emotional distress and chronic life stress. A review of the literature on the role of psychosocial factors in diabetes can be found in Sections 1.5.2 and 1.5.3 of Chapter 1. Our observation that depressive symptoms were elevated in people with T2D replicates previous research (Ali, Stone, Peters, Davies, & Khunti, 2006; Mezuk, Eaton, Albrecht, & Golden, 2008 etc.) and is important in light of the evidence that depression in diabetes is associated with a greatly increased risk of CVD (Lin et al., 2010; Scherrer et al., 2011 etc.) and increased mortality risk (Hofmann et al., 2013; Park et al., 2013). The participants with diabetes also reported higher levels of hostility and low levels of optimism than the control group. There was a trend for greater loneliness in the diabetes group. Additionally, the finding that individuals with diabetes reported greater financial strain could be reflective of chronic stress in everyday life.

Taken together, the results suggest a profile of psychosocial adversity in the T2D group that is likely to promote heightened allostatic load. However, it must be acknowledged that this was not directly assessed in the present study. To investigate the relationships between these various psychosocial factors and their impact on the disturbances across the multiple biological systems in the diabetes and control groups was beyond the scope of this PhD thesis. The following chapter will address this issue on a small scale by focusing on one psychosocial factor and investigating associations with responsivity and recovery across the laboratory session in the diabetes group.

Additionally, the available health behaviour data for the groups were analysed. We found that participants with diabetes were more likely to smoke and reported greater

levels of sleep disturbance than controls. As previously reported the diabetes group were less active and spent longer periods of time in engaging in sedentary behaviour (Hamer et al., 2013). The groups differed in alcohol consumption, but interestingly the healthy control group was more likely to drink excessively than the diabetes group. More participants with diabetes reported no alcohol consumption in the previous week. This is in keeping with the so-called 'sick quitter effect', that is that individuals with a longstanding illness are known to consume less alcohol (Ng Fat, Cable, & Shelton, 2015). The differences between the groups in loneliness and financial strain could also offer an explanation, as the drinking of alcohol often occurs in social setting and alcohol is expensive to purchase.

Smoking and BMI were controlled for in all the stress reactivity and recovery analyses as these factors have previously been shown to affect stress responsivity (e.g. Evans et al., 2012; Jones et al., 2012; Phillips, Der, Hunt, & Carroll, 2009; Phillips et al., 2012; Steptoe & Wardle, 2005), though the direction of the relationship across studies has not been consistent. Physical activity, alcohol consumption and sleep have also been shown to impact physiological responses to laboratory stress (Evans et al., 2012; Hamer, Taylor, & Steptoe, 2006; Heffner et al., 2012). However, sensitivity analysis including the other health behaviours measures as covariates did not alter the pattern of results.

3.6.7. Intercorrelations between the different systems

Measures of allostatic load have been criticized for bringing together an arbitrary set of biomarkers, assuming that extreme values load on an underlying unitary construct. Recent factor analytic studies indicate that a single common factor underlies variation across autonomic, neuroendocrine, inflammatory, and metabolic processes (Booth,

Starr, & Deary, 2013; McCaffery et al., 2012). Another concern is that different studies use different biomarkers to measure allostatic load and as such there is no standardised approach as to what factors to include (Beckie, 2012). Despite this issue, allostatic indices of dysregulation across multiple biological systems have been shown to have greater predictive value for health outcomes than any single biological predictor alone in multiple studies (Juster et al., 2010; Karlamangla et al., 2014; Seeman et al., 2001). In the current study the majority of the factors correlated together and the intercorrelations between responses in the different biological systems provide support for the value of this approach.

3.6.8. Limitations

The study was cross-sectional, so no causal conclusions can be drawn. It is possible that heightened allostatic load precedes the development of T2D and is a mechanism through which psychosocial factors contribute to diabetes risk. As discussed in Chapter 1, there are direct effects of inflammation on β cells (Kahn et al., 2014), and meta-analytic evidence indicates that heightened inflammation predicts diabetes onset in initially healthy populations (Wang et al., 2013). With regards to cardiovascular measures, high BP is recognised to be a risk factor for T2D (Emdin et al., 2015; International Diabetes Federation, 2015) and long-term cortisol excess as seen in Cushing's syndrome (Newell-Price et al., 2006) and in glucocorticoid-treated patients (Clare & Thurby-Hay, 2009) increases susceptibility for hyperglycaemia and manifest T2D. Pathological (Clayton et al., 2011) and experimental (Connell et al., 1987) exposure to excessive cortisol is related to metabolic disturbances such as hypertension, abnormal glucose metabolism and central obesity, all of which are risk factors for T2D (Anagnostis et al., 2009). Studies of heightened dynamic allostatic load in people with

insulin resistance but no diabetes would help to elucidate whether heightened allostatic load precedes the development of diabetes.

The alternative is that allostatic load is a manifestation of diabetes that is secondary to the abnormalities of glucose metabolism. The cardiovascular, neuroendocrine, and inflammatory responses we observed may be significant for the broader health consequences of diabetes. Disturbances of cortisol regulation are apparent in CHD and depression (Brotman et al., 2007; Stetler & Miller, 2011), and it has been argued that glucocorticoids may contribute to the development of cognitive impairment in people with diabetes (Strachan, Reynolds, Frier, Mitchell, & Price, 2009). Chronic systemic inflammation is also involved in CVD, dementia, and depression (Hansson, 2005; Slavich & Irwin, 2014). There is evidence that inflammation might play a role in linking T2D with increased incidence of CHD. For example, the atherosclerotic plaques of people with diabetes have a higher expression of inflammatory receptors and proteins and greater inflammatory cell infiltration compared to people without T2D (Burke et al., 2004; Marfella et al., 2006; Moreno et al., 2000). In population studies inflammatory factors have been more strongly correlated with sub-clinical atherosclerosis in people with T2D than those without the condition (Metcalf et al., 2000). Additionally, heightened concentrations of inflammatory markers have also been prospectively related to cardiac events in people with T2D (e.g. Kengne, Czernichow, Stamatakis, Hamer, & Batty, 2013; Kengne, Batty, Hamer, Stamatakis, & Czernichow, 2012; Lowe et al., 2014 etc.). A more detailed review of this literature can be found in Section 1.7.4.1 of Chapter 1.

Our results are consistent with the possibility that some of the comorbidities of T2D arise from the disruption of the multiple systems involved in allostatic load rather than being direct consequences of impaired glucose regulation. The dynamic aspects of

multisystem dysregulation in allostatic load have not been studied prospectively in relation to long-term health outcomes. However, other measures of allostatic load have been associated with a range of adverse health outcomes. For example, in the MacArthur cohort of older American adults at 3 year follow-up high allostatic load was associated with an increased risk of CVD (Seeman et al., 2001) and after a 7 year follow-up allostatic load was associated with all-cause mortality independent of socio-demographic factors and baseline health status. Subsequent prospective research has also found an association between high allostatic load and future all-cause mortality (Crimmins et al., 2009; Karlamangla et al., 2006). Higher allostatic load has been associated prospectively with disability and functional limitations (Gruenewald et al., 2009; Karlamangla et al., 2002; Read & Grundy, 2014) as well as cognitive impairment in older adults (Karlamangla et al., 2014). Individual components such as reduced cardiovascular post-stress recovery, blunted stress reactivity, and increased IL-6 during acute stress are also associated with future adverse health outcomes (Chida & Hamer, 2008; de Rooij, 2013; Phillips, 2011).

In addition to the cross-sectional design, other limitations of this study are that the diabetes group was more ethnically diverse than the control group. There were 28 (20%) ethnic minority participants in the diabetes group, whereas the controls were all of white ethnicity. Adding ethnicity as a factor to the analyses did not alter the pattern of results so it was not included in the final models. However, T2D prevalence is known to vary significantly by ethnicity, with diagnoses reported to be over 2 times more common in South Asian and Black groups (Tillin et al., 2013). With our predominately white sample we were unable to tease out effects of ethnicity on group responsivity and recovery from stress. Additionally, the assessment of stress responses was carried out over a single session in this study. Although the stress tasks used in this study have been

shown to have robust reproducibility over repeated administrations (Hamer, Gibson, Vuononvirta, Williams, & Steptoe, 2006), it is plausible that our results reflect situational rather than chronic processes. Further, we did not measure glucose and insulin responses across the mental stress session and we therefore were unable to assess the potential effect of these processes on reactivity and recovery in the other biological systems and whether this differs by group status. The study was limited by using self-report measures to assess all of the psychological factors and the majority of the health behaviours. More thorough assessment of psychosocial factors such as depression could be improved by assessing emotional distress using a clinical interview. Our measure of alcohol consumption was a quite rough estimate could have been affected by retrospective bias. A thorough calculation of weekly alcohol consumption (we were unable to assess alcohol volume and quantity) would have improved the current study.

3.6.9. Conclusion

Despite these limitations, our observations provide fresh evidence to link epidemiological studies implicating stress-related processes with biological dysfunction in T2D. The patterns of cardiovascular and cholesterol responses exemplify the disturbances of reactivity and recovery noted in McEwen's model of allostatic load (McEwen, 1998). It has been posited that high allostatic load leads to prolonged responses due to delayed shutdown of physiological reactivity, so post-stress recovery is impeded. Blunted reactivity may also occur, resulting in compensatory hyperactivity in other mediating pathways (Miller et al., 2007). The allostatic load concept synthesizes diverse findings concerning stress-related dysregulation across cardiovascular, neuroendocrine, metabolic, and inflammatory systems. Our findings highlight the

importance of moving beyond glucose regulation to address a range of disturbances across multiple systems. Although individual pathways can be targeted by pharmacotherapy, the allostatic approach implies that systems interact in a dynamic fashion. Interventions that affect both brain and body are likely to be particularly beneficial. Two promising candidates are physical activity and stress modification, both of which are implicated in diabetes (Sluik et al., 2012). Development of the allostatic approach to T2D may open new avenues for pharmacological and social-behavioural approaches to management and prevention.

4. Study 2: Hostility and physiological responses to acute stress in T2D

4.1. Overview

The study presented in Chapter 3 assessed physiological responses to laboratory stress, as well as self-reported measures of psychosocial stress in everyday life in people with diabetes and matched healthy controls. The results of this study suggest that people with T2D experience disturbances in physiological stress reactivity and post-stress recovery and report greater psychosocial distress than controls. In this chapter, rather than comparing those with diabetes to healthy individuals, the focus is on the diabetes group alone. Psychosocial factors are known to impact responses to stress and in the study presented below the effect of a psychosocial factor of interest on physiological stress reactivity in people with diabetes will be assessed. Hostility was selected as the psychosocial factor of choice and justification of this and an introduction to the study will be presented below. This will be followed by the results of the study and discussion of the findings.

Note: The results presented in this chapter have been published in Hackett, Lazzarino, Carvalho, Hamer, & Steptoe, (2015). For the purpose of this PhD additional analyses were conducted assessing associations between hostility and cardiac index and HRV stress responsivity.

4.2. Introduction

In Chapter 3 we found that in comparison to age sex and income matched controls, the participants with diabetes reported significantly higher levels of depression and hostility and lower levels of optimism. Depression is the most well researched psychosocial factor in the diabetes field and numerous prospective studies and meta-analyses have been conducted associating depression with new-onset diabetes and poorer outcomes in individuals with an existing diagnosis (Anderson, Freedland, Clouse, & Lustman, 2001; Mezuk, Eaton, Albrecht, & Golden, 2008; Rotella & Mannucci, 2013; Scherrer et al., 2011). Other psychosocial factors such as hostility and optimism¹ have received less attention.

Hostility is a trait that is typically conceptualised as a negative cynical attitude towards others, with a propensity for anger or aggression (Cook & Medley, 1954). Several studies have identified hostility as an independent risk factor for all-cause mortality (Klabbers, Bosma, Akker, Kempen, & Eijk, 2012). In particular, hostility has been suggested to play a role in CVD.

Results from a meta-analysis of prospective cohort studies indicate that hostility is associated with an increased risk of CVD in initially healthy populations, as well as poorer prognosis in CVD patients (Chida & Steptoe, 2009b). There is evidence that acute episodes of anger can trigger MI and sudden cardiac death (Mostofsky, Maclure, Tofler, Muller, & Mittleman, 2013). In addition to cardiac events, hostility has been implicated in the long-term development of coronary atherosclerosis. Prospective associations between hostility and carotid atherosclerosis, as indexed by intima-media

¹ Disclosure: Although it does not form part of this thesis it should be noted that a manuscript on optimism and physiological stress responses in this sample is currently under review for publication. I am a co-author on this manuscript.

thickness, have been reported in both male and female samples (Matthews, Owens, Kuller, Sutton-Tyrrell, & Jansen-McWilliams, 1998; Pollitt et al., 2005).

Despite the growing evidence linking hostility to ill health, the underlying mechanisms involved are not well understood. One possibility is that the relationship is mediated through behavioural pathways. Hostility may lead to adverse health behaviours, such as poor diet, sedentary lifestyle, smoking and excessive alcohol consumption (Siegler et al., 2003) all of which are established risk factors for CVD. However, findings from the majority of studies remain significant after adjusting for health behaviours (Chida & Steptoe, 2009b; Klabbers et al., 2012). Thus, it may be that direct biological mechanisms are involved.

In epidemiological studies, hostility has been linked with disturbances across multiple biological systems. High levels of hostility have been associated with autonomic dysfunction (Thomas, Nelesen, & Dimsdale, 2004; Virtanen et al., 2003), inflammation (Marsland, Prather, Petersen, Cohen, & Manuck, 2008; Ranjit et al., 2007) and increased platelet activation (Markovitz, Matthews, Kiss, & Smitherman, 1996).

Acute mental stress testing is another research strategy that is used to investigate the biological concomitants of hostility. As discussed in Section 1.6.2 of Chapter 1 mental stress testing involves the measurement of biological responses to acute challenges. This method allows detailed dynamic responses to be studied under controlled conditions, reducing the impact of other factors that may confound associations (Steptoe & Poole, 2010).

The majority of research in the field has investigated cardiovascular responses to acute stress. Meta-analytic results indicate that heightened cardiovascular stress responsivity is associated with an increased risk of future CVD (Chida & Steptoe, 2010)

and hostility has been associated with heightened cardiovascular stress responses in healthy participants (Chida & Hamer, 2008).

As mentioned in Chapter 1, CHD has been characterised as an inflammatory condition. Heightened IL-6 concentrations have been prospectively associated with future CVD and poor outcomes in existing CVD patients (Danesh et al., 2008; Libby et al., 2011). Additionally, positive associations between circulating IL-6 concentrations and hostility have been observed (Marsland et al., 2008; Ranjit et al., 2007).

Excessive glucocorticoid action is associated with cardiovascular risk factors such as central obesity (Incollingo Rodriguez et al., 2015; Rosmond, Dallman, & Björntorp, 1998), insulin resistance (Reynolds & Walker, 2003) and hypertension (Collomp et al., 2016; Whitworth, Brown, Kelly, & Williamson, 1995). Cortisol is involved in regulating inflammation through activation of the glucocorticoid receptor, leading to inhibition of inflammatory cytokine production by monocytes (Raison & Miller, 2003). However, prolonged exposure to heightened cortisol levels may result in dysregulation of this system manifested through insufficient glucocorticoid signaling (Raison & Miller, 2003). Hostility has been associated with flattening of cortisol rhythms in some studies (Ranjit et al., 2009; Sjögren, Leanderson, & Kristenson, 2006). Evidence indicates that low cortisol responders have significantly higher cytokine responses to acute stress (Kunz-Ebrecht, Mohamed-Ali, Feldman, Kirschbaum, & Steptoe, 2003). Thus, diminished cortisol levels may facilitate heightened inflammation associated with ill health.

Despite this evidence, few studies have investigated inflammatory and neuroendocrine mechanisms in relation to hostility and the majority of research has been conducted with healthy samples. To our knowledge only one small study has investigated acute stress responses in a sample at high risk for coronary events (Brydon

et al., 2010). In this study, more hostile individuals with advanced CAD had heightened SBP and DBP responses to mental stress tasks. Hostility was also positively associated with IL-6 and negatively correlated with cortisol concentrations during post stress recovery.

The additional risk of CVD in people with T2D is largely unexplained. Therefore, it is possible that personality factors could potentially play a role in linking the conditions. Hostility is not well researched in relation to T2D. There is a small amount of evidence that hostility is associated with outcomes such as fasting glucose and insulin resistance (Shen et al., 2008; Zhang et al., 2006) as well as prevalent T2D (Williams et al., 2011). Additionally, anger, a related construct to hostility has been prospectively associated with new onset T2D in two large cohort studies (Abraham et al., 2015; Golden et al., 2006). A more detailed review of the literature looking at the relationship between hostility and diabetes can be found in Section 1.5.2.4 of Chapter 1. Considering the evidence as a whole, it is plausible that hostility plays a role in T2D and that it may contribute to the increased risk CVD in people with the condition.

Considering the excess risk of CVD in this population and the lack of research relating hostility and inflammatory and neuroendocrine stress responses we investigated the relationship between hostility and SBP, DBP, heart rate, cardiac index, HRV, IL-6 and cortisol responses to laboratory stress in a sample of T2D individuals. As discussed in greater detail in Section 1.7.4 of Chapter 1, in epidemiological studies raised IL-6 levels have been prospectively associated with CVD development (Danesh et al., 2008) and poorer outcomes in CVD patients (Libby et al., 2011). Inflammation is involved in the pathogenesis of T2D, and IL-6 and CRP are the most widely studied markers in the field. Meta-analytic results indicate that heightened IL-6 rather than CRP is a stronger predictor of subsequent diabetes in initially healthy samples (Wang et al., 2013) and that

concentrations of IL-6 are elevated in T2D patients (Pickup, 2004). We predicted that participants with greater hostility scores would have greater cardiovascular and IL-6 responses to acute stress. Neuroendocrine dysfunction is suggested to play a role in T2D and results from the comparative study presented in Chapter 3 indicate that cortisol stress responsivity is blunted in T2D (Steptoe et al., 2014). We predicted that more hostile individuals would have more diminished cortisol responses to stress.

4.3. Method

The method for the current study can be found in Chapter 2. To reiterate in brief this was a study of 140 individuals with T2D who underwent psychophysiological stress testing. Stress responsivity and post-stress recovery was measured across multiple biological systems in this sample. Cynical hostility was measured using the 10 item Cook Medley Cynical Hostility Scale (Cook & Medley, 1954). Total scores ranged from 0-10 with higher scores indicating greater hostility. Further information on this hostility measure can be found in section 2.3.4.1 of Chapter 2.

4.4. Statistical analysis

As in Chapter 3, the cardiovascular measures were averaged into 5-minute means for baseline, the two tasks, and the two recovery periods. The two task trials were subsequently averaged. The pattern of cortisol over the laboratory session was analysed using individual values, and also by computing cortisol AUC with respect to ground using procedures described by Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, (2003).

Responses to mental stress testing were analysed using repeated measures analysis of variance. Subjective stress, cardiovascular variables and IL-6 were analysed

across four trials (baseline, task, 45 min and 75 mins post-stress), and cortisol across five trials (baseline, task, 20 min, 45 min and 75 min post-task). Associations with hostility were analysed using multiple regression. Multivariable linear regressions on baseline values of SBP, DBP, heart rate, cardiac index, HRV and IL-6, and regressions on responses following stress were carried out. Cortisol was analysed using individual values and AUC to investigate total cortisol output across the whole session. For analyses of associations with baseline values, hostility was entered into the regression models along with age, sex, BMI, smoking, household income, time of laboratory testing, oral anti-diabetic medication and beta blockers. These covariates were chosen because previous research has indicated these factors might influence physiological function (Jones et al., 2012; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Roy, Steptoe, & Kirschbaum, 1994; Steptoe et al., 2002) and preliminary analyses indicated that these variables were correlated with the physiological responses assessed in this study.

Associations of hostility with stress reactivity and recovery involved regressions onto changes between baseline and task or post-task values, and included the baseline level of the dependent variable as an additional covariate. We conducted preliminary analyses to check whether other factors influenced the relationship between hostility and physiological function. We investigated whether there was a relationship between HbA1c and hostility as well as responses to stress. These analyses were non-significant and are therefore not presented. We also investigated whether hostility interacted with sex, but found no significant associations with physiological responses, so interaction terms were not included in the final models. The majority of our sample was obese, so we investigated whether BMI interacted with hostility but found no significant associations with physiological responses. Our sample included 28 (20%) non-white

individuals. Adding ethnicity as a factor to the analyses did not alter the results, so it was not included in the models described here. Depressed mood was also assessed in the study and was significantly correlated with hostility ($p < .001$). We investigated whether hostility interacted with depression, but found no significant associations with physiological responses. Additionally, adding depression as an extra covariate did not affect the pattern of results. Therefore depression was not included in the final models. As participants were taking medication at the time of testing, we assessed whether anti-diabetic medication and beta-blockers interacted with hostility. Hostility did not interact with anti-diabetics, but we found a significant interaction between beta-blockers and hostility for some of the cardiovascular responses. However, inclusion of this interaction term did not affect the pattern of physiological responses so this variable was not retained for the final analyses.

Results are presented as unstandardised regression coefficients (B) with 95% CIs using continuous hostility scores as the predictor variable. Significant effects from the regression analyses are illustrated by comparing high and low hostility groups defined by a median split (cut-off ≥ 4) using analysis of covariance. All analyses were conducted using SPSS version 22 (SPSS, Chicago, IL, USA).

4.5. Results

4.5.1. Participant characteristics

The sample consisted of 140 people (88 men and 52 women) with T2D. Participant characteristics are detailed in Table 4.1. Participants were aged 63.71 ± 7.00 years on average and were predominately white with relatively low incomes. BMI ranged from 19.2 - 47.80 and the average BMI was in the obese range (BMI >30). Levels of HbA1C were less than 6.5% in 29.9% of the sample, between 6.5 – 7.5% in 41%, and over 7.5%

in 29.1% of participants. Hostility scores averaged 3.77 ± 2.8 and were not related to age, sex, ethnicity, BMI, waist circumference, smoking or medication use at the time of testing (p 's $> .136$). However, there was an association with household income ($\chi^2 = 8.08$, $p = 0.018$). Hostility was greater among participants with household incomes under £40,000 (mean 4.13 ± 2.95) than among participants with incomes over £40,000 (2.81 ± 2.22).

Table 4.1 Participant characteristics

Characteristics	Mean \pm SD or N (%)
Age (years)	63.71 \pm 7.00
Sex (% men)	88 (62.9%)
Ethnicity (% white)	112 (80%)
Current smoker (% yes)	20 (14.4%)
BMI (kg/m ²)	30.75 \pm 5.72
Waist (cm)	105.50 \pm 13.49
Household Income	
< £40K (approx. \$33,286)	95 (71.4%)
> £40K (approx. \$66,573)	38 (28.6%)
Cook Medley Cynical Hostility (10 item)	3.77 \pm 2.8
CES-D	11.85 \pm 8.9
HbA1c (%)	7.25 \pm 1.42
Oral anti-diabetic	109 (80.1%)
Injectable anti-diabetic and insulin	15 (11.0%)
Beta-Blockers	16 (11.8%)

4.5.2. Responses to stress

Details of participants' subjective and biological responses to stress are presented in Table 4.2. We found significant main effects of trial for SBP, DBP, heart rate, cardiac index, HRV, IL-6, cortisol and subjective stress levels (p 's < .001). The tasks elicited substantial cardiovascular reactions, with an average rise of 23.27 ± 15.89 mmHg in SBP and 12.51 ± 7.00 mmHg in DBP. Although BP returned towards baseline during the post-task period, both SBP and DBP remained elevated above baseline levels at 45 and 75 min after tasks. We found that heart rate also increased significantly in response to the tasks, with an average rise of 4.56 ± 4.67 bpm. With regards to cardiac index, values did not increase significantly in response to the tasks and values fell over the recovery period with an average decrease of 0.51 ± 0.58 L/min/m² at 45 minutes and 0.53 ± 0.64 L/min/m² at 75 minutes post-task respectively. HRV fell in responses to the tasks and rose again over the recovery period. IL-6 increased following the tasks with a notable delay consistent with previous stress studies (Steptoe, Hamer, & Chida, 2007), reaching the highest values at 75 mins post-task. The pattern of response was different for cortisol, levels fell significantly in response to the tasks with an average decrease of 1.29 ± 0.08 nmol/l immediately post-task and 2.3 ± 0.13 nmol/l 20 minutes post-task. There were marked individual differences in this stress response, with changes in cortisol ranging from 0.23 to -6.54 nmol/l post-task and from -0.44 to -12.28 nmol/l at 20 minutes post-task. Participant's subjective stress levels increased during the tasks and returned to low levels during recovery. There were no significant relationships between hostility and any of the subjective stress ratings (p 's > .05).

Table 4.2 Subjective and biological responses to stress (means \pm standard errors)

	Baseline	Task	20 mins	45 mins	75 mins
Subjective Stress	1.49 \pm 0.08 ^a	4.49 \pm 0.13 ^b		1.53 \pm .08 ^a	1.43 \pm .08 ^a
SBP (mmHg)	126.08 \pm 1.16 ^a	149.35 \pm 1.76 ^b		134.24 \pm 1.74 ^c	137.05 \pm 1.46 ^d
DBP (mmHg)	71.74 \pm 0.87 ^a	84.25 \pm 1.07 ^b		78.04 \pm 1.27 ^c	79.51 \pm 1.18 ^d
Heart rate (bpm)	71.77 \pm 1.04 ^a	76.33 \pm 1.04 ^b		70.18 \pm 1.04 ^c	70.15 \pm 1.02 ^d
Cardiac index (L/min/m ²)	4.12 \pm 0.09 ^a	4.12 \pm 0.09 ^a		3.62 \pm 0.08 ^b	3.59 \pm 0.08 ^b
HRV (s)	0.042 \pm 0.003 ^a	0.037 \pm 0.002 ^b		0.047 \pm 0.003 ^c	0.49 \pm 0.003 ^d
IL-6 (pg/ml)	2.08 \pm 0.11 ^a	2.07 \pm 0.11 ^a		2.18 \pm 0.12 ^a	2.31 \pm 0.12 ^b
Cortisol (nmol/l)	10.03 \pm 0.47 ^a	8.74 \pm 0.39 ^b	7.74 \pm 0.34 ^c	6.89 \pm 0.36 ^d	7.17 \pm 0.49 ^c

^{a, b, c} Values in rows with different superscripts are significantly different from one another ($p < 0.05$).

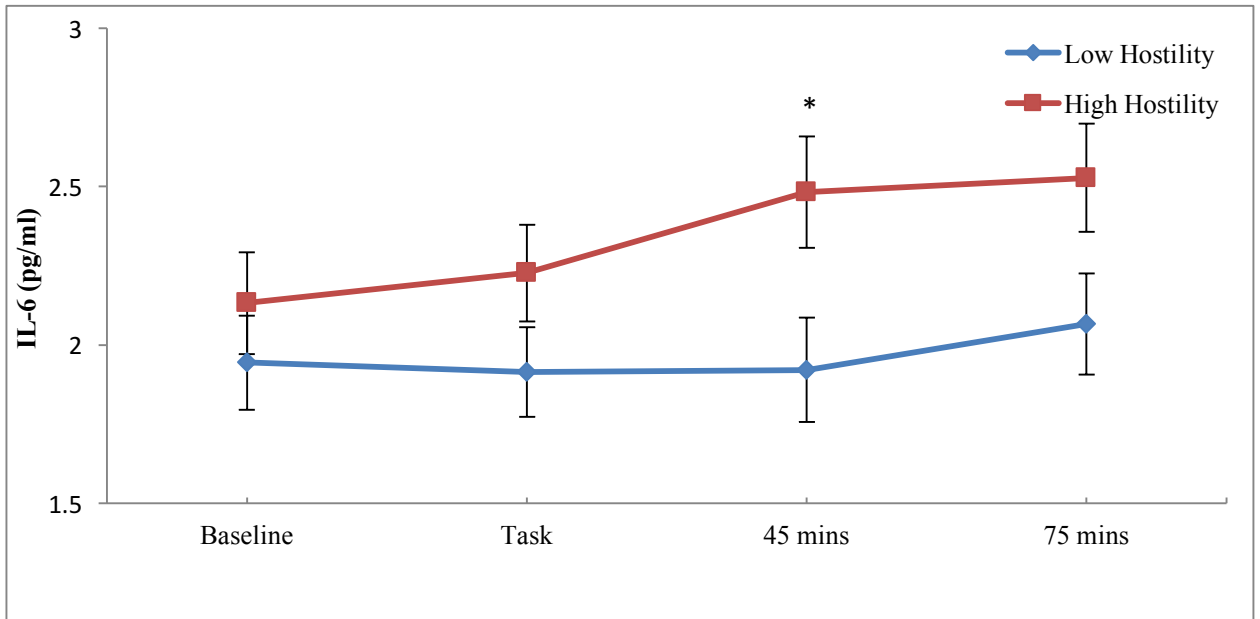
4.5.3. Hostility and biological responses to stress

There was no association between hostility and baseline levels of SBP, DBP, heart rate, cardiac index and HRV (B values between -0.376 and -0.016 and p 's > 0.076).

Similarly, cardiovascular responses to the task or recovery from the tasks were not related to hostility (B values between -0.0826 and 0.236 and p 's > 0.113). There was no association between hostility and baseline plasma IL-6 concentrations ($B = -0.015$, $C.I. = -0.095$ to 0.064 , $p = 0.703$). However, regressions on the change in IL-6 between baseline and 45 minutes post-task ($B = 0.082$, $C.I. = 0.032$ to 0.132 , $p = 0.002$) and 75 min post-task ($B = 0.076$, $C.I. = 0.021$ to 0.131 , $p = 0.007$) show larger increases in more hostile participants. These effects were independent of baseline IL-6, age, sex, BMI, smoking, household income, time of testing, beta-blockers and oral anti-diabetic medications. The association between hostility and IL-6 levels over the laboratory session is illustrated Figure 4.1, where participants in the study have been divided into high and low hostility groups. Greater hostility was associated with larger plasma IL-6 increases following stress.

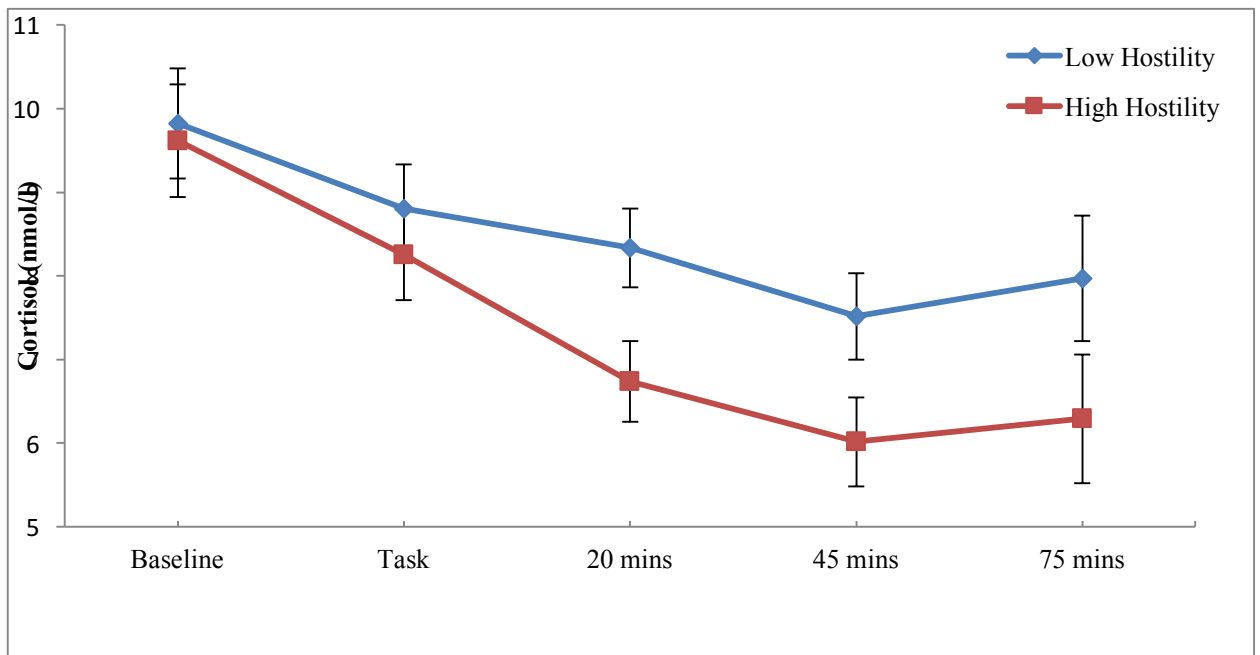
In the analyses of cortisol, there was again no association with hostility at baseline ($B = -0.002$, $C.I. = -0.016$ to 0.011 , $p = 0.747$). However, cortisol concentration at 20 minutes post-task ($B = -0.017$, $C.I. = -0.027$ to -0.006 , $p = 0.002$), 45 minutes post-task ($B = -0.018$, $C.I. = -0.032$ to -0.005 , $p = 0.010$) and 75 minutes after tasks ($B = -0.023$, $C.I. = -0.037$ to -0.009 , $p = 0.002$) was lower in more hostile individuals after adjustment for covariates. The association between hostility and cortisol was further examined using the cortisol AUC measure. There was an inverse association between hostility and cortisol AUC ($B = -26.69$, $C.I. = -41.39$ to -11.98 , $p < 0.001$). The difference in cortisol levels between participants with high and low hostility scores is illustrated in Figure 4.2. Cortisol levels declined across the laboratory session in both groups. However, higher hostility was associated with a significantly greater decrease in cortisol output over the testing period.

Figure 4.1 IL-6 stress responses for high hostility and low hostility groups over the laboratory session



Values are adjusted for age, sex, BMI, smoking, household income, beta-blockers and oral anti-diabetic medications. Error bars are standard error of mean.

Figure 4.2 Cortisol stress responses for high hostility and low hostility groups over the laboratory session



Values are adjusted for age, sex, BMI, smoking, household income, beta-blockers and oral anti-diabetic medications. Error bars are standard error of mean.

4.5.4. Inter-correlation between IL-6 and cortisol

In light of the associations between hostility and IL-6 and cortisol responses to stress, we assessed the inter-correlations between IL-6 and cortisol. The change in IL-6 in responses to the tasks at 45 minutes and 75 minutes was significantly negatively correlated with cortisol AUC ($r = -.35$ and $-.38$, $p's < 0.001$) and with all individual cortisol measurements over the laboratory session (r values between $-.19$ and $-.29$, all $p's < .05$).

4.6. Discussion

4.6.1. Results summary

This study investigated the relationship between hostility and cardiovascular, inflammatory and neuroendocrine responses to acute stress in people with T2D. We predicted that participants with greater hostility scores would be more responsive to stress. The main finding is that greater hostility was associated with elevated IL-6 responses to acute stress. By contrast, cortisol output following stress was diminished to a greater extent in more hostile individuals. These associations were independent of baseline values, age, sex, BMI, smoking, household income, anti-diabetic medications and beta-blockers. Contrary to prediction, we did not observe any associations between hostility and cardiovascular responses.

4.6.2. Earlier studies of hostility and acute stress responsivity

IL-6 responses to stress were significantly elevated in T2D subjects with greater hostility ratings. This result corroborates previous work from Professor Steptoe's group in which IL-6 was elevated following acute stress in more hostile patients with CAD

(Brydon et al., 2010). Only one other study has investigated inflammatory stress responses in relation to hostility. Brummett *et al.* (Brummett et al., 2010) examined the effects of hostility on IL-6 responses to an emotional recall stressor in 525 healthy participants, but found no association.

This discrepancy in findings may reflect variation in the study population. The current investigation and the Brydon *et al.* (Brydon et al., 2010) study assessed IL-6 responsivity in two high risk phenotype samples, whereas Brummett *et al.* (Brummett et al., 2010) used a healthy participant group. It may be that heightened inflammatory stress responses are only associated with hostility in groups with an increased propensity for CVD. Further studies will be required to assess the impact of the study population on the presence of an association between hostility and inflammation. Nevertheless, the results of the current analysis suggest that more hostile T2D individuals may be susceptible to stress-induced inflammation.

We observed no relationship between hostility and cardiovascular responses to stress in this T2D sample. This result is paradoxical as a considerable body of evidence indicates that heightened cardiovascular stress responsivity is associated with hostility in healthy individuals (Bongard, al' Absi, & Lovallo, 1998; Chida & Hamer, 2008; Girdler, Jammer, & Shapiro, 1997; Suarez, Kuhn, Schanberg, Williams, & Zimmermann, 1998). Indeed, in our previous analysis of CAD patients, greater hostility was associated with increased SBP and DBP responses to laboratory stress (Brydon et al., 2010). The lack of association seen in the present analysis cannot be attributed to the intensity of stressor used, as both subjective stress ratings and cardiovascular measures increased significantly in response to the task. It is unlikely that the current study was underpowered to detect cardiovascular effects. We used the same laboratory procedure as our study of 34 CAD patients (Brydon et al., 2010) and associations have been

reported in other analyses with much smaller sample sizes than the present study (Bongard et al., 1998; Brydon et al., 2010). Our analysis also took account statistically of medications and a number of previous studies have found no effect of beta-blockers on cardiovascular responses to stress (Mills & Dimsdale, 1991). However, we cannot rule out the possibility that the null association observed was attributable to medication, as the T2D participants continued to take beta-blockers and anti-diabetic medications at the time of testing.

We found that cortisol output following stress was attenuated in T2D participants with greater hostility scores. The observed inverse relationship between cortisol AUC and hostility is consistent with the findings of our previous analysis in which cortisol levels were reduced post-stress in more hostile CAD subjects (Brydon et al., 2010). It is plausible that decreased cortisol levels may have facilitated the elevated IL-6 responses observed in more hostile subjects in both studies. However, this relationship has not been consistently observed. In a study of 52 healthy men high levels of hostility were associated with heightened cortisol responses to an anagram task, but only in those who simultaneously experienced harassing comments from the experimenter (Suarez et al., 1998). The task used in the present analysis was designed to elicit general stress responses, whereas the task in the Suarez *et al.* (Suarez et al., 1998) study was designed to provoke hostile reactions and this may account for the diverging findings.

4.6.3. Cortisol and inflammation in T2D

Our results observed in a laboratory environment, offer the possibility that the negative impact of hostility on health could be mediated in part through stress-related dysregulation of the neuroendocrine and inflammatory systems. Cortisol levels declined

significantly throughout the laboratory session in all participants, which may be indicative of neuroendocrine dysfunction in individuals with T2D. As discussed in greater detail in the main literature review in Chapter 1 T2D is a recognised complication of long-term cortisol excess (Clayton et al., 2011) and there is emerging evidence that diurnal cortisol secretion may be altered in T2D (Champaneri et al., 2012). Additionally, cortisol plays a pivotal role in many physiological processes relevant to diabetes (Di Dalmazi et al., 2012). Neuroendocrine dysfunction may play a role in diabetes is through circadian disruption. Circadian rhythms are regulated at the hypothalamic level by the suprachiasmatic nuclei. It has been suggested that disturbances in circadian rhythms may act on T2D through the alteration of glucose metabolism. Indeed, experimental work indicates that circadian disruption heightens both fasting and postprandial plasma glucose levels through inadequate pancreatic insulin secretion (Buxton et al., 2012).

Despite the literature highlighting the role of neuroendocrine dysfunction in T2D, little research has assessed dynamic physiological stress responses in this population. The participants in the present study were part of a larger trial comparing biological responses to stress in individuals with T2D and healthy controls (Stephoe et al., 2014). Results from this study presented in Chapter 3 indicate that participants with diabetes have blunted cortisol responses to stress compared to healthy individuals. The associations between blunted stress reactivity and health were discussed at length in Chapter 3. However, it is important to reiterate for the purposes of the present chapter that blunted cortisol responses to stress have been associated with health risk factors in relatively healthy samples (Phillips et al., 2013) and have been found in patients with CAD (Nijm et al., 2007; Waller et al., 2016). The current study adds to this by

suggesting that greater levels of hostility exaggerate disturbances in neuroendocrine function in people with T2D.

Cortisol is also involved in the regulation of inflammation and chronic exposure to psychosocial stress results in increased cortisol secretion (Miller et al., 2007). Cortisol typically has an inhibitory effect on pro-inflammatory cytokine production. However, long-term heightened cortisol concentrations may result in dysregulation of this system manifested through insufficient glucocorticoid signaling (Raison & Miller, 2003). In this case, reduced cortisol levels may have a permissive effect on inflammatory markers. In the current investigation, hostility was inversely associated with cortisol output over the laboratory session. Other evidence indicates that high cortisol responders have significantly smaller cytokine responses to acute stress (Kunz-Ebrecht et al., 2003). Our findings suggest that more hostile T2D people show insufficient glucocorticoid signalling to inhibit inflammatory responses under stress due to decreased hormone release. This decreased cortisol production may have contributed to the heightened IL-6 stress responses observed in more hostile participants.

The acute changes observed in this study offer the possibility that inflammation may be one of the mechanisms through which hostility confers an increased risk for ill health. Although we found no association between hostility and baseline IL-6 in our sample, large cohort studies have reported a relationship (Marsland et al., 2008; Ranjit et al., 2007). The study by Marsland *et al.*, (2008) had a sample size of 885. The effect sizes reported in this study for components of the Cook Medley Hostility Scale ranged from .13 - .16 at a significance level of $p < .005$. Using this information and assuming a normal bivariate distribution and two tailed test with an alpha of 0.05 a post-hoc power calculation using G power software suggested that the current study power of 33% to detect a correlation of .13 and power of 47% to detect a correlation of .16. These

calculations suggest that the present study was under powered to detect basal differences in IL-6, which might explain why the association between IL-6 and hostility only emerged when induced by stress. In epidemiological studies raised IL-6 levels have been prospectively associated with CVD development (Danesh et al., 2008) and poorer outcomes in CVD patients (Libby et al., 2011). Inflammation also plays a role in the pathogenesis of T2D. Heightened circulating IL-6 levels are predictive of T2D development in initially healthy samples (Wang et al., 2013) and concentrations are elevated in T2D patients (Pickup, 2004).

It is possible that hostility might potentially contribute to the increased risk of CVD in people with T2D through dysregulated stress-related inflammatory pathways. In this way hostility may contribute to insulin resistance and dyslipidaemia as elevated IL-6 concentrations inhibit AMP-activated protein kinase, an enzyme involved in insulin-stimulated fatty-acid oxidation, down-regulating gene transcription of proteins involved in insulin-stimulated glucose transport and lipid uptake in adipose tissue (Pickup, 2004). However, prospective studies will be required to test this pathway.

4.6.4. Limitations

Many of the limitations of the present study were discussed in detail in Chapter 3 as both studies were drawn from the same dataset. The majority of participants were of white European origin, with the remainder consisting of other ethnic groups. Adding ethnicity as a factor to the analyses did not alter the pattern of results so it was not included in the final models. It is important to reiterate that T2D prevalence is more common in non-white groups (Tillin et al., 2013). This therefore limits the generalisability of these results. Most of the participants were taking medications at the time of testing. As before, although the statistical analyses took account of medication,

an effect of medication on stress reactivity cannot be excluded. The study was cross-sectional in nature so it is not possible to infer causality. Longitudinal research is needed to elucidate the degree to which trait hostility and changes in hostility over time are associated with inflammatory, neuroendocrine and cardiovascular processes, as well as negative health outcomes in people with T2D. The study was limited by the use of a self-report measure to assess hostility. The assessment of observable hostile behaviour could provide a different perspective in understanding the relationship between hostility and stress reactivity.

The stress tasks used may not have been optimal for studying hostility. Social or interpersonal stressors may provoke greater reactions in hostile individuals (Suarez et al., 1998). Other studies of hostility and physiological stress reactivity (Anderson, Linden, & Habra, 2005; Lai & Linden, 1992; Suarez et al., 1998) have used standardised harassment (Hokanson & Shetler, 1961) to provoke hostile reactions. In these studies participants are exposed to verbal harassment using standardised statements (e.g. “You’re making too many mistakes, so try harder”) at regular intervals when undertaking the stress tasks. Whether our results would be exaggerated or unaffected by standardised harassment is unknown. An experimental manipulation where some individuals are exposed to harassment during the tasks and others are able to complete the task without commentary would shed light on this.

4.6.5. Conclusion

Despite these considerations, the results suggest that responses to stress are dysregulated in more hostile individuals with T2D. We observed greater IL-6 stress responses and diminished cortisol output over the laboratory session in more hostile individuals with T2D independent of covariates. It is possible that heightened stress-

induced inflammation may increase the risk for CVD in this population. However, further studies are required to confirm this pathway.

5. The Whitehall II study: Introduction and methods

5.1. Introduction

This chapter will provide an overview of the Whitehall II study and the use of this secondary dataset in the context of this PhD. Data from this longitudinal cohort is used in the studies presented in Chapters 6 and 7. The main aim of the Whitehall analyses was to assess the relationship between diurnal cortisol secretion and diabetes. A cross-sectional analysis and prospective analysis was conducted to fulfil this aim. A brief overview of the Whitehall II cohort study will be presented, followed by the procedures used to obtain Whitehall data and the methods used for the cortisol and diabetes studies.

5.2. The Whitehall II study

The Whitehall II study is a prospective cohort of London-based civil servants. The study was originally established to explore the relationships between SES, stress and CVD (Marmot & Brunner, 2005). The cohort of 10,308 participants (3414 women and 6895 men) was initially recruited between 1985 and 1988 from 20 civil service departments. The participants were aged between 35 and 55 at phase 1 of the study (Marmot & Brunner, 2005). Since the first phase of data collection, clinical data and questionnaire measures have been collected from the cohort every 2-5 years. This includes measures of SES, biological, psychosocial and behavioural factors. Attrition rates and the timing of the data collection phases of the study can be seen in Table 5.1. Over time the research themes of the study have moved beyond SES, stress and CVD to focus more broadly on circumstances that affect health and wellbeing in an ageing cohort. Key areas of focus in the study today include depression and chronic disease as well as cognitive and physical functioning. Data collection is set to continue with this cohort until 2030 (Sanchez, 2016). Ethical approval for the Whitehall II study was

obtained from the University College London Medical School committee on the ethics of human research. Informed consent for involvement in the study was obtained from all participants.

Table 5.1 Data collection phases of the Whitehall II study

Phase	Dates	Type	N of participants
1	1985-1988	Clinical assessment / questionnaire	10,308
2	1989-1990	Questionnaire	8,132
3	1991-1994	Clinical assessment / questionnaire	8,815
4	1995-1996	Questionnaire	8,628
5	1997-1999	Clinical assessment / questionnaire	7,870
6	2001	Questionnaire	7,355
7	2002-2004	Clinical assessment / questionnaire	6,967
8	2006	Questionnaire	7,173
9	2007-2009	Clinical assessment / questionnaire	6,761
10	Feb-Mar 2011	Clinical assessment / questionnaire	277 (Pilot*)
11	2012-2013	Clinical assessment / questionnaire	6,318
12	2015-2016	Clinical assessment / questionnaire	In progress

Adapted from Sanchez, 2016

*Pilot study for new measures introduced at phase 11

5.3. Procedure to obtain data from the Whitehall II group

The Whitehall II study is housed in the Department of Epidemiology and Public Health at UCL where I was based during my PhD. However, as I was a member Professor Steptoe's Psychobiology group rather than the Whitehall II study group I had to go through formal procedures to obtain the data used in Chapters 6 and 7. A pdf of the Whitehall II policy on data sharing can be found on the Whitehall II study website (see

Sanchez, 2016). In brief, the data sharing model follows a gated-access approach such that the Whitehall data are available for use subject to approval by the Whitehall study team. The application for data sharing has to include the scientific hypotheses of the proposed study, a detailed study background as well as the planned scientific outputs. A list of the required variables needed for the project must also be submitted and justification for the variables requested must be given. Only proposals that are deemed ethical, rigorous and of high scientific quality by the Whitehall II team qualify for data-sharing. I first applied for phase 7 data for the cross-sectional analysis that is presented in Chapter 6. During the course of my PhD the phase 11 data was collected and following data cleaning it became available for data sharing to select researchers. I used this data for the prospective analysis presented in Chapter 7. The Whitehall II team ideally require 1-2 scientific outputs from every data sharing application. I fulfilled this requirement by publishing the two studies presented in Chapters 6 and 7 (Hackett, Kivimäki, Kumari, & Steptoe, 2016; Hackett, Steptoe, & Kumari, 2014).

5.4. Methods and measures

5.4.1. Participants

5.4.1.1. *Participants for the cross-sectional analysis*

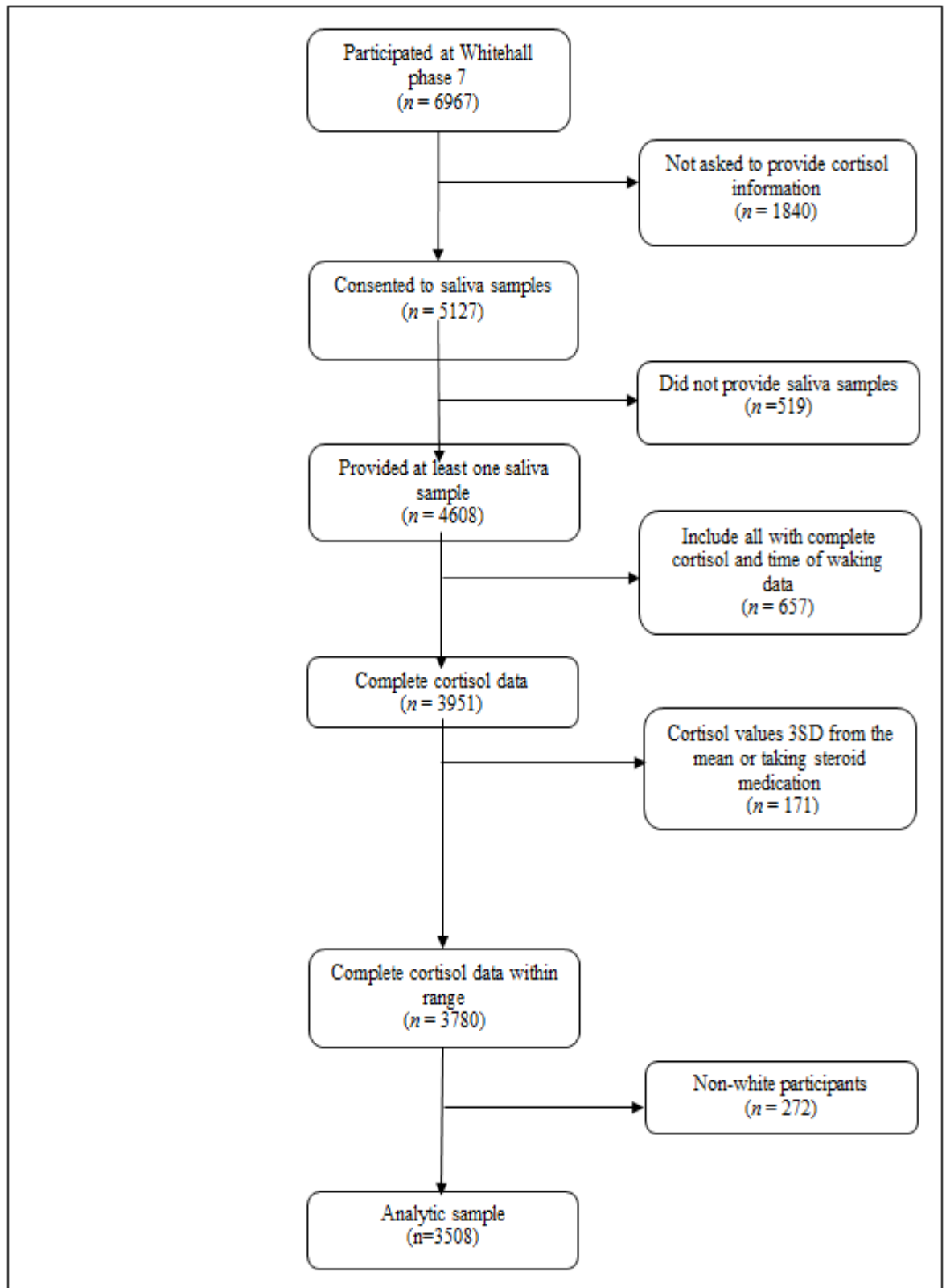
Data for the study presented in Chapter 6 was taken from phase 7 (2002–2004) of the Whitehall II study. A flowchart of the participants included and excluded from the cross-sectional analysis can be seen in Figure 5.1. The total number of participants at phase 7 was 6967, and of these, 6484 had a clinical assessment. Saliva collection for the assessment of cortisol was instigated partway through phase 7 and of those participants that were asked to collect saliva samples, 90.1% (n=4608) returned samples. This group

had fewer participants in the lowest civil service employment grades compared with phase 1 of the study: however, this difference was small. The analysis was restricted to those with complete information on waking time (as self-reported on the day of sample collection), cortisol measures and diabetes status. This left a final sample of 3508 participants

5.4.1.2. *Participants for the prospective analysis*

Data for the study presented in Chapter 7 was taken from phase 7 (2002–2004) and phase 11 (2012–2013) of the Whitehall II study. The majority of the participants that were retained for the cross-sectional analysis (n= 3508) were included in the prospective study, with the exception of participants who had prevalent diabetes at phase 7 (n =238) who were excluded. This left a final sample of 3270 participants for the prospective study.

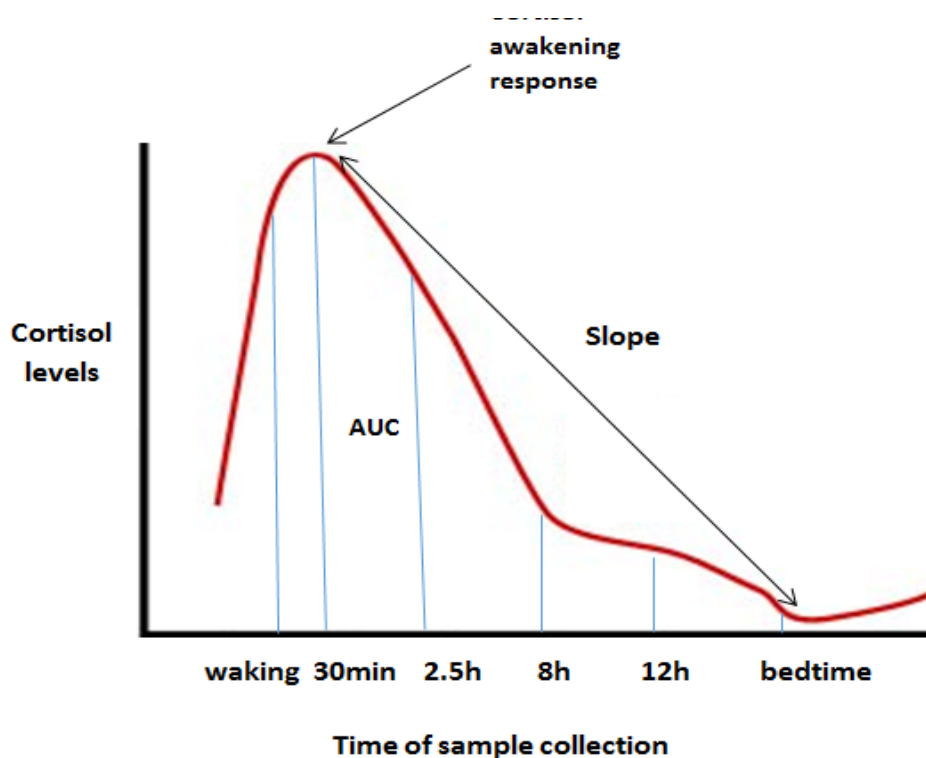
Figure 5.1 Flow diagram of participants included and excluded from the cross-sectional analysis



5.4.2. Cortisol collection and analysis

The cortisol was collected at phase 7 (2002-2004) of the Whitehall II study. Participants were provided with a set of salivettes and were asked to take six samples over the course of a normal weekday (Monday-Friday) at waking, after 30 minutes, 2.5 hours, 8 hours, and 12 hours, and at bedtime. Figure 5.2 shows the timing of the sample collection and relevant cortisol parameters (figure not to scale). The participants were instructed not brush teeth or consume any food or beverages for 15 minutes prior to sample collection. An instruction booklet was used to record information on the day of sampling including wake time, time each sample was taken, and stressful events. The salivettes and booklet were returned by post. Salivettes were centrifuged at 3000g for 5 min resulting in a clear supernatant of low viscosity. Salivary cortisol levels were measured using a commercial chemiluminescence immunoassay (CLIA; IBL Hamburg, Germany). The lower concentration limit of the assay was 0.44 nmol/litre and the intra- and inter-assay coefficients of variance were less than 8%. Any sample over 50 nmol/litre was re-analysed.

Figure 5.2 Cortisol collection in the Whitehall II study



5.4.3. Assessment of T2D and IGT in the Whitehall II study

Type 2 diabetes was defined as a fasting glucose ≥ 7.0 mmol/l or a 2-h post load glucose ≥ 11.1 mmol/l during the OGTT performed at the Whitehall clinical assessment or by reported doctor diagnosed diabetes, or the use of diabetes medication (American Diabetes Association, 2012). Impaired fasting glucose (IFG) was classified as a fasting glucose between 5.6 and 6.9 mmol/litre (American Diabetes Association, 2012). For the purposes of the OGTT, participants provided a venous blood sample 8 hours after fasting and at 2 hours post administration of a 75g glucose solution. Blood glucose was measured using the glucose oxidase method (Cooper, 1973) on a YSI MODEL 2300 STAT PLUS Analyzer (YSI Corporation, Yellow Springs, OH; mean coefficient of variation 1.4–3.1%) (Astles, Sedor, & Toffaletti, 1996). For the cross-sectional analysis presented in Chapter 6 there were 238 participants with prevalent diabetes in the sample and of these 126 had known diabetes (confirmed by report of doctor diagnosis and diabetic medication) at the beginning of phase 7. A further 112 participants with diabetes were identified by the OGGT carried out at the Whitehall clinical assessment at phase 7.

5.4.4. Demographic measures

For both the cross-sectional and the prospective analyses we used the characteristics of the participants at the time cortisol was collected (phase 7, 2002–2004). Participant demographic characteristics were assessed by self-report and included information on age, sex, and current or most recent civil service employment grade, which was used as a measure of social position in this cohort.

5.4.5. Health measures

Again for both the cross-sectional and prospective analyses we used measures as assessed at the time of cortisol sample collection (phase 7, 2002–2004). Smoking status was assessed by self-report and defined as current smokers vs. non-current smokers (Badrick, Kirschbaum, & Kumari, 2007). BMI was assessed by the objective measurement of height and weight at the Whitehall clinical assessment. Height was assessed using a stadiometer with the head in the Frankfort plane, and weight was assessed using a portable digital scale (Tanita, Yiewsley, Middlesex, UK). BMI was calculated as weight (in kilograms)/height (in meters) squared. For presentation purposes in the cross-sectional analysis in Chapter 6, BMI was categorised as obese ($\geq 30 \text{ kg/m}^2$) or non-obese ($< 29.9 \text{ kg/m}^2$). At the clinical assessment it was recorded whether participants had a history of CHD. Participants also provided details of current medication use and these were subsequently coded using the British National Formulary (Joint Formulary Committee, 2013). Cardiovascular medication usage was defined as the use of beta-blockers, anti-hypertensives, lipid lowering drugs, nitrates or anti-platelet medications.

Fatigue has been previously associated with alterations in diurnal cortisol secretion in the Whitehall II study (Kumari, Badrick, Chandola, et al., 2009), and fatigue is common in individuals with T2D (Fritschi & Quinn, 2010). Therefore, in the cross-sectional analysis presented in Chapter 6 we assessed differences in fatigue by diabetes status. Fatigue was assessed using the vitality subscale of the Short Form-36 (Ware & Sherbourne, 1992). The questionnaire assesses self-reported fatigue in the past 4 weeks with items such as ‘did you feel full of life’ and ‘did you have a lot of energy’. Responses were rated on a 6 point scale ranging from 1 ‘all of the time’ to 6 ‘none of the time’. The items were summed such that that a higher score was indicative of higher

vitality and lower fatigue. Scores could range from 0-100 and a cut point of 50 was used to define fatigue, as suggested previously (Ware & Sherbourne, 1992). Using this cut-off a binary score (fatigued yes/no) was created. I was unable to assess the internal consistency (Cronbach's α) of this scale as I did not have access to the individual items of the questionnaire as part of my data sharing agreement with the Whitehall II group.

6. Study 3: Cross-sectional association of diurnal cortisol patterns with T2D in the Whitehall II study

6.1. Overview

This chapter concerns the findings from the first analysis of the Whitehall II dataset. As discussed in Chapter 1 the HPA axis is thought to play a role in T2D. However, the evidence to date for an association between diurnal cortisol patterns and T2D is equivocal. The aim of this study was to cross-sectionally examine associations of cortisol patterns throughout the day with T2D status in 3508 participants from the Whitehall II cohort. Details on the study method can be found in Chapter 5. A brief overview of the study background is presented followed by the results of this study and a discussion of the findings.

Note: Some of the results presented in this chapter have been published in Hackett, Steptoe, & Kumari, (2014). For the purpose of this PhD additional analyses were conducted assessing the association between cortisol AUC and diabetes status. Sensitivity analyses including self-reported stress on the day of sample collection are also presented.

6.2. Introduction

Cortisol plays a pivotal role in many physiological processes relevant to diabetes (Di Dalmazi et al., 2012). Chronic over-activation of the HPA axis can lead to dysregulated cortisol output (McEwen, 1998). As cortisol is essential for life any deviation from the optimal range is thought to have deleterious effects and neuroendocrine dysfunction has been implicated in diabetes. Pathological (Clayton et al., 2011) and experimental

(Connell et al., 1987) exposure to excessive cortisol has been related to diabetes risk factors such as central obesity and hypertension, and prolonged hypercortisolism as seen in as seen in Cushing's syndrome (Lacroix et al., 2015; Newell-Price et al., 2006) and in glucocorticoid-treated patients (Clare & Thurby-Hay, 2009) increases susceptibility for hyperglycemia and manifest T2D.

The role of cortisol in T2D was discussed in section 1.7.3 of Chapter 1 but to summarize for the purposes of this chapter; the initial research investigating the link between diabetes and cortisol provided mixed evidence for an association (e.g. Asfeldt, 1972; Bruehl et al., 2007; Chiodini et al., 2007). However, these early studies were limited by the use of various single time point cortisol measurements, as well as small sample sizes and participants selected from convenience samples. Increasingly, the marked diurnal patterning in the release of cortisol, rather than single cortisol sample assessment has been the focus of large-scale HPA axis research (Adam & Kumari, 2009; Collomp et al., 2016). The daily cortisol pattern is typically characterised by high cortisol levels on waking, followed by a rise that reaches a peak 30 minutes after waking (termed the CAR) and subsequent decline across day (Adam & Kumari, 2009). Another parameter of interest is total cortisol output over the day. This can be calculated using AUC (Pruessner et al., 2003). An illustration of the different components of daily cortisol release can be found in Figure 1.5 in Chapter 1.

HPA axis dysregulation is thought to cause reduction in the amplitude of the diurnal cortisol pattern, or a flatter slope in cortisol across the day (Adam & Kumari, 2009). Flattening of the diurnal cortisol slope can be driven by low cortisol output on waking and/or higher evening cortisol concentrations. In brief, flatter slope in cortisol over the day has been associated with diabetes-related outcomes such as central adiposity (Kumari, Chandola, Brunner, & Kivimaki, 2010) and an increased risk of

cardiovascular death (Kumari et al., 2011), yet the reported associations between diurnal cortisol patterns and T2D are equivocal.

The studies that have investigated the cross-sectional associations between components of daily cortisol secretion and diabetes status were discussed in Section 1.7.3.3 of Chapter 1. However, it is important to reiterate these findings for the purposes of the present chapter. In the MESA cohort, in the overall sample participants with T2D were found to have a significantly lower CAR than those without diabetes and in women those with T2D had a significantly higher AUC than controls (Champaneri et al., 2012). Bruehl *et al.*, similarly observed a blunted CAR in participants with T2D, but found no association of T2D with slope in cortisol across the day or AUC (Bruehl et al., 2009). In contrast, Lederbogen *et al.* observed a flattened slope in diurnal cortisol secretion among those with diabetes, but detected no association with the AUC (Lederbogen et al., 2011). Whereas, Vreeburg *et al.* observed no association between diabetes status and the CAR or diurnal cortisol slope or cortisol AUC (Vreeburg et al., 2009).

The possible reasons for these diverging findings were discussed previously. In brief, it is possible that differences in participant characteristics or in the number and timing of cortisol samples between studies may have contributed to these mixed results. We therefore sought to examine the association of diurnal cortisol secretion with diabetes status in sample of 3508 community-dwelling men and women of the Whitehall II study. We assessed 5 components of daily cortisol output in this study: waking cortisol, the CAR, the cortisol slope across the day, bedtime cortisol and cortisol AUC. Based on previous research we predicted that individuals with T2D would have a greater CAR, flatter slope in cortisol across the day and a greater cortisol output over the day as indexed by cortisol AUC.

6.3. Statistical analysis

Participants with cortisol values outside 3 SD from the mean and those taking steroid medications were removed from the analyses (n=171). Despite this, cortisol data were skewed and were therefore logged for analysis. The CAR was calculated by subtracting cortisol measured at time 1 (waking) from cortisol measured at time 2 (+30 min). Conventionally, analyses are restricted to samples that are collected within 10 min of waking (sample 1 taken >10 min, n=646) because of a reduced CAR in those with longer delays (Kudielka, Broderick, & Kirschbaum, 2003). We did not see a difference in sample delays by diabetes status so all participants were retained. The majority of participants (n= 3395, 96.8%) took cortisol sample 2 on time. We did not see a difference in late sample 2 collection by diabetes status so this was not included as a covariate in the analyses. The method used to calculate the diurnal slope in cortisol secretion has been previously described (Kumari, Badrick, Chandola, et al., 2009; Kumari et al., 2010, 2011). In brief, the slope of the decline in cortisol levels over the day was calculated by regressing cortisol values on time after waking for samples 1 (waking), 2 (+2.5 h), 4 (+8 h), 5 (+12 h), and 6 (bedtime). Because it is suggested that the CAR and slope in cortisol secretion are under different neurobiological control systems (Adam & Kumari, 2009), sample 2 was not included to ensure that the CAR does not obscure the slope calculation. Lower (more negative) slopes indicate a more rapid decline in cortisol levels, whereas slope values closer to zero reflect flatter diurnal rhythms. AUC with respect to ground was calculated using procedures described by Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, (2003). The time of the 6th cortisol sample differed between participants as they were instructed to take this sample at bedtime. Therefore the mean time interval (251 minutes) between cortisol sample 5 and the bedtime sample was used for the AUC calculation.

Descriptive and clinical characteristics of the sample were compared using *t*-tests for continuous variables and chi-square tests for categorical variables. Associations between prevalent diabetes and the cortisol measures were analysed using linear regression. Multivariable linear regressions using waking cortisol, CAR, slope, bedtime cortisol and AUC as outcome variables were performed to analyse associations with prevalent diabetes. Age, sex, grade of employment, smoking, waking time, late saliva sample 1 collection, fatigue, BMI, cardiovascular medication and history of CHD were included as covariates in all analyses. Sensitivity analysis including self-report stress on the day of cortisol collection as an extra covariate is also presented. Participants with missing covariate information were excluded from the analyses. Previous research has shown sex differences in the relationship between cortisol and diabetes status (Champaneri et al., 2012). Therefore, we investigated whether diabetes status interacted with sex, but found no significant associations with cortisol measures, so interaction terms were not included in the final models. A non-linear relationship between BMI and slope has been found previously in the Whitehall II cohort (Kumari et al., 2010). We investigated whether the pattern of results changed including BMI as a quadratic term. As the results were robust to controlling for the non-linear effects of BMI, only BMI as a continuous variable was included in the final models. Of the Whitehall II participants who provided cortisol data 92.8% (n=3508) were of white ethnicity. We investigated whether prevalent diabetes interacted with ethnicity in the current sample. This interaction term was significant for slope ($p < .001$) and bedtime cortisol ($p = 0.015$). The direction of the interaction term was negative for these cortisol variables. Therefore, we limited the present analysis to individuals of white ethnicity. Results are presented as unstandardised regression coefficients (B) with 95% CIs. The slope

estimates were generated using MLWin version 2.10 beta 6, all other analyses were conducted using SPSS version 22 (SPSS, Chicago, IL, USA).

6.4. Results

We restricted our analysis to those with complete information on time of waking, cortisol measures and diabetes status. This resulted in 3508 participants. The characteristics of participants included and excluded from the analysis are displayed in Table 6.1. The groups significantly differed in diabetes prevalence. However, this effect did not remain when non-white participants were removed from the excluded group ($p = 0.380$, data not shown). The group with complete cortisol data were younger, more likely to be male and had fewer participants in the lowest civil service employment grades. They were less likely to take cardiovascular medication and have a history of CHD than the phase 7 group who did not provide saliva samples.

Table 6.1 Participant characteristics at phase 7 of the Whitehall II study

	Participants included in the cortisol analyses (n= 3508)	Participants excluded from the analyses (n= 3459)	P value
Sex (% men)	2636 (75.1%)	2257 (65.3%)	< 0.001
Mean age (SD)	61.04 (5.94)	61.44 (6.06)	= 0.005
Smoker (% yes)	230 (6.6%)	274 (8.0%)	= 0.056
Employment grade (% lowest)	271 (7.7%)	489 (14.6%)	< 0.001
Mean BMI (SD)	26.68 (4.29)	26.84 (4.49)	= 0.131
Fatigued (% yes)	682 (19.5%)	700 (21.3%)	= 0.068
Cardiovascular medication (% yes)	1005 (28.6%)	1135 (33.1%)	< 0.001
History of CHD (% yes)	467 (13.7%)	570 (17.2%)	< 0.001
T2D (% yes)	238 (6.78%)	309 (8.9%)	< 0.001

Data presented as means \pm standard deviations and numbers (%)

The characteristics of the participants who provided cortisol samples are displayed in Table 6.2. Two hundred and thirty eight (6.78%) participants had prevalent diabetes at the time of saliva collection. The group with diabetes were older on average and were more likely to be in the lowest civil service employment grades. They were more likely to be obese, have a history of CHD and take cardiovascular medicine than those without diabetes. Cortisol collection measures, such as waking time on day of sampling did not differ by diabetes status.

Table 6.2 Participant characteristics at the time of cortisol assessment by diabetes status

	N of participants	No diabetes (n= 3270)	Prevalent Diabetes (n= 238)	P value
Mean age (SD)	3508	60.85 (5.89)	63.64 (5.99)	< 0.001
Sex (% men)	3508	2455 (75.1%)	181 (76.1%)	= 0.816
Smoker (% yes)	3506	212 (6.5%)	18 (7.6%)	= 0.522
Employment grade (% lowest)	3498	237 (7.3%)	34 (14.3%)	< 0.001
Obese (% yes)	3494	567 (17.4%)	80 (33.8%)	< 0.001
Fatigued (% yes)	3491	625 (19.2%)	57 (24.1%)	= 0.075
Late saliva collection (% yes)	3508	597 (18.3%)	49 (20.6%)	0.386
Cardiovascular medication (% yes)	3508	861 (26.3%)	144 (60.5%)	< 0.001
History of CHD (% yes)	3398	407 (12.8%)	60 (26.2%)	< 0.001

Data presented as means \pm standard deviations and numbers (%)

The average CAR in the sample was 7.33 (SD =11.575). As shown in

	Prevalent Diabetes (n= 238)	No diabetes (n= 3270)	Unadjusted P value	Model 1 P value	Model 2 P value
Waking cortisol (nmol/l)	16.32 (7.74)	15.82 (7.18)	0.383		
CAR (nmol/l)	7.35 (10.64)	7.54 (10.96)	0.923		
Slope (nmol/l)	-0.125 (0.022)	-0.129 (0.023)	0.002	0.014	0.017

per h)					
Bedtime cortisol (nmol/l)	2.59 (2.57)	2.34 (2.95)	0.002	0.020	0.046
AUC (nmol/l)	116.4 (40.81)	110.88 (40.23)	0.042	0.013	0.014

Table 6.3 the CAR did not differ by diabetes status ($B = 0.002$, $C.I. = -0.036$ to 0.039 , $p = 0.923$). The average diurnal slope estimated from the hierarchical linear model was -0.1290 nmol/l per h ($SD=0.023$). Participants with diabetes had a flatter slope in cortisol across the day than those without diabetes ($B = 0.004$, $C.I. = 0.001$ to 0.007 , $p = 0.014$). This association was robust to adjustment for age, sex, grade of employment, smoking, waking time, late saliva collection, fatigue, BMI, cardiovascular medication and history of CHD. A flatter slope in cortisol patterns across the day can be due to low waking values or high evening values of cortisol. We examined the association of these cortisol measures with diabetes status. While participants with diabetes had higher waking levels on average compared to those without diabetes, this difference was not significantly different ($B = 0.014$, $C.I. = -0.018$ to 0.046 , $p = 0.383$). In contrast, cortisol measures at bedtime differed significantly between the groups. Participants with diabetes had significantly greater bedtime cortisol values than those without diabetes controlling for covariates ($B = 0.063$, $C.I. = 0.010$ to 0.117 , $p = 0.020$). This suggests that raised evening cortisol levels accounted for the difference in slope between the two groups. Looking at cortisol AUC, the participants with diabetes had higher cortisol concentrations on average over the day than those without diabetes. This difference between the groups was significant and was robust to adjustment for covariates ($B = 7.74$, $C.I. = 1.667$ to 13.81 , $p = 0.013$). Sensitivity analysis including stress on the day of testing (model 2 below) as an extra covariate did not change the pattern of results.

Table 6.3 Mean (SD) values of cortisol measure by diabetes status at phase 7

	Prevalent Diabetes (n= 238)	No diabetes (n= 3270)	Unadjusted P value	Model 1 P value	Model 2 P value
Waking cortisol (nmol/l)	16.32 (7.74)	15.82 (7.18)	0.383		
CAR (nmol/l)	7.35 (10.64)	7.54 (10.96)	0.923		
Slope (nmol/l per h)	-0.125 (0.022)	-0.129 (0.023)	0.002	0.014	0.017
Bedtime cortisol (nmol/l)	2.59 (2.57)	2.34 (2.95)	0.002	0.020	0.046
AUC (nmol/l)	116.4 (40.81)	110.88 (40.23)	0.042	0.013	0.014

Model 1 adjusted for age, sex, smoking, grade of employment, waking time, fatigue, late saliva sample 1 collection, BMI, CVD medication, and history of CHD.

Model 2 additional adjustment for self-reported stress on the day of sample collection

6.5. Discussion

6.5.1. Results summary

This study investigated the cross-sectional association between components of the diurnal cortisol profile and diabetes status in a large population of community dwelling adults. To our knowledge this is the largest study to date to assess these associations. We found that the slope in cortisol across the day was flatter in those with compared to those without T2D. Our data suggest that the flat slope in cortisol in individuals with T2D is due to raised late evening cortisol levels rather than depressed morning levels. We also found that participants with diabetes have greater daily cortisol output on average than those without diabetes. These findings were robust to adjustment for a range of covariates. No association emerged for the CAR.

6.5.2. Earlier studies of daily cortisol secretion and diabetes status

Previous reports of the association between cortisol secretion and diabetes status are mixed (Bruehl et al., 2009; Champaneri et al., 2012; Lederbogen et al., 2011; Vreeburg et al., 2009). In the present study, we observed a flattened diurnal cortisol slope in participants with T2D. This corroborates the results of Lederbogen *et al.*, who found an association between diabetes status and flatter daily cortisol profiles in 979 individuals from a community cohort (Lederbogen et al., 2011). Similar to our analysis, individuals with T2D were observed to have raised evening cortisol concentrations compared to controls without diabetes. Elevated late-night cortisol levels have been suggested as a diagnostic criterion for Cushing's syndrome (Carroll, Raff, & Findling, 2009). We removed participants with very high cortisol concentrations from our analysis, which would serve to exclude individuals with Cushing's syndrome. Our findings were also independent of obesity, which is strongly associated with the disorder. Indeed, raised late-night salivary cortisol levels have been previously described in individuals with diabetes but without Cushing's syndrome (Liu, Bravata, Cabaccan, Raff, & Ryzen, 2005).

In contrast to our findings, Vreeburg *et al.*, found no association between T2D and diurnal cortisol slope in 491 individuals without psychopathology from the NESDA cohort (Vreeburg et al., 2009). Participants in the study provided four saliva samples within an hour of waking and two late-evening samples. The additional samples collected in the late morning and afternoon in the present investigation may account for the diverging findings, as we were better able to define the shape of the diurnal cortisol curve. It is possible that the lack of information on late morning and afternoon cortisol levels reduced the ability of Vreeburg *et al.* to examine the curvilinear nature of the decline in cortisol across the day (Adam & Kumari, 2009).

We failed to find an association between diabetes status and the CAR. This result is in contrast to the findings of Bruehl *et al.* (Bruehl *et al.*, 2009) and Champaneri *et al.* (Champaneri *et al.*, 2012) and who observed a blunted CAR in T2D individuals relative to controls. The reasons for the inconsistent results are unclear. However, our study is considerably larger than previous studies and consisted of a well-defined group of white community dwelling individuals. In contrast, the study by Bruehl *et al.* (Bruehl *et al.*, 2009), was limited by low participant numbers and a lack of adjustment for potential confounding factors. Champaneri *et al.*, investigated the association between diabetes status and cortisol secretion in a cohort of over a 1000 individuals (Champaneri *et al.*, 2012). However, the sample used was ethnically diverse and over 60% of the participants were of Hispanic origin. The present study was unpowered to detect the potential ethnic differences in the association between cortisol secretion and T2D and this may account for the differing findings between the studies.

Waking cortisol was not related to diabetes status in the current analysis. We have previously reported a relationship between fatigue and lower cortisol on waking (Kumari, Badrick, Chandola, *et al.*, 2009) and fatigue is a common complaint among individuals with diabetes (Fritschi & Quinn, 2010). Fatigue was independently associated with the diurnal cortisol slope and bedtime cortisol in the present study ($p=0.010$ and $p=0.017$ respectively; data not shown). However, the association between T2D and these cortisol measures was robust to adjustment for this factor.

We found that participants with T2D had a higher daily cortisol output on average (as indexed by AUC) in comparison to those without diabetes. This association was robust to adjustment for covariates. Of the previous studies that have investigated the association between cortisol AUC and diabetes status only one study has detected an relationship (Champaneri *et al.*, 2012). However, in the MESA cohort there was a sex

difference in the association such that women with T2D had a significantly higher cortisol AUC than controls. No associations were found for men (Champaneri et al., 2012). In the current study, there was no interaction by sex for AUC ($p = 0.263$). The majority of participants in this study were male (75.1%) and we had a much greater sample size than the Champaneri *et al* study. These factors may have improved our ability to detect an association between diabetes and AUC in both sexes.

6.5.3. Mechanisms linking T2D and cortisol

The causes of a flattened slope in diurnal cortisol and greater cortisol AUC are unknown and the mechanisms by which T2D is related to the HPA axis also remain to be elucidated. As mentioned previously cortisol plays a pivotal role in many physiological processes relevant to diabetes. It directly reduces insulin sensitivity and decreases insulin secretion by acting through glucocorticoid receptors, which are expressed on pancreatic β -cells. It triggers hepatic gluconeogenesis, promotes lipolysis and the release of fatty free acids into the circulation and the accumulation of triglycerides in adipose tissue (Di Dalmazi et al., 2012). Obesity is common in T2D and visceral adipose tissue expresses high levels of glucocorticoid receptors (Pou et al., 2007). It has been hypothesised that adipocytes are a source of cortisol. Research has shown that transgenic mice overexpressing 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), the enzyme activating the inactive form of glucocorticoids, have increased adipose levels of corticosterone (Masuzaki et al., 2001). Increased 11 β -HSD1 activity in human visceral adipose tissue has been associated with symptoms of the metabolic syndrome (Walker & Andrew, 2006). Thus, obesity offers one possible mechanism through which T2D might be associated with alterations in cortisol secretion.

Participants with diabetes in our sample were significantly more likely to be obese and obesity has been linked with HPA dysregulation (for a review see Incollingo Rodriguez et al., 2015). In the Whitehall II cohort obesity has been associated with a flattened slope in diurnal cortisol secretion (Kumari et al., 2010). As previously reported (Kumari et al., 2010), BMI as a continuous measure was not associated with the slope in diurnal cortisol secretion or AUC. However, obesity was independently associated with the diurnal cortisol slope and bedtime cortisol. Despite this the relationship between diabetes status and these cortisol measures was robust to adjustment for obesity (sensitivity analysis data not shown).

Inflammation is another pathway through which T2D might be related to alterations in HPA axis function. As mentioned previously, inflammatory cytokines are involved in the pathogenesis of T2D. Circulating cytokine levels are elevated in diabetic individuals (Pickup, 2004) and heightened concentrations are predictive of T2D development in initially healthy samples (Wang et al., 2013). Cortisol is involved in the regulation of inflammation (Di Dalmazi et al., 2012) and circadian rhythms are regulated at the hypothalamic level by the suprachiasmatic nuclei. It has been suggested that circadian control is an important aspect of hypothalamic-immune communication, and that glucocorticoids may dysregulate the immune response via circadian-immune communication (Arjona & Sarkar, 2008). It is also possible that disturbances in circadian rhythms may act on T2D through the alteration of glucose metabolism. Experimental work indicates that circadian disruption increases both fasting and postprandial plasma glucose concentrations through inadequate pancreatic insulin secretion (Buxton et al., 2012). Additional research is needed to examine whether changes in inflammation and alterations in glucose metabolism may underlie the association between flatter slopes in diurnal cortisol section and T2D.

Another mechanism that may explain the relationship between diurnal cortisol slope and T2D is psychosocial stress. As discussed at length in Chapter 1, results from meta-analyses and longitudinal studies indicate that psychosocial factors increase the risk of developing T2D (Pouwer et al., 2010) and contribute to disease progression in diabetic individuals (Chida & Hamer, 2008). Cortisol levels are elevated by exposure to stress (Miller et al., 2007) and the flattening of the diurnal cortisol slope has been associated with both acute and chronic stress factors (Adam, Hawkley, Kudielka, & Cacioppo, 2006; Collomp et al., 2016). Acute stress assessed by stressful events on the day of saliva sampling was not associated with the slope in cortisol, evening cortisol or AUC in the current analysis and our findings remained independent of acute stress. However, it is possible that the findings could be attributed to long-term changes in circadian regulation as a result of chronic stress in people with T2D. Additional research is needed to examine whether chronic stress factors may underlie the association of flattened diurnal cortisol slopes with T2D.

6.5.4. Limitations

In the present study we assessed cortisol across the day in a large community-based sample. The participants with T2D were well-characterised and we were able to use data from the larger cohort study to adjust for a number of potentially confounding factors in our analysis. However, our findings should be interpreted in light of some limitations. The Whitehall II study is an occupational cohort of civil servants and as such, our sample is not representative of the general population. For example, there is an SES gradient in diabetes, such that people from lower SES groups are more likely to get the condition (HSCIC, 2014). Although grade of employment is an appropriate measure of SES in this cohort, people in the civil service are in paid work receiving a regular

income so these results may not apply to those who are unemployed or are receiving benefits. Furthermore, the civil service departments that took part in the Whitehall II study were all based in London and there is a recognized divide in rural versus urban prevalence of diabetes (International Diabetes Federation, 2015). Due to ethnic differences in the pathogenesis of T2D (Tillin et al., 2013) we restricted the current analysis to white individuals. We investigated whether prevalent diabetes interacted with ethnicity for the wider phase 7 cohort and the interaction term was significant for bedtime cortisol and slope but not for the other cortisol measures. However, we were under powered to investigate differences by ethnicity as the larger cohort study is predominately of white ethnicity. Therefore, the present results may not generalise to other populations.

T2D was assessed by self-report of doctor diagnosis, use of diabetic medications or OGGT rather than clinical diagnosis. We also lacked data on the duration of T2D, which may be related to neuroendocrine function. The associations observed in the analyses were small. However, these patterns are thought to be representative of chronic differences that are present on an everyday basis. Under these circumstances, even modest effects may contribute to substantial accumulated differences in cortisol output over time. Studies specifically designed to test the association between T2D and neuroendocrine function would provide richer data. The cross-sectional design makes it impossible to draw causal conclusions about the temporal relationship between aberrant cortisol output and diabetes. The longitudinal analysis presented in the following chapter sheds some light on this issue.

Cortisol was assessed over a single day, whereas Champaneri *et al.*, assessed diurnal cortisol output over 3 consecutive days (Champaneri et al., 2012). Champaneri *et al.* found that participants with diabetes had a lower CAR, whereas we detected no

associations for the CAR in the present analysis. It has been suggested that single day assessment of cortisol may obscure the CAR to situational rather than chronic correlates (Hellhammer et al., 2007). Furthermore, the night release of cortisol was not assessed and therefore it was not possible to evaluate total 24 hour circadian cortisol exposure. We only measured free cortisol levels in the study and did not assess glucocorticoid receptor function. As mentioned previously in a sub-analysis of the Diabetes Study glucocorticoid receptor function was found to be altered in people with diabetes in comparison to controls (Carvalho et al., 2015). It is possible that the findings reflect reduced episodic cortisol release which has been reported to modify the regulation of glucocorticoid sensitive genes (Stavreva et al., 2009). We relied on self-report for the timing of sample collection, whereas other studies have used salivettes with ‘track caps’ to objectively assess the timing of cortisol sample collection (Champaneri et al., 2012). Our prevalence of ‘late’ reporting was similar to previously reported rates (Kudielka et al., 2003) and evidence suggests that participants are generally accurate in their recording of this information (Dockray, Bhattacharyya, Molloy, & Steptoe, 2008). We did not see a difference in sample delays by diabetes status so all participants were retained for the final analysis. However, over 600 individuals in this study took the waking sample late (> 10 minutes after waking). To limit confounding we included late saliva sample 1 collection as a covariate in all our analyses.

6.5.5. Conclusion

Despite these considerations, our findings indicate that flat slopes in salivary cortisol, raised evening levels of cortisol and greater cortisol AUC are associated with T2D in non-clinical population of middle-aged men and women. The mechanisms by which these associations occur remain to be determined. It is possible that neuroendocrine

dysfunction may be related to the pathophysiology of T2D. However, longitudinal studies are required to assess the prognostic properties of cortisol secretion for T2D.

7. Diurnal cortisol, future diabetes and impaired glucose metabolism in the Whitehall II study

7.1. Overview

The study presented in Chapter 6 assessed the cross-sectional associations between components of diurnal cortisol secretion and diabetes status at phase 7 (2002-2004) of the Whitehall II cohort. The results of this study suggest that people with diabetes have a flatter slope in cortisol, raised evening cortisol levels and greater cortisol AUC over the day in comparison to controls. This chapter builds upon these findings by assessing the prospective association of diurnal cortisol secretion with future glucose disturbance in 3270 participants from the Whitehall II cohort. Participants who were normoglycaemic at the time of cortisol collection (phase 7) were re-examined at phase 11 (2012-2013). Details on the study method can be found in Chapter 5. A brief

overview of the study background is presented followed by the results of this study and a discussion of the findings.

Note: Some of the results presented in this chapter have been published in Hackett, Kivimäki, Kumari, & Steptoe, (2016). For the purpose of this PhD additional analyses were conducted assessing the association between cortisol AUC and future disturbances in glucose metabolism.

7.2. Introduction

Type 2 diabetes is characterised by hyperglycaemia resulting from insulin resistance and β -cell dysfunction (American Diabetes Association, 2012). As discussed in Chapter 1 the hyperglycaemia of T2D develops gradually (Tabák et al., 2009) and evidence suggests that the health risk accompanying raised glucose is continuous (Emerging Risk Factors Collaboration et al., 2010). An illustration of glucose and insulin trajectories over time can be seen in Figure 1.1 of Chapter 1. Due to the increasing health risk associated with rising glucose concentrations intermediate states of hyperglycaemia that are higher than normal but do not meet the diagnostic criteria for T2D have been defined (American Diabetes Association, 2012). These ‘pre-diabetes’ states are significant as individuals with glucose concentrations in this range have an elevated risk of developing T2D and diabetes complications (Morris et al., 2013; Tabák et al., 2012).

As mentioned previously in this thesis cortisol plays a role in many processes relevant to T2D (Di Dalmazi et al., 2012). Several studies have investigated the cross-sectional association between daily cortisol secretion and diabetes, but the findings have

been mixed. In Chapter 6 the results from the largest study to date in this area were presented. In this analysis of 3508 individuals from the Whitehall II cohort, we found that participants with T2D had a flatter slope in cortisol across the day. This corroborates the findings of Lederbogen *et al.*, who observed an association between flatter daily cortisol profiles and T2D in a community cohort (Lederbogen *et al.*, 2011). In both studies, individuals with T2D had significantly higher evening cortisol levels compared with controls (Chapter 6; Lederbogen *et al.*, 2011). Participants with diabetes were also found to have greater daily cortisol output (as indexed by AUC) on average than those without diabetes. Of the previous studies that have investigated the association between cortisol AUC and diabetes status only one study has detected an relationship (Champaneri *et al.*, 2012). However, in the MESA cohort there was a sex difference in the association such that women with T2D had a significantly higher cortisol AUC than controls. No associations were found for men in this study (Champaneri *et al.*, 2012). In the cross-sectional analysis presented in Chapter 6 we detected no interaction by sex for AUC.

However, diverging findings from the analysis presented in Chapter 6 have also been reported in the literature. Champaneri *et al.* (Champaneri *et al.*, 2012) and Bruehl *et al.* (Bruehl *et al.*, 2009) found a blunted CAR in individuals with T2D relative to controls, but no association for cortisol slope. Whereas, Vreeburg *et al.* (Vreeburg *et al.*, 2009), found no association between any component of the diurnal cortisol curve and T2D.

Longitudinal evidence relating neuroendocrine dysfunction with IFG (a form of pre-diabetes) or T2D is sparse. In the LASA cohort morning and evening salivary cortisol were measured in 998 initially healthy people. Raised evening cortisol was

associated with future T2D in female participants, but no associations were found for men (Schoorlemmer et al., 2009).

In the MESA cohort changes over time in cortisol parameters were assessed in people with prevalent diabetes and controls rather than looking at whether changes in cortisol predict new onset diabetes (Spanakis et al., 2016). In this study of 580 people, of whom 90 had T2D, no statistically significant change in cortisol parameters was found in the participants with T2D compared with controls over 6 years follow-up. This offers the possibility that alterations in cortisol may precede diabetes onset.

To date no study has examined the relationship between the complete diurnal cortisol profile and incident T2D in an initially healthy population. We therefore sought to examine these associations in the Whitehall II cohort. In keeping with our cross-sectional findings we hypothesised that a flatter diurnal cortisol slope, raised evening cortisol levels and greater cortisol AUC at phase 7 (2002-2004) would predict new onset IFG and T2D at phase 11 (2012-2013).

7.3. Method

The method for the current study can be found in Chapter 5. To reiterate in brief we used data from phase 7 (2002–2004) and phase 11 (2012-2013) of the Whitehall II study. Salivary cortisol was assessed from 6 samples over the course of a normal day at phase 7. Participants who had prevalent diabetes at phase 7 (n=238) were excluded from the analysis giving a final sample of 3270 participants at phase 11. We assessed whether components of diurnal cortisol secretion were predictive of new onset IGT and T2D at phase 11. New onset T2D cumulating from the end of phase 7 (2002-2004) to phase 11 (2012-13) was defined as a fasting glucose ≥ 7.0 mmol/l or by reported doctor

diagnosed diabetes, or diabetes medication usage. IFG was classified as a fasting glucose between 5.6 and 6.9mmol/l (American Diabetes Association, 2012).

7.4. Statistical analysis

We removed participants with cortisol values outside 3SD from the mean and those taking steroid medications from the analyses (n=171). Despite this, cortisol data were skewed and were logged for analysis. The CAR was calculated by subtracting cortisol at time 1 (waking) from cortisol at time 2 (30 minutes after waking). Analyses are conventionally limited to samples collected within 10 minutes of waking (sample 1 taken ≥ 10 minutes, n=579) because of reduced CAR in those with longer delays (Kudielka et al., 2003). We did not find a difference in sample delays by new onset diabetes or IFG so all participants were retained. Participants were asked to refrain from eating 15 minutes before sample collection and there was high adherence to this protocol. We checked whether eating between sample 1 and 2 affected the pattern of results. We did not find a relationship between eating behaviour on the morning of sampling and new onset T2D or IFG so all samples were retained for analysis. The slope of the decline in cortisol levels over the day was calculated by regressing cortisol values on time after waking for samples 1 (waking), 2 (2.5 hours), 4 (8 hours), 5 (12 hours), and 6 (bedtime) (Hackett et al., 2014; Kumari et al., 2011). It is thought that the CAR and slope are under different neurobiological control systems (Adam & Kumari, 2009). Therefore sample 2 was not included to ensure the CAR did not obscure the slope calculation. More negative slopes indicate a more rapid decline in cortisol levels, whereas slope values closer to zero reflect flatter diurnal rhythms. AUC with respect to ground was calculated using procedures described by Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, (2003). The time of the 6th cortisol sample differed

between participants as they were instructed to take this sample at bedtime. Therefore the mean time interval (251 minutes) between cortisol sample 5 and the bedtime sample was used for the AUC calculation. Descriptive characteristics of the sample were compared using univariate ANOVA for continuous variables and chi-square tests for categorical variables. Z-scores (mean =0; SD =1) were created for waking cortisol, the CAR, slope, evening cortisol and AUC. The associations between the cortisol measures and new onset diabetes or IFG at phase 11 were assessed using logistic regression. Age, sex, grade of employment, smoking, BMI, IFG at phase 7, cardiovascular medication and history of CHD were included as covariates in all analyses. Participants with missing covariate information were excluded from the analyses. A nonlinear relationship between BMI and cortisol slope has been found previously in the Whitehall II cohort (Kumari et al., 2010). Therefore BMI was categorised using the cut-point of 23 where the relationship between slope and BMI changes. Including BMI as a continuous variable did not change the pattern of results. Sex differences in the relationship between cortisol and diabetes have been reported previously (Champaneri et al., 2012; Schoorlemmer et al., 2009). We investigated whether sex interacted with the cortisol measures but found no significant associations, so interaction terms were not included in the final models and men and women were analysed in combination. Results are presented as adjusted OR with 95% CIs. The slope estimates were generated using MLWin version 2.10 beta 6, all other analyses were conducted using SPSS version 22 (SPSS, Chicago, IL, USA).

7.5. Results

We restricted our study group to those with complete information on cortisol measures and removed participants with prevalent diabetes at phase 7 (n=238) giving a final

sample of 3270. Participants with prevalent diabetes were removed from the excluded group for comparative purposes (n=179). The characteristics of participants included and excluded from the analyses are displayed in Table 7.1. The group included in the analyses were more likely to be male and had fewer participants in the lowest civil service employment grades. They were less likely to smoke, take cardiovascular medication and have a history of CHD than the excluded group.

Table 7.1 Characteristics of participants included and excluded from the analyses

	Phase 7 without complete cortisol information (n= 1458)	Participants included in the analyses (n=3270)	P value
Sex (% men)	1006 (69.0%)	2455 (75.1%)	< 0.001
Mean age (SD)	60.77 ± 5.96	60.85 ± 5.89	= 0.699
Smoker (% yes)	128 (8.9%)	212 (6.5%)	= 0.013
Employment grade (% lowest)	197 (13.9%)	237 (7.3%)	< 0.001
Mean BMI (SD)	26.78 ± 4.38	26.52 ± 4.18	= 0.058
Cardiovascular medication (% yes)	449 (31.3%)	861 (26.3%)	< 0.001
History of CHD (% yes)	253 (18.1%)	407 (12.8%)	< 0.001

Data are presented as means ± standard deviations and numbers (percentages)

For the purposes of the study, the participants were divided into three groups based on their glucose status at phase 11. The characteristics of the groups are displayed in Table 7.2. Two hundred and ten (6.4%) participants had new onset diabetes and 518 (15.8%) individuals had IFG at phase 11. The individuals with diabetes were older, more likely to be male and more likely to be the lowest civil service employment grades compared with normoglycaemic individuals. They had higher BMI and were more likely to have a history of CHD and take cardiovascular medication than normoglycaemic or IFG participants. Late saliva collection and unadjusted cortisol values did not significantly differ by glucose status.

Table 7.2 Characteristics of participants at the time of cortisol assessment (2002-2004) by glucose status at phase 11 (2012-2013)

	Normoglycaemic (n=2542)	IFG (n=518)	Incident diabetes (n= 210)	p value*
Mean age (SD)	60.94 ± 5.90	60.13 ± 5.72	61.45 ± 6.03	= 0.005
Sex (% men)	1862 (73.2%)	433 (83.6%)	160 (76.2%)	< 0.001
Smoker (% yes)	172 (6.8%)	25 (4.8%)	15 (7.1%)	= 0.433
Employment grade (% lowest)	189 (7.4%)	20 (3.9%)	28 (13.5%)	< 0.001
Mean BMI (SD)	26. 18 ± 4.13	27.16 ± 3.76	29.18 ± 4.74	< 0.001
Cardiovascular medication (% yes)	626 (24.6%)	146 (28.2%)	89 (42.4%)	< 0.001
History of CHD (% yes)	299 (12.1%)	62 (12.4%)	46 (22.9%)	< 0.001
Waking cortisol (nmol/l)	15.99 ± 7.2	15.19 ± 7.07	15.41 ± 7.07	= 0.066
CAR (nmol/l)	7.35 ± 10.9	8.65 ± 11.31	7.04 ± 10.61	= 0.102
Slope across the day (nmol/l per h)**	-0.1304 ± 0.022	-0.1288 ± 0.022	-0.1274 ± 0.023	= 0.083

Bedtime cortisol (nmol/l)	2.33 ± 3.03	2.36 ± 2.83	2.42 ± 2.07	= 0.895
AUC (nmol/l)***	110.19 ± 40.05	114.69 ± 41.54	109.89 ± 38.72	= 0.067

Data are presented as means ± standard deviations and numbers (percentages)

* p values refer to an overall comparison of the three groups

**To calculate the slope cortisol values were log-transformed

Table 7.3 shows the ORs for incident diabetes and combined diabetes and IFG per 1 SD

		Incident Diabetes (n=210)		IFG or Incident Diabetes (n= 728)	
Waking cortisol (nmol/l)	Unadjusted	0.95 (C.I. 0.83 – 1.09)	<i>p</i> = 0.446	0.91 (C.I. 0.84 – 0.99)	<i>p</i> = 0.022
	Adjusted	0.98 (C.I. 0.84 – 1.13)	<i>p</i> = 0.745	0.93 (C.I. 0.85 – 1.01)	<i>p</i> = 0.064
CAR (nmol/l)	Unadjusted	0.95 (C.I. 0.83 – 1.09)	<i>p</i> = 0.498	1.08 (C.I. 0.99 – 1.17)	<i>p</i> = 0.070
	Adjusted	0.93 (C.I. 0.79 – 1.07)	<i>p</i> = 0.318	1.04 (C.I. 0.95 - 1.13)	<i>p</i> = 0.350
Slope across the day (nmol/l per h)	Unadjusted	1.13 (C.I. 0.98 – 1.30)	<i>p</i> = 0.086	1.09 (C.I. 1.01 - 1.19)	<i>p</i> = 0.038
	Adjusted	1.15 (C.I. 0.99 - 1.33)	<i>p</i> = 0.075	1.12 (C.I. 1.02 - 1.22)	<i>p</i> = 0.015
Bedtime cortisol (nmol/l)	Unadjusted	1.15 (C.I. 0.99 – 1.32)	<i>p</i> = 0.055	1.05 (C.I. 0.97 – 1.15)	<i>p</i> = 0.209
	Adjusted	1.18 (C.I. 1.01 - 1.37)	<i>p</i> = 0.035	1.10 (C.I. 1.01 - 1.20)	<i>p</i> = 0.044
AUC (nmol/l)	Unadjusted	0.99 (C.I. 0.99 – 1.01)	<i>p</i> = 0.714	1.00 (C.I. 1.00 – 1.01)	<i>p</i> = 0.069
	Adjusted	1.00 (C.I. 0.87 - 1.16)	<i>p</i> = 0.973	1.09 (C.I. 0.99 - 1.19)	<i>p</i> = 0.096

increase in scores of the cortisol measures. We found no association between the CAR and incident diabetes (*p* = 0.318). For slope across the day we observed a trend for a flatter slope in participants with new onset diabetes (*p* = 0.075). A flattened cortisol slope can be due to low waking or high evening cortisol values. No association was observed between waking cortisol and T2D (*p* = 0.745). In contrast, evening cortisol was predictive of incident diabetes (*p* = 0.035). This association was robust to adjustment for all covariates. Participants with new onset diabetes had higher evening cortisol values (\bar{x} = 2.43, *SD* = 2.07) than participants without diabetes (\bar{x} = 2.34, *SD* =

2.99) controlling for covariates. We found no association between cortisol AUC and incident diabetes ($p = 0.973$).

Table 7.3 OR of incident diabetes and combined incident diabetes and IFG among 3270 individuals from phase 7-phase 11 by z scores of cortisol measures

		Incident Diabetes (n=210)		IFG or Incident Diabetes (n= 728)	
Waking cortisol (nmol/l)	Unadjusted	0.95 (C.I. 0.83 – 1.09)	$p = 0.446$	0.91 (C.I. 0.84 – 0.99)	$p = 0.022$
	Adjusted	0.98 (C.I. 0.84 – 1.13)	$p = 0.745$	0.93 (C.I. 0.85 – 1.01)	$p = 0.064$
CAR (nmol/l)	Unadjusted	0.95 (C.I. 0.83 – 1.09)	$p = 0.498$	1.08 (C.I. 0.99 – 1.17)	$p = 0.070$
	Adjusted	0.93 (C.I. 0.79 – 1.07)	$p = 0.318$	1.04 (C.I. 0.95 - 1.13)	$p = 0.350$
Slope across the day (nmol/l per h)	Unadjusted	1.13 (C.I. 0.98 – 1.30)	$p = 0.086$	1.09 (C.I. 1.01 - 1.19)	$p = 0.038$
	Adjusted	1.15 (C.I. 0.99 - 1.33)	$p = 0.075$	1.12 (C.I. 1.02 - 1.22)	$p = 0.015$
Bedtime cortisol (nmol/l)	Unadjusted	1.15 (C.I. 0.99 – 1.32)	$p = 0.055$	1.05 (C.I. 0.97 – 1.15)	$p = 0.209$
	Adjusted	1.18 (C.I. 1.01 - 1.37)	$p = 0.035$	1.10 (C.I. 1.01 - 1.20)	$p = 0.044$
AUC (nmol/l)	Unadjusted	0.99 (C.I. 0.99 – 1.01)	$p = 0.714$	1.00 (C.I. 1.00 – 1.01)	$p = 0.069$
	Adjusted	1.00 (C.I. 0.87 - 1.16)	$p = 0.973$	1.09 (C.I. 0.99 - 1.19)	$p = 0.096$

Adjusted for age, sex, smoking, grade of employment, BMI greater than 23, cardiovascular medication, history of CHD and IFG at phase 7.

To further explore the relationships between the cortisol measures and future glucose status and in particular the trend for a flattened slope in those with T2D, we analysed the prospective association between cortisol measures and glucose status in the combined group of participants with new onset diabetes or IFG at phase 11. Again no associations were detected between the CAR or the AUC and incident diabetes or IFG ($p = 0.35$ and $p = 0.096$, respectively). However, slope was significantly predictive of new onset diabetes or IFG at phase 11 controlling for covariates ($p = 0.015$). Participants with incident diabetes or IFG had flatter slope in cortisol across the day at baseline ($\bar{x} = -0.128$, $SD = 0.023$) compared with normoglycaemic controls ($\bar{x} = -$

0.130, $SD = 0.022$). No significant association was found between waking cortisol and incident diabetes or IFG ($p = 0.064$), but evening cortisol was associated with future diabetes or IFG ($p = 0.044$) adjusting for covariates. Participants with diabetes or IFG had higher evening cortisol values ($\bar{x} = 2.40$, $SD = 2.67$) than normoglycaemic individuals ($\bar{x} = 2.33$, $SD = 3.02$) controlling for covariates. It is possible that raised cortisol levels later in the day contributed to the difference in slope between the two groups.

7.6. Discussion

7.6.1. Results summary

This study investigated the longitudinal association between components of the diurnal cortisol profile and future T2D and IFG in a sample of community-dwelling adults. We found that raised evening cortisol levels were predictive of new onset T2D approximately 9 years later. For slope across the day we observed a trend for a flatter slope in participants with incident diabetes. We found that a flattened diurnal cortisol slope at phase 7 was predictive of the combined outcome of future IFG or T2D at phase 11. Evening cortisol was also associated with future diabetes or IFG. No associations emerged for the CAR, waking cortisol or the AUC.

7.6.2. Comparison to previously published work

To our knowledge this is the first study to investigate the prospective associations between the diurnal cortisol profile and future T2D as well as IFG. However, the association between morning and evening cortisol and future T2D has been investigated previously in the LASA cohort (Schoorlemmer et al., 2009). Our finding that evening cortisol at phase 7 is predictive of new onset diabetes at phase 11 corroborates and

extends the results of that study. In the LASA cohort, raised evening cortisol concentrations were associated with future T2D but only in female participants. We checked for an interaction between sex and cortisol measures but found no significant associations. Our study is considerably larger than the LASA cohort study and this may account for our ability to detect an association between evening cortisol and incident diabetes in both sexes. Raised evening cortisol has been associated with T2D in cross-sectional studies (Lederbogen et al., 2011) including the study presented in Chapter 6 (Hackett et al., 2014). The present study adds to these findings by showing that evening cortisol is not only elevated in people with prevalent diabetes but is predictive of new onset diabetes in initially healthy individuals. No associations between waking cortisol and incident T2D were detected in the study. This finding is in keeping with the LASA study (Schoorlemmer et al., 2009) and the cross-sectional work presented in Chapter 6 (Hackett et al., 2014).

No associations emerged for the CAR in the present study. The lack of a prospective association with the CAR corroborates the results from our cross-sectional analysis (Chapter 6; Hackett et al., 2014). We observed a borderline significant result for a flatter cortisol slope in participants with new onset T2D. Expanding this analysis to a broader category of glucose disturbance we found that participants with IFG or T2D had a flatter cortisol slope compared with normoglycaemic controls. The analysis of the T2D group alone may have been underpowered to detect a significant effect. This result builds upon the work presented in Chapter 6 (Hackett et al., 2014) and suggests that a flatter slope in cortisol might also be predictive of future disturbances in glucose metabolism in an initially healthy population.

Cortisol AUC was not associated with future diabetes or the combined IGT and diabetes outcome in the current study. In the cross-sectional analysis presented in

Chapter 6, participants with diabetes were found to have a higher cortisol AUC than controls without the condition. In the MESA cohort the association of T2D with longitudinal changes in components of the daily cortisol secretion curve was assessed (Spanakis et al., 2016). Over 6 years of follow-up, change in AUC was not found to be significantly different those with diabetes versus controls. Similarly to this research this suggests that AUC may not have good predictive value over time.

7.6.3. Mechanisms linking T2D and cortisol

Cortisol plays an important role in many processes relevant to IFG and T2D. One major function of cortisol is to raise glucose through gluconeogenesis. By acting via glucocorticoid receptors, which are expressed on β -cells cortisol directly reduces insulin sensitivity and decreases insulin secretion. Cortisol can induce lipolysis and the release of fatty free acids into the bloodstream and the build-up of triglycerides in adipose tissue (Di Dalmazi et al., 2012). Both these factors are associated with an increased risk of diabetes although it is unclear whether these associations are causal (Pankow et al., 2004; Silva et al., 2011). Changes in diurnal cortisol secretion have been associated with disorders that are possible complications of T2D and impaired glucose metabolism. Previous work in this cohort has shown that flatter diurnal cortisol rhythms and raised evening cortisol levels are predictive of cardiovascular mortality (Kumari et al., 2011).

The reasons for the alterations in cortisol secretion observed in this study are unknown and the mechanism by which T2D and IFG are related to the HPA axis remains unclear. The findings of the earlier studies in this thesis suggest that stress may be important. An increasing body of literature suggests that psychosocial stress factors increase the risk of T2D (Pouwer et al., 2010). Stress stimulates the HPA axis inducing

cortisol release (Miller et al., 2007) and acute and chronic stressors have been linked with a flatter cortisol slope across the day and raised evening cortisol concentrations (Adam et al., 2006; Collomp et al., 2016). As reported in Chapter 6 acute stress on the day of saliva sampling was not associated with slope or evening cortisol in this sample. The association between evening cortisol and future diabetes and the association between slope and the combined IGT and T2D outcome was robust to adjustment for acute stress ($p = 0.029$ and $p=0.035$ respectively). However, it is plausible that our results could be due to long-term changes in circadian regulation as a result of chronic stress which predisposed participants to future IFG and T2D.

Obesity is another potential pathway that could link cortisol and diabetes. Previous work in the Whitehall II cohort has associated obesity with a flatter cortisol slope and raised evening cortisol concentrations (Kumari et al., 2010). Obesity is risk factor for T2D (Guh et al., 2009) and weight loss is the treatment of choice for pre-diabetes (Tabák et al., 2012). Visceral adipose tissue expresses high concentrations of glucocorticoid receptors (Pou et al., 2007) and it is thought that adipocytes are a source of cortisol. In Chapter 6 research on the enzyme 11 β -HSD1 which regulates glucocorticoid metabolism at the tissue level was presented. In brief, mice overexpressing 11 β -HSD1 have heightened adipose tissue levels of corticosterone (Masuzaki et al., 2001) and in humans increased 11 β -HSD1 activity has been associated with features of the metabolic syndrome (Walker & Andrew, 2006). Inhibitors of 11 β -HSD1 are being trialled as a potential treatment for T2D (Rosenstock et al., 2010). BMI was associated with cortisol slope and evening cortisol, and was predictive of new onset T2D, as well as the broader category of glucose disturbance in our analysis. Additionally, participants with new onset IFG and T2D had higher BMIs than the normoglycaemic individuals in our sample. Despite this the relationship between future

glucose status and these cortisol measures was robust to adjustment for BMI. This suggests that in this cohort obesity is not the mechanism by which diurnal cortisol secretions increases the risk of T2D and IFG.

Abnormal daily cortisol rhythms may disrupt immune and inflammatory processes. Inflammatory cytokines play a role in T2D and heightened cytokine concentrations are predictive of T2D development (Wang et al., 2013). Cortisol plays a role in regulating inflammation and it has been suggested that glucocorticoids might dysregulate immunity via circadian-immune communication (Arjona & Sarkar, 2008). As well as changes in immune and inflammatory processes, it is possible that disturbances in circadian rhythms may act on IFG and T2D through the alteration of glucose metabolism. More information on this pathway can be found in section 6.5.3 of Chapter 6.

7.6.4. Limitations

Our results should be interpreted in light of some limitations. Many of these shortcomings were addressed in Chapter 6 but to reiterate in brief for the present study the Whitehall II study is an occupational cohort of civil servants and the cohort participants are predominately of white ethnicity. Due to ethnic differences in the pathogenesis of T2D (Tillin et al., 2013) the current analysis was restricted to white individuals. Therefore, the findings presented in this chapter are not necessarily generalizable to other populations. Glucose was only measured at the Whitehall clinical waves. Therefore it was not possible to assess when exactly incident IFG and T2D occurred in the follow-up period. The effects observed in our analyses were small. However, these patterns are thought to be representative of chronic differences that are present on an everyday basis. Our findings suggest that even modest differences in daily

cortisol secretion may have negative effects on glucose status over time. We only assessed cortisol over a single day and it is suggested that this may obscure the CAR to situational rather than chronic correlates (Hellhammer et al., 2007). This may have contributed to our inability to detect an association between the CAR and future glucose status in the present analysis. This is an observational study so we are not able to determine causal relationships, and other factors such as food intake, chronic stress or other psychological factors may have contributed to or independently driven the association between cortisol and later glucose status. We do not have information on the amount or type of food participants ate on the day of cortisol collection. The night release of cortisol was not assessed and therefore we could not evaluate total 24-hour circadian cortisol exposure. We relied on self-report for the timing of sample collection as evidence suggests that people are usually accurate in reporting this information (Dockray et al., 2008). The prevalence of ‘late’ collection was comparable to previously reported rates (Kudielka et al., 2003).

7.6.5. Conclusion

Despite these considerations, our findings indicate that diurnal cortisol secretion is associated with future T2D and impaired glucose metabolism in a non-clinical population. It is plausible that neuroendocrine dysfunction is related to the pathophysiology of T2D, but the precise mechanisms through which changes in cortisol secretion impairs glucose metabolism remain to be determined.

8. Discussion

8.1. Overview

This PhD consisted of four studies with an overarching aim to assess the associations between psychological wellbeing, stress-related biological processes and diabetes. Two different methods of investigation were used in this thesis to fulfil this aim: acute laboratory stress testing and naturalistic monitoring in a large epidemiological cohort. A body of evidence has highlighted the involvement of psychosocial stress in the pathogenesis of CVD. Considering the heightened risk of CVD in people with T2D, the

literature concerning the role of psychosocial stress in T2D has grown in recent years. However, gaps in understanding remain particularly with regards to the biological pathways linking psychosocial factors and diabetes.

In Study 1 (presented in Chapter 3) the notion that people with T2D experience chronic allostatic load, manifest as dynamic disturbances in reactivity to and recovery from stress across multiple biological systems, coupled with heightened experience of chronic life stress was tested by comparing people with diabetes to matched controls. Study 2 (presented in Chapter 4) focused on the diabetes group alone assessed the impact of hostility (a psychosocial factor of interest) on physiological stress reactivity. Studies 3 and 4 (presented in Chapters 6 & 7) assessed neuroendocrine disturbances in diabetes using Whitehall II study data. Study 3 assessed whether daily cortisol output differs between people with and without diabetes cross-sectionally. Following on from this Study 4 used a prospective approach to assess whether components of daily cortisol output are linked to future diabetes in an initially healthy sample. In this chapter the hypotheses and findings of the four studies presented in this thesis will be briefly summarised and the contribution of these studies to the literature will be highlighted. The limitations of this thesis, implications of this work and ideas for future research will also be discussed.

8.2. Main findings and their implications

8.2.1. Study 1: Comparing physiological responses to stress in people with and without diabetes

In Study 1 (presented in Chapter 3) the notion that people with T2D experience chronic allostatic load, manifest as dynamic disturbances in reactivity to and recovery from stress across multiple biological systems, coupled with heightened experience of

chronic life stress was tested. The background to this study was that stress has been suggested to be involved in the pathogenesis of diabetes (Pouwer, 2009; Pouwer, Kupper, & Adriaanse, 2010), both as a predictor of new onset diabetes and as a prognostic factor in people with an existing T2D diagnosis (see sections 1.5 and 1.6 of Chapter 1). However, the diverse associations between stress-related processes and diabetes reported in these studies are only partly accounted for by lifestyle factors, suggesting that direct psychobiological pathways may be involved.

Allostatic load is the process by which repeated or sustained stimulation of the stress system is thought to lead to ‘wear and tear’ on the body. This can result in dysregulated responsivity to stress which in turn can increase vulnerability to disease over time (McEwen & Wingfield, 2003). Few studies have investigated allostatic load in relation to diabetes (Carlsson et al., 2011; Mattei et al., 2010) and these studies have only assessed measures taken at rest. In Study 1 the dynamic aspect of allostatic load was assessed by conducting an experimental comparison of acute laboratory stress responses in 140 people with diabetes and 280 controls. It was hypothesised that diabetes group would experience disturbances in cardiovascular (SBP, DBP, heart rate, cardiac index and HRV), metabolic (as indexed by cholesterol), neuroendocrine (as indexed by cortisol), and inflammatory (as indexed by IL-6) measures compared with the controls. Group differences in several psychosocial factors outside the laboratory were also assessed.

The patterning of responsivity and recovery in the T2D group was consistent with the concept of allostatic load and most of the hypotheses were supported. Post-stress recovery was attenuated in the diabetes group in SBP and DBP, heart rate, cardiac index and total cholesterol, together with blunted stress reactivity in SBP, DBP heart rate, cardiac index, cortisol, and cholesterol concentration. Plasma IL-6 concentration

was higher in people with T2D, so that although the increases following mental stress were smaller than those of controls, absolute levels remained higher. The diabetes group had higher baseline cortisol than controls in the laboratory. These effects were independent of covariates and were evident in both men and women. Additional sensitivity analyses adjusting for health behaviours did not alter the pattern of results. Contrary to hypothesis no associations were detected for HRV. With regards to psychosocial stress, participants with diabetes had more depressive and hostile symptoms, and reported greater chronic stress in terms of financial strain when compared with the healthy control group.

The review of the published literature conducted for Chapter 1 found no previous studies that have examined the dynamic responses to mental stress across multiple biological systems in T2D. Therefore this study has added something new to the field by addressing a gap in the literature. Disturbances in stress reactivity and post-stress recovery reflected by blunted stress-reactivity and poorer recovery post-stress were detected for SBP, DBP, heart rate and cardiac index in the diabetes group. Although ‘inadequate responses’ are a recognised component of allostatic load (McEwen, 1998) much research has focused on heightened stress reactivity and its association with health risk (Phillips et al., 2013). Study 1 has contributed to the field by showing blunted stress reactivity in a diseased population. More broadly, that both heightened and blunted responses have been associated with health risk in the literature adds credence to the theory of allostatic load which suggests that exaggerated responses, prolonged responses and diminished responses represent significant deviation from the physiological norm and therefore are potentially damaging to health (McEwen & Wingfield, 2003; McEwen, 1998).

Contrary to prediction no associations were found for HRV. The reason why we detected blunting in the diabetes participants for the other cardiovascular measures but not for HRV is unclear but the most likely explanation is that the measure of HRV was weak. We found that cortisol stress reactivity was blunted in the diabetes group in comparison to the controls and that IL-6 was heightened in the participants with diabetes throughout the laboratory session. The regulatory role of cortisol on inflammation could account for these findings (Miller et al., 2007). The blunting of cortisol may have a permission effect on inflammatory markers such as IL-6, allowing concentrations to rise (Miller et al., 2007; Raison & Miller, 2003). The high IL-6 concentration is likely related to the role of inflammation in T2D (Pickup, 2004; Tilg & Moschen, 2008).

Moving on from the disturbances in biological stress reactivity, we also observed differences between the diabetes and control groups in self-reported emotional distress and chronic life stress. Taken together, the result is a profile of psychosocial adversity in the T2D group that is likely to promote heightened allostatic load. However, it must be acknowledged that this was not directly assessed in Study 1.

The limitations of Study 1 were discussed in detail in Chapter 3; however it is important to reiterate here that the cross-sectional nature of the study makes it impossible to draw causal conclusions. It is possible that heightened allostatic load precedes the development of T2D and is a mechanism through which psychosocial factors contribute to diabetes risk. The alternative explanation for the results is that allostatic load is a manifestation of diabetes that is secondary to the abnormalities of glucose metabolism. The cardiovascular, neuroendocrine, and inflammatory responses we observed may be significant for the broader health consequences of diabetes.

These cross-sectional data need to be corroborated by further research. Despite this consideration (and others), I believe that the findings presented in Study 1 are novel and have significantly added to the literature by showing for the first time that people with diabetes may experience chronic allostatic load as indexed by disturbances in reactivity and recovery from stress across multiple biological systems, coupled with heightened experience of chronic life stress.

8.2.2. Study 2: Hostility and physiological responses to acute stress in T2D

In Study 2 (presented in Chapter 4) rather than comparing those with diabetes to healthy individuals, the focus was on the diabetes group alone. This study built upon the findings of Study 1 by assessing the effect of hostility (a psychosocial factor of interest) on physiological stress reactivity in people with diabetes. Hostility has been associated with an increased risk of CVD in initially healthy populations, as well as poorer prognosis in CVD patients (Chida & Steptoe, 2009b) but has received less attention in relation to T2D (see Section 1.5.2.4 of Chapter 1 for an overview). Additionally, despite the evidence linking hostility to ill health, the underlying mechanisms involved are not well understood. Considering these gaps in the literature and the fact that psychosocial factors are known to impact responses to stress, hostility was selected as an interesting psychosocial factor to investigate.

The majority of previous research in the field investigated cardiovascular responses to acute stress. Hostility has been associated with heightened cardiovascular stress responses in healthy participants (Chida & Hamer, 2008) and meta-analytic results indicate that heightened cardiovascular stress responsivity is associated with an increased risk of future CVD (Chida & Steptoe, 2010). Few studies have investigated

inflammatory and neuroendocrine mechanisms in relation to hostility and the majority of research has been conducted with healthy samples.

Considering the excess risk of CVD in people with T2D and the lack of research relating hostility and inflammatory and neuroendocrine stress responses we investigated the relationship between hostility and SBP, DBP, heart rate, cardiac index, HRV, IL-6 and cortisol responses to laboratory stress in a sample of T2D individuals. Taking the findings of Study 1 into consideration, it was hypothesized that participants with of high levels of hostility would show an exaggerated pattern of disturbed stress responsivity across multiple biological systems.

The main finding of the study was that greater hostility was associated with elevated IL-6 responses to acute stress in people with T2D. By contrast, cortisol output following stress was diminished to a greater extent in more hostile individuals. These associations were independent of a range of covariates. Contrary to prediction, no associations between hostility and cardiovascular responses were observed.

The IL-6 and cortisol findings corroborates previous work from Professor Steptoe's group in which IL-6 was elevated and cortisol blunted following acute stress in more hostile patients with CAD (Brydon et al., 2010). It is plausible that decreased cortisol levels may have facilitated the elevated IL-6 responses observed in more hostile subjects in both studies. The fact that the participants of Study 2 and the participants of Brydon *et al.*, study and were drawn from diseased populations might account for the commonalities in the results.

Contrary to hypothesis no relationship between hostility and cardiovascular responses to stress were detected in this T2D sample. Whereas in the Brydon *et al.*, study more hostile individuals with advanced CAD had heightened SBP and DBP responses to mental stress tasks. This result was unexpected and is paradoxical

considering the evidence that hostility is associated with heightened cardiovascular responses to stress in healthy individuals (e.g. Bongard, al' Absi, & Lovallo, 1998; Chida & Hamer, 2008; Girdler, Jammer, & Shapiro, 1997; Suarez, Kuhn, Schanberg, Williams, & Zimmermann, 1998). The lack of association between hostility and cardiovascular responses to stress could not be attributed to the intensity of stressor used, as both subjective stress ratings and cardiovascular measures increased significantly in response to the task and it is unlikely that the study was underpowered. Considering the findings of Study 1 it is apparent that healthy individuals and people with T2D differ in cardiovascular stress responsivity. Therefore, it is plausible that associations between hostility and biological reactivity could differ between the groups too. However, this notion was not directly assessed, as Study 2 focused on the participants with diabetes alone.

The limitations of Study 2 were discussed in Chapter 4. The main limitation was the cross-sectional nature of the study. Longitudinal research is needed to elucidate the degree to which trait hostility and changes in hostility over time are associated with inflammatory, neuroendocrine and cardiovascular processes, as well as negative health outcomes in people with T2D. The study was further limited by use of a self-report measure to assess hostility as the assessment of observable hostile behaviour could provide a different perspective in understanding the relationship between hostility and stress reactivity. Additionally, the stress tasks used may not have been optimal for studying hostility, as social or interpersonal stressors may provoke greater reactions in more hostile individuals (Suarez et al., 1998).

Despite these considerations (and others discussed in Chapter 4) I believe this study offers a novel contribution to the literature for several reasons. Firstly, hostility is suggested to play a role in the pathogenesis of CVD and is under-researched in relation

to T2D. Secondly, the relationship between hostility and acute stress reactivity in inflammatory and neuroendocrine factors is not well-researched. Thirdly, this study builds upon the findings of Study 1 and suggests that psychosocial factors experienced outside the laboratory can exaggerate disturbances in stress reactivity and recovery in people with T2D. The findings that more hostile individuals with T2D had greater IL-6 stress responses and diminished cortisol output over the laboratory session offers the possibility that disturbances in stress-responsivity may increase the risk for CVD in this population. However, further studies are required to confirm this pathway.

8.2.3. Study 3: Cross-sectional association of diurnal patterns in salivary cortisol with T2D in the Whitehall II study

Moving on from acute laboratory stress testing, Study 3 (presented in Chapter 6) took a naturalistic monitoring approach by assessing the association of cortisol patterns throughout the day with T2D status in 3508 participants from the Whitehall II cohort.

The background to this study was that neuroendocrine dysfunction has been suggested to play a role in diabetes. However, early studies in the field were limited by the use of various single time point cortisol measurements, as well as small sample sizes and participants selected from convenience samples (e.g. Asfeldt, 1972; Bruehl et al., 2007; Chiodini et al., 2007). Increasingly, the marked diurnal patterning in the release of cortisol, rather than single cortisol sample assessment has been the focus of large-scale HPA axis research (Adam & Kumari, 2009; Collomp et al., 2016). Again the findings from the studies that have investigated the cross-sectional associations between components of daily cortisol secretion and diabetes status are mixed. For example, in the MESA cohort, in the overall sample participants with T2D were found to have a significantly lower CAR than those without diabetes and in women those with T2D had

a significantly higher AUC than controls (Champaneri et al., 2012). Bruehl *et al.*, similarly observed a blunted CAR in participants with T2D, but found no association of T2D with slope in cortisol across the day or AUC (Bruehl et al., 2009). In contrast, Lederbogen *et al.* observed a flattened slope in diurnal cortisol secretion among those with diabetes, but detected no association with the AUC (Lederbogen et al., 2011),. Whereas, Vreeburg *et al.* observed no association between diabetes status and the CAR or diurnal cortisol slope or cortisol AUC (Vreeburg et al., 2009).

Considering this previous research, five components of daily cortisol output were assessed in this study: waking cortisol, the CAR, the cortisol slope across the day, bedtime cortisol and cortisol AUC. Based on previous research we predicted that individuals with T2D would have a greater CAR, flatter slope in cortisol across the day and a greater cortisol output over the day as indexed by cortisol AUC.

We found that the slope in cortisol across the day was flatter in those with compared to those without T2D. The data suggest that the flat slope in cortisol in individuals with T2D is due to raised late evening cortisol levels rather than depressed morning levels. We also found that participants with diabetes have greater daily cortisol output on average (cortisol AUC) than those without diabetes. These findings were robust to adjustment for a range of covariates. Contrary to hypothesis no association emerged for the CAR. These findings were in agreement with some but not all of the previous studies. It is possible that differences in participant characteristics or in the number and timing of cortisol samples between studies may have contributed to the diverging findings across studies.

Study 3 makes a significant contribution to the literature as it is the largest study to date to examine the association of diurnal cortisol secretion with diabetes. Furthermore, the components of the daily cortisol curve were well defined in this study

as participants took 6 cortisol samples over the course of a normal day and the participants with T2D were well-characterised. Although this study demonstrated an association between components of the diurnal cortisol curve and diabetes status, the causes of a flattened slope in diurnal cortisol and greater cortisol AUC are unknown and the mechanisms by which T2D is related to the HPA axis also remain to be elucidated. In the context of this thesis, psychosocial stress offers a particularly interesting pathway linking disturbances in cortisol secretion with T2D. However, this mechanism was not assessed in this thesis, despite the fact that the participants of the Whitehall II study have data on psychosocial factors. Other limitations of the analysis were detailed in Chapter 6. The main limitation was the cross-sectional design which makes it impossible to draw causal conclusions about the temporal relationship between aberrant cortisol output and diabetes. Study 4 presented in Chapter 7 and summarised below sheds some light on this issue.

8.2.4. Study 4: Diurnal cortisol patterns, future diabetes and impaired glucose metabolism in the Whitehall II cohort

Study 4 (presented in Chapter 7) built upon the findings of Study 3 by assessing the prospective association of diurnal cortisol secretion with future glucose disturbance in 3270 participants from the Whitehall II cohort. Participants who were normoglycaemic at the time of cortisol collection (phase 7: 2002-2004) were re-examined at phase 11 (2012-2013). Future IGT as well as future overt T2D were assessed as evidence suggests that the health risk accompanying raised glucose is continuous (Emerging Risk Factors Collaboration et al., 2010).

Longitudinal evidence relating neuroendocrine dysfunction with future IFG or T2D is sparse. In the LASA cohort morning and evening salivary cortisol were

measured in 998 initially healthy people. Raised evening cortisol was associated with future T2D in female participants, but no associations were found for men (Schoorlemmer et al., 2009). Study 4 makes a significant contribution to the literature as no previous study has examined the relationship between the complete diurnal cortisol profile and incident T2D in an initially healthy population. In keeping with the findings of Study 3 we hypothesised that a flatter diurnal cortisol slope, raised evening cortisol levels and greater cortisol AUC at phase 7 (2002-2004) would predict new onset IFG and T2D at phase 11 (2012-2013).

We found that raised evening cortisol levels were predictive of new onset T2D approximately 9 years later. For slope across the day we observed a trend for a flatter slope in participants with incident diabetes. We found that a flattened diurnal cortisol slope at phase 7 was predictive of the combined outcome of future IFG or T2D at phase 11. Evening cortisol was also associated with future diabetes or IFG. As predicted in keeping with the cross-sectional findings no associations emerged for the CAR or waking cortisol. However, contrary to hypothesis no associations emerged for the AUC.

The finding that evening cortisol at phase 7 is predictive of new onset diabetes at phase 11 corroborates and extends the results of Schoorlemmer *et al* (Schoorlemmer et al., 2009). In the LASA cohort, raised evening cortisol concentrations were associated with future T2D but only in female participants. We checked for an interaction between sex and cortisol measures but found no significant associations. Our study is considerably larger than the LASA cohort study and this may account for our ability to detect an association between evening cortisol and incident diabetes in both sexes. We observed a borderline significant result for a flatter cortisol slope in participants with new onset T2D. Expanding this analysis to a broader category of glucose disturbance we found that participants with IFG or T2D had a flatter cortisol slope compared with

normoglycaemic controls. The analysis of the T2D group alone may have been underpowered to detect a significant effect. This result builds upon the work presented in Chapter 6 (Hackett et al., 2014) and suggests that a flatter slope in cortisol might also be predictive of future disturbances in glucose metabolism in an initially healthy population. The lack of association detected for AUC was unexpected. It may be that average cortisol concentrations begin to rise following the development of overt diabetes but that this component of daily cortisol output does not have predictive value in initially normoglycaemic individuals.

The limitations of Study 4 were presented in Chapter 7. One limitation was that glucose was only measured at the Whitehall clinical waves and therefore it was not possible to assess when exactly incident IFG and T2D occurred in the follow-up period. Additionally, the effects observed in our analyses were small. Despite these considerations (and others), these findings are novel and contribute to the literature in this field by demonstrating for the first time that diurnal cortisol secretion is associated with future T2D and impaired glucose metabolism in a non-clinical population. It is plausible that neuroendocrine dysfunction is related to the pathophysiology of T2D, but the precise mechanisms through which changes in cortisol secretion impairs glucose metabolism remain to be determined.

8.2.5. Overall summary of the findings of studies in this thesis

Taken together, some but not all hypotheses were supported by the data. I believe this work has shed light on some previously unexplored associations and that in combination these studies contribute to the literature linking diabetes with poor psychosocial wellbeing and alterations in stress-related biological processes.

8.3. Methodological issues and limitations

The findings presented in this thesis have to be interpreted in light of their limitations. The shortcomings of the individual studies were discussed at the end of each chapter. Therefore, in this section only the most important issues will be mentioned and more general, over-arching limitations will be discussed.

8.3.1. The study samples

This thesis used several different samples. The samples for Study 1 and 2 were taken from the Diabetes Study carried out by the Psychobiology Group at UCL. Study 1 was a comparative study of 140 people with doctor diagnosed T2D and 280 age- sex- and income-matched controls. In Study 2 rather than using the comparative sample, the 140 participants with diabetes were assessed alone. In Study 3 and 4 the participants (n= 3508 and n= 3270 respectively) were taken from a sub-sample of the Whitehall II epidemiological cohort at phase 7 of the study (2002-2004).

One advantage of the participants assessed for the different studies in thesis was that diabetes diagnosis was well delineated in each sample. In the Diabetes Study the participants with T2D were recruited from either diabetic outpatient clinics or were referred from their GP with doctor diagnosed T2D. The controls used for Study 1 were diabetes free and the mean HbA1c in this group was not in the diabetic range. In the Whitehall II study participants either had self-reported doctor diagnosed diabetes, were taking diabetic medications or had an objective assessment of IGT or diabetes at the Whitehall clinical assessment. Furthermore, the age of the participants in different studies was similar (mean age across studies early 60s) at the time of biological sample assessment.

However, these participant samples were not without limitations. In the Diabetes Study the inclusion criteria were strict. Enrolment was restricted to patients without a history or previous diagnosis of CHD, inflammatory diseases, allergies or mood disorders. Participants were recruited from both diabetic outpatient clinics and primary care practices. The number of eligible participants at the outpatient clinics was low due to the high number of co-morbidities in this population. Only 14 (10%) participants in the study were recruited from outpatient clinics. Therefore, the recruitment strategy was changed to recruit participants from primary care practices in the Camden area in London. However, the study response rate was difficult to assess as primary care clinics differed in their requirements surrounding the confidentiality of patient data and the sending of recruitment letters. In some clinics paper records were hand searched, in others the practice shared the addresses of eligible participants and in the remainder the practice themselves searched and sent out the recruitment letters independently. These varying strategies meant that in many practices it was unknown how many potentially eligible participants there were or many letters were sent out before an interested individual contacted the study team at UCL.

Another shortcoming of the recruitment strategy that applies to Study 1 is that the recruitment method differed for the participants with diabetes and the healthy controls. As mentioned previously the healthy controls were drawn from the Heart Scan Study, an earlier study which was carried out at the Psychobiology Group at UCL. The Heart Scan Study participants were recruited from the Whitehall II cohort rather than directly from the community as the participants with diabetes were. As mentioned previously Whitehall II is not a representative cohort and the participants were selected from 20 civil service departments across London. Therefore the healthy controls are not representative of people living without diabetes in the Camden area.

A general limitation of recruiting the participants with diabetes from the borough of Camden is that the gap between the number of individuals with diagnosed T2D and the number of expected cases with T2D is wide and significantly higher compared to London and England as a whole (Camden Joint Strategic Needs Assessment, 2014). This suggests that there may be a significant number of people with undiagnosed diabetes in the borough who would not have been reached with the recruitment strategy used in this study.

Selection bias can distort the results if potential participants with certain patterns of association are more/less likely to be recruited and selected for a study. Eighteen primary care practices agreed to take part in the research out of the 36 practices approached. Of the 18 practices that agreed to take part, 16 of these provided participants. Despite the fact that the study response rate could not be accurately assessed, the participants recruited for the Diabetes Study were blind to the study hypothesis so it is unlikely that selection bias could have accounted for the participants' willingness to take part. Furthermore, as we excluded participants who had a diagnosed mood disorder it is unlikely that people with a higher vulnerability to stress were more prone to be selected, rendering them artificially more frequent in the Diabetes Study than they are in the general population. Indeed the higher rate of self-reported life stress in the participants with diabetes in comparison to the healthy controls would be expected considering the literature in this field.

In the Diabetes study fluency in English was required to successfully complete the questionnaire booklet and to participate in the laboratory protocol. Since non-English speaking participants were not included in the study, the sample is not entirely representative of the community from which participants were drawn. In Camden,

government data indicates that Bengali is the primary spoken language of 13% of the borough's population (Office for National Statistics, 2011).

The majority of the participants in the Diabetes Study were of white ethnicity (80%) and all of the participants selected for the Whitehall analyses presented in this thesis were also white. This limitation has been highlighted in the discussion section of the individual chapters. However, it is important to mention this limitation again particularly in relation to Diabetes Study as this was primary data, rather than secondary analysis of an existing dataset. In Camden an estimated 35% of the population are from a black or ethnic minority background (Office for National Statistics, 2011). In most parts of the borough Bangladeshis form the largest minority group (Office for National Statistics, 2011). Research from the tri-ethnic Southall and Brent Revisited cohort study indicates that people of South Asian and African Caribbean origin living in London have a significantly greater risk of diabetes than white ethnic groups (Tillin et al., 2013). Adding ethnicity as a factor to the analyses did not alter the pattern of results reported in Study 1 & 2 so it was not included in the final models. Nevertheless, with this predominately white sample we were unable to tease out effects of ethnicity on responsivity and recovery from stress and this is a limitation of the work presented in this thesis. The limitation also applies to the Whitehall II findings presented in Study 3 & 4 as only white participants were included in the analyses.

8.3.2. Alternative biological measures

The limitations of the biological measures used in this thesis such as the weak measure of HRV and the shortcomings of allostatic load have been discussed in the respective chapter discussions. This section concerns alternative measures that could have been used in this thesis.

It is plausible that several stress-related biological pathways might play role in both CVD and T2D. BP and other markers of cardiovascular function, cholesterol and inflammation have well-established roles in the pathogenesis of CVD. There is also evidence that disturbances in daily cortisol regulation are related to CVD. Study 1 showed that participants with diabetes have alterations in stress responsivity and recovery across cardiovascular (BP, heart rate, cardiac index, HRV), inflammatory (as indexed by IL-6), metabolic (as indexed by cholesterol) and neuroendocrine (as indexed by cortisol) systems compared with controls. Study 3 & 4 showed that diurnal cortisol patterns differ in people with diabetes compared to controls and that disturbances in daily cortisol secretion are predictive of new onset diabetes in initially healthy people.

The biomarkers used in this thesis were selected due to their associations with stress and CVD and also for ease of measurement. However, there are other potentially relevant biological systems that could play a role in linking the conditions that were not assessed. Glucose and insulin are known to be responsive to stress in healthy individuals (Nowotny et al., 2010; Picard, Juster, & McEwen, 2014) and in people with T2D acute psychophysiological stress may alter glucose control (Faulenbach et al., 2012). However, stress reactivity and post-stress recovery in insulin and glucose were not assessed in the Diabetes Study, primarily because these measures were not available in the Heart Scan Study. Comparison of responses across multiple biological systems was a major aim of the Diabetes Study and had these responses been included we would have been unable to compare them in people with T2D and controls. However, glucose metabolism is a critical issue in T2D and at the time the Heart Scan Study was conducted the focus was on healthy individuals rather than on people with T2D. In future research it would be interesting to assess stress responsivity and post-stress

recovery in insulin and glucose in people with T2D and also to compare these responses in people with T2D and controls.

Cortisol was assessed in response to laboratory stress in the Diabetes Study and over the course of a normal day in the Whitehall analyses, but in recent years there has been interest in assessing cortisol from the hair shaft as a retrospective indicator of average cortisol exposure over the past months (Stalder & Kirschbaum, 2012). Indeed, two studies have found that hair cortisol concentrations are elevated in people with CVD (Manenschijn et al., 2013; Pereg et al., 2011) and high levels of hair cortisol have been correlated with 3.2 fold increased risk of T2D in a community sample (Manenschijn et al., 2013). Furthermore, naturalistic monitoring was used to assess diurnal cortisol rhythms but we could have applied this method to BP as well. BP varies significantly over the day so continuous ambulatory blood pressure readings would complement measures taken in the laboratory (Mallion, Baguet, Siché, Tremel, & De Gaudemaris, 1999). Additionally, ambulatory measures are often more reliable predictors of patient outcomes than measures taken in a clinical or laboratory setting (Sheikh, Sinha, & Agarwal, 2011) due to the ‘white coat effect’, a transient elevation in BP exhibited in response to observation during measurement. Although the baseline BP reading was taken 30 minutes into the rest period in the Diabetes Study it is possible that this effect could have impacted the resting measurement in the study. With regards to inflammation, only one measure of inflammation was assessed in the Diabetes Study, other markers such as CRP which are known to play a role in T2D (Wang et al., 2013) would have been interesting to assess. The Whitehall participants have data on inflammatory markers and it is plausible that inflammation is a mechanism linking changes in diurnal cortisol output with diabetes. However, this notion was not one of the original Study 3 & 4 hypotheses and was not included in the Whitehall data sharing

application. Therefore, inflammatory markers from the Whitehall II study could not be assessed in this thesis. Other possible avenues of exploration in this field include telomere length which has been associated with both T2D and CVD (Salpea & Humphries, 2010), and diabetes-related genetic processes (Swerdlow, 2016). The factors mentioned here should not be considered an exhaustive list of alternative biological measures, but have been included as an indication of other possibilities within this field.

8.3.3. Causality

Three out of the 4 studies presented in this thesis were cross-sectional which precludes any inference about causality. This means for example, in Study 1 it is possible that heightened allostatic load precedes the development of T2D and is a mechanism through which stress can contribute to diabetes risk or that allostatic load is a manifestation of diabetes that is secondary to the abnormalities of glucose metabolism. In Study 2 which was also cross-sectional, hostility was only assessed at one time point. Longitudinal studies could help elucidate whether the relationship between hostility and stress-reactivity in people with T2D changes over the life-span and whether this relationship influences outcomes in this population.

Study 3 was also cross-sectional but formed the basis for the longitudinal analysis in Study 4. Although Study 4 was a prospective analysis this does not assure that there is a causal relationship between components of diurnal cortisol secretion and future diabetes. For example, the issue of reverse causality cannot be completely ruled out, such that it is possible that some of the participants included could have had undiagnosed diabetes or IGT at baseline, as this condition develops slowly over many years (Tabák et al., 2012). However, it is unlikely that this was an issue in

Study 4 as participants with overt diabetes at baseline were excluded and IGT at the time of cortisol assessment was included as a covariate in all the analyses. Nevertheless, the study was observational so it is difficult to draw causal conclusions and adjusting for potentially confounding factors does not excluded the possibility that unmeasured or poorly measured factors account for the relationship. Additionally, controlling for determinants of diabetes in this study such as BMI or cardiovascular medications does not mean that these factors are unimportant. Merely controlling for these factors does not tease out the complexities of the relationships between these processes and diurnal cortisol secretion and future T2D.

Naturally this issue also applies to the other studies. For example in Study 1, sensitivity analyses showing that the group differences in stress responsivity are robust to adjustment for health related factors such as physical activity or alcohol consumption does not mean these factors are irrelevant. Lifestyle factors offer an important means of intervention to alter stress-related processes and will be discussed later on in this chapter. Furthermore, the self-report measures of alcohol consumption or poor sleep could be a source of residual confounding through imperfect measurement.

8.3.4. Limitations of acute laboratory stress testing and naturalistic monitoring

The advantages and limitations of acute laboratory stress testing were discussed in Section 1.6.2 of Chapter 1. To reiterate in brief, the advantage of psychophysiological laboratory testing is that it allows detailed dynamic responses to be studied under controlled conditions, reducing the impact of other factors that may confound associations. However, there are several shortcomings to this method such as the fact

that only acute short-term biological responses can be recorded and that the stress tasks are often arbitrary and may bear little resemblance to everyday life.

The stroop and mirror tracing tasks that were used in the Diabetes Study were selected because they have previously been shown to stimulate similar appraisals of involvement and engagement from participants across the social gradient and have been used in a number of previous studies in the Psychobiology Group (Steptoe et al., 2002). However, these tasks are not without limitations. The stroop task was computer-based and as the age of the participants ranged from 50-75 years some of the older participants may have had limited experience using a computer. This could have impacted performance and ratings of perceived stress and task difficulty. Experience was unlikely to have impacted performance or participant ratings of the mirror tracing task. However, the shortcoming of using the combination of the stroop and the mirror tracing tasks is that there was no socially evaluative element to the stress session, which could have potentially altered the pattern of responses or elicited greater cortisol stress responses (Dickerson & Kemeny, 2004). Furthermore, for the purposes of Study 2 the stress tasks used may not have been optimal for studying hostility. Social or interpersonal stressors may provoke greater reactions in hostile individuals (Suarez et al., 1998).

Psychophysiological testing is often complemented by naturalistic and ambulatory monitoring methods. The advantages and limitations of naturalistic monitoring were discussed in Section 1.6.2 of Chapter 1. In brief, naturalistic studies involve the sampling of biological variables during everyday life, thus circumventing some of the problems with laboratory studies concerning ecological validity (Steptoe & Poole, 2010). Another advantage is that naturalistic monitoring can be incorporated into large epidemiological studies such as the monitoring of diurnal cortisol in the Whitehall II cohort. However, naturalistic monitoring is not without weakness. Participants in

these studies are generally aware that they are being monitored and may alter their behaviour. Additionally, the devices used in naturalistic studies may be perceived as intrusive and therefore participants might not adhere to the study protocol regarding their use and there are limits to the number of biological processes which can be measured due to technical restrictions.

The limitations of cortisol sample collection in the Whitehall II were mentioned in the discussion sections of Chapters 6 & 7. In brief, cortisol was assessed over a single day and it has been suggested that single day assessment of cortisol may obscure the CAR to situational rather than chronic correlates (Hellhammer et al., 2007). Furthermore, the night release of cortisol was not assessed and therefore it was not possible to evaluate total 24 hour circadian cortisol exposure. We relied on self-report for the timing of sample collection, whereas other studies have used salivettes with ‘track caps’ to objectively assess the timing of cortisol sample collection (Champaneri et al., 2012). However, the prevalence of ‘late’ reporting was similar to previously reported rates (Kudielka et al., 2003) and evidence suggests that participants are generally accurate in their recording of this information (Dockray et al., 2008).

8.4. Suggestions for future research

Each of the studies presented in this thesis have highlighted gaps in the literature and demonstrate the need for more research in this field. Suggestions for future research were provided in each of the study chapters. However, it is important to mention these again here. For Studies 1 and 2 the most obvious recommendation would be for longitudinal research to be conducted. For Study 1 it is unclear whether allostatic load precedes diabetes development or whether these disturbances are secondary to alterations in glucose metabolism. Studies of heightened dynamic allostatic load in

people with insulin resistance but no diabetes would help shed light on this issue. With regards to Study 2 longitudinal research is needed to elucidate the degree to which trait hostility and changes in hostility over time are associated with inflammatory, neuroendocrine and cardiovascular processes, as well as negative health outcomes in people with T2D.

Different stress tasks could also be used in future when looking at the relationship between hostility and acute laboratory stress reactivity as the tasks used in Study 2 used may not have been optimal for studying hostility. Other studies of hostility and physiological stress reactivity (Anderson, Linden, & Habra, 2005; Lai & Linden, 1992; Suarez et al., 1998) have used standardised harassment (Hokanson & Shetler, 1961) to provoke hostile reactions. Whether our results would be exaggerated or unaffected by standardised harassment is unknown. An experimental manipulation where some individuals are exposed to harassment during the tasks and other are able to complete the task without commentary would help clarify this.

Study 3 formed the basis for the longitudinal analysis performed in Study 4. Data collection with the Whitehall II cohort is set to continue until 2030 (Sanchez, 2016). This offers several possibilities for future research. It would be interesting to track changes in cortisol parameters over time as cortisol was only assessed at one time point in Studies 3 & 4. Also it would be interesting to compare cortisol trajectories in those who developed diabetes and those who do not. Furthermore it would be interesting to know whether aspects of diurnal cortisol secretion are linked with outcomes in people with T2D, as flatter slopes in cortisol and heightened evening cortisol concentrations have been shown to predict cardiovascular mortality in this cohort (Kumari et al., 2011). Another line of research would be to investigate the mechanisms through which changes in cortisol secretion impairs glucose metabolism as

these remain to be determined. In particular in the context of this thesis it would be interesting to know the impact that measures of psychosocial stress have upon diurnal cortisol output in people with T2D and whether these have an impact on the longitudinal relationship between diurnal cortisol output and future diabetes. The Whitehall participants have data on psychosocial measures and research has linked factors such as psychological distress and work stress with future diabetes in this cohort (Heraclides, Chandola, Witte, & Brunner, 2012; Virtanen et al., 2014). However, assessing whether psychosocial factors contribute to disturbances in daily cortisol secretion and in turn contribute to T2D was not one of the original Study 3 & 4 hypotheses and was not included in the Whitehall data sharing application. Therefore, psychosocial factors from the Whitehall II study could not be assessed in this thesis, but offer an interesting possibility to assess in the future.

8.5. Implications

The findings of Study 1 are consistent with the broad theory that people with diabetes have greater allostatic load as indexed by disturbances across multiple biological systems combined with greater reports of psychosocial stress in everyday life which may exaggerate disturbances in stress related processes. Study 2 builds upon these results by suggesting that hostility (a psychosocial stress factor) in individuals with diabetes might exaggerate disturbances in biological stress responsivity in this population. Study 3 & 4 suggest that diurnal cortisol secretion is altered in people with T2D and disturbances in daily cortisol output may predispose initially healthy people to IGT and overt diabetes. This begs the question whether modifying psychosocial stress or cortisol would be beneficial for people with diabetes. The following sections will review the evidence to date in both of these areas.

8.5.1. Modifying psychosocial stress in people with T2D

To date the majority of research in this area has investigated whether the treatment of depression in diabetes has a therapeutic impact on physical outcomes in T2D. A Cochrane review in 2012 included 19 RCTs investigating both psychological and pharmacological interventions for depression in patients with diabetes (Baumeister, Hutter, & Bengel, 2012). Psychological intervention studies showed a beneficial effect on short, medium and long-term depression severity and had a good impact on depression remission compared to usual care. However, the effect of psychological intervention on glycaemic control was mixed and inconclusive. With regards to the pharmacological interventions, there was a moderate effect of anti-depressant medication on short-term depression severity and depression remission, and interestingly the pharmacological trials significantly improved glycaemic control in the short term as well. But no study has assessed the relationship between depression treatment and glycaemic control in the longer term. Taking the evidence together it appears that depression treatment is moderately effective in diabetes, but only pharmacological trials have shown a consistent improvement in glycaemic control.

Mindfulness-based interventions for modifying psychosocial stress factors have also been tested in people with diabetes (Noordali, Cumming, & Thompson, 2015). They have been found in several studies to have psychological benefits, lowering depression, anxiety stress, and diabetes-distress symptoms in people with diabetes. However, the evidence for the effectiveness of these interventions on glycaemic control is mixed. Out of the 7 studies that assessed HbA1c as a marker of glycaemic control, four interventions lowered HbA1c levels, but the three largest studies reported no change in HbA1c (Noordali et al., 2015). Mindfulness-based intervention in diabetes is

a new field, and much of the research is exploratory. It may be that the short follow-up periods of many studies were not sufficient to observe significant changes in HbA1c.

There is limited evidence that the treatment of psychosocial factors can reduce the risk of adverse outcomes in people with diabetes (Adriaanse & Pouwer, 2015). The Prevention of Suicide in Primary Care Elderly Collaborative Trial RCT was used to investigate whether depression management would decrease mortality in diabetes (Bogner, Morales, Post, & Bruce, 2007). Depressed people with diabetes who were assigned to the intervention group (an individualized case management approach) had significantly lower mortality rates than controls over 5-year follow-up (HR 0.49, 95% CI 0.24 – 0.98). However, this study has been criticized with regards to study design and analysis, with suggestions that the methods may not have been appropriate (Thombs & Ziegelstein, 2008). The impact of mindfulness interventions on CVD outcomes in people with diabetes has not yet been examined (Noordali et al., 2015).

In sum, there is little evidence as yet that the treatment of psychosocial factors in diabetes has a therapeutic benefit for adverse outcomes such as CVD. However, pharmacological interventions have been shown to improve glycaemic outcomes in the short-term and hyperglycaemia is linearly associated with increased CVD risk (Emerging Risk Factors Collaboration et al., 2010; Goff et al., 2007). Additionally, both psychological and pharmacological treatments as well as mindfulness-based interventions appear to have beneficial effects on psychosocial factors in people with diabetes. Despite the limited effectiveness of these treatments on overt CVD outcomes, there have been calls that the psychological well-being in people with diabetes should be a priority for its own sake (Jones, Vallis, & Pouwer, 2015).

8.5.2. Lifestyle interventions to prevent diabetes

Lifestyle factors offer a possible indirect pathway linking psychosocial factors with future health risk and could also moderate or mediate the relationship between psychosocial factors and outcomes in people with an existing health condition such as T2D (Abraham et al., 2015; Farvid et al., 2014). Although the analyses presented in this thesis were robust to adjustment for lifestyle factors it is important to reiterate that does not necessarily indicate that these factors are unimportant. It is well known that psychosocial factors affect health behaviours, for example both obesity and physical inactivity increase the risk of developing diabetes and both of these factors are known to moderate depressive symptoms (Cooney et al., 2013; Fabricatore et al., 2011). In turn in people with overt diabetes, depression adversely effects adherence to recommended diet and exercise regimens (Katon et al., 2004; Katon et al., 2010). Furthermore, both psychosocial and lifestyle factors impact biological systems that are implicated in disease. Therefore, merely controlling for these factors does not tease out the complexities of the relationships between these processes.

The lack of evidence that altering psychosocial stress in people with existing diabetes offers the possibility that targeting individuals before T2D onset rather than after diagnosis might be a more optimal strategy. The research on lifestyle interventions in people before diabetes onset and in those with overt diabetes was presented in Section 1.5 of Chapter 1. In brief, intensive programs targeting lifestyle factors such as diet, physical activity and weight management have been shown to prevent T2D onset in people with and without pre-diabetes (Knowler et al., 2002; Li et al., 2008; Uusitupa et al., 2009). The Diabetes Prevention Programs are recognised as some of the most effective lifestyle interventions for preventing chronic disease. Since these interventions modify CVD risk factors such high BMI and BP (Look AHEAD

Research Group et al., 2013), they in turn should have an impact on CVD outcomes in people with T2D. However, lifestyle interventions to reduce CVD in people with T2D have been largely disappointing (see Fox et al., 2015 for a recent review). Again this suggests that targeting people before the development of diabetes might be a better strategy for intervention.

8.5.3. Interventions to modify cortisol

Study 4 presented in this thesis has shown for the first time that components of daily cortisol have predictive value for new onset IGT or T2D. However, changes in diurnal cortisol secretion have been prospectively associated with diseases other than diabetes. For example, previous work in the Whitehall II cohort has shown that flatter diurnal cortisol rhythms and raised evening cortisol levels are predictive of cardiovascular mortality (Kumari et al., 2011). In a study of 250 participants who underwent coronary artery bypass graft surgery, pre-surgical flattening of the daily cortisol slope was associated with an increased risk of future adverse cardiac events and all-cause mortality (Ronaldson et al., 2015). A flattened cortisol slope across the day has also been associated with decreased survival in a range of cancers including breast cancer (Sephton, Sapolsky, Kraemer, & Spiegel, 2000), lung cancer (Sephton et al., 2013), renal cell carcinoma (Cohen et al., 2012) and ovarian cancer (Schrepf et al., 2015). Raised evening cortisol has been linked with earlier mortality in breast cancer patients (Sephton et al., 2000) and a one standard deviation increase in night time cortisol was associated with a 46% greater likelihood of death in ovarian cancer patients (Schrepf et al., 2015).

Taken together the findings of these studies suggest that dysregulation of components of daily cortisol secretion curve can lead to negative health outcomes over

time. This has led to an emerging literature of studies that have attempted to change daily cortisol patterns in patient populations. One intervention that has been applied in this regard is physical activity (Collomp et al., 2016). When reviewing the literature only one intervention study with the aim to alter cortisol through exercise in people with T2D was found. In this study of older Indian individuals with T2D, 73 participants who undertook 90 minutes of guided yoga practice daily for three months were compared with 70 controls who received no intervention (Beena & Sreekumaran, 2013). At the end of the study, fasting serum cortisol levels fell significantly in the intervention group with no corresponding change detected in the controls. However, it should be acknowledged that this study had many limitations, including a lack of group randomisation (participants who were interested in yoga were selected for the intervention), no control variables included in the analysis and the fact that cortisol was only measured at baseline and at 3 months from a single sample.

Several exercise intervention studies that have included cortisol as an outcome measure have been carried out in people with cancer (Banasik, Williams, Haberman, Blank, & Bendel, 2011; Saxton et al., 2014; Vadiraja et al., 2009). One study of 88 breast cancer outpatients compared a 6 week yoga program of 3 hour long classes per week (n=44) to supportive therapy for 15 minutes every 10 days (n=44) (Vadiraja et al., 2009). After the 6 week period, participants from the yoga group were found to have significant decreases in morning cortisol and cortisol AUC in comparison to controls with a parallel reduction in perceived stress, anxiety and depression. In another intervention study with breast cancer survivors, participants who were randomized to an 8 week yoga course reported decreased morning and evening cortisol concentrations and improved emotional well-being scores in comparison to controls (Banasik et al., 2011). However, this study was limited by a small sample size of 18 participants. Other

studies with breast cancer patients have included yoga as part of a mindfulness based intervention, but the impact of the individual components of these varying interventions on cortisol parameters is difficult to assess (Carlson, Speca, Patel, & Goodey, 2004; Cruess et al., 2000; Matousek, Pruessner, & Dobkin, 2011).

Additionally, cardiovascular exercise interventions have been shown to have a beneficial effect on cortisol output in a study of 85 breast cancer patients (Saxton et al., 2014). However, this study used a combined exercise and diet intervention so the impact of physical activity alone on cortisol parameters is difficult to ascertain.

Moving on from studies in cancer patients, one study in the area has compared the effect of different types of exercise on cortisol parameters in participants with the metabolic syndrome (Corey et al., 2014). In this RCT of 171 people, 72 were assigned to yoga and 64 underwent a stretching intervention for 6 months. Saliva was assessed at 4 time points over the course of three days at baseline and at 6 months. At follow-up, the stretching group had greater reductions in waking and bedtime cortisol compared to the yoga group. In the stretching group, increases in perceived social support following the intervention were related to improved cortisol dynamics. The authors suggest that although yoga is carried out in group settings the focus is on the individual whereas the stretching intervention was more interactive. This offers the possibility that social support may mediate the effects of exercise on improvements in cortisol.

Taken together, there is an emerging literature assessing the impact of exercise on cortisol parameters in patient groups. However, the studies conducted in this area thus far have been limited by small samples and in most cases a lack of inclusion of covariates in the statistical analysis. Furthermore, it is unclear which exercise is most beneficial and whether the changes in cortisol parameters are due to exercise or increasing social support. There is a dearth of evidence in patients with diabetes with

only one limited study conducted to date. It is important to reiterate that negative psychosocial factors that are common in T2D may also decrease motivation for health lifestyle choices in this population as discussed previously in this thesis. This is a consideration when planning exercise interventions with this population. On a final note, one might question whether it is too early to consider intervening with cortisol as research on the role of cortisol in disease is yet to be fully understood. Nevertheless, it is an interesting possibility to consider intervention in this area.

8.6. Final conclusions

This thesis has presented the findings from four studies which have used a mixture of acute stress laboratory testing and naturalistic monitoring methods. I believe each of the studies presented have added something new to the field. Study 1 was the first trial to compare dynamic responses to acute laboratory stress across multiple biological systems in people with diabetes and healthy controls. The findings of this study suggest that people with T2D experience dysregulation across several biological systems in responsivity and recovery from stress. The patterning of these results is in keeping with the concept of heightened allostatic load. The participants with diabetes also reported greater levels of life stress outside of the laboratory environment in comparison to controls which is in keeping with previous literature in this field. Hostility is under-researched in relation to diabetes and Study 2 demonstrated that hostility might exaggerate disturbances in stress reactivity in people with T2D. Study 3 & 4 used secondary data from the Whitehall II cohort to assess the relationship between diurnal cortisol secretion and diabetes. Study 3 was the largest cross-sectional study to date to assess the association between daily cortisol output and diabetes status. The disturbances in various cortisol parameters detected in the participants with T2D cross-

sectionally formed the basis for Study 4 which was a prospective investigation of the relationship between daily cortisol output and future disturbances in glucose metabolism in an initially healthy population. This was the first study to assess the longitudinal relationship between the complete diurnal cortisol profile and future glucose status. The results of this study suggest that raised evening cortisol levels are predictive of new onset diabetes and that a flatter slope in cortisol across the day and raised evening cortisol levels are predictive of future IGT and T2D.

9. Peer reviewed publications

1. Puig-Perez, S., **Hackett, R. A.**, Lazzarino, A. I., Carvalho, L. A., Hamer, M., & Steptoe, A., (under review), Optimism moderates psychophysiological responses to stress in people with type 2 diabetes. *Biological Psychology* [Impact factor 3.403]

2. **Hackett, R. A.**, & Steptoe, A., (accepted). Psychosocial factors in type 2 diabetes: Effect on diabetes risk and cardiovascular complications. *Current Cardiology Reports*. [Impact factor 1.925]
3. **Hackett, R. A.**, Kivimaki, M., Kumari, M., & Steptoe, A., (2016). Diurnal cortisol patterns, future diabetes and impaired glucose metabolism in the Whitehall II cohort. *Journal of Clinical Endocrinology and Metabolism*, 101, (2), 619–625. [Impact factor 6.209]
4. Endrighi, R., Hamer, M., **Hackett, R. A.**, Carvalho, L.A., Jackson, S. E., Wardle, J. & Steptoe, A., (2015) Effect of short-term weight loss on mental stress-induced cardiovascular and pro-inflammatory responses in women, *Stress*, 18, (5), 602–606. [Impact factor 3.252]
5. **Hackett, R. A.**, Lazzarino, A. I., Carvalho, L. A., Hamer, M., & Steptoe, A., (2015), Hostility and physiological responses to acute stress in people with type 2 diabetes, *Psychosomatic Medicine*, 77(4), 458-466. [Impact factor 3.968]
6. Beinart, N., **Hackett, R. A.**, Graham, C. D., Weinman, J., & Ostermann, M. (2015). Mood and illness experiences of adults with cystinosis. *Renal failure*, (0), 1-5. [Impact factor 0.944]
7. Carvalho, L.A., Urbanova, L., Hamer, M., **Hackett, R. A.**, Lazzarino, A. I., & Steptoe, A., (2015), Blunted glucocorticoid and mineralocorticoid sensitivity to stress in people with diabetes, *Psychoneuroendocrinology*, 51, 209-218. [Impact factor 4.944]
8. Steptoe A., **Hackett R. A.**, Lazzarino A. I., Bostock S., La Marca R., Hamer M., (2014), Disruption of multisystem responses to stress in type

- 2 diabetes: Investigating the dynamics of allostatic load. *Proc. Natl. Acad. Sci.*, 111(44), 15693-15698. [Impact factor 9.674]
9. **Hackett, R. A.**, Steptoe, A. & Kumari, M., (2014), Association of diurnal patterns in salivary cortisol with type 2 diabetes in the Whitehall II study, *Journal of Clinical Endocrinology and Metabolism*, 99(12), 4625-4631. [Impact factor 6.209]
 10. Hamer, M., **Hackett, R.**, Bostock, S., Lazzarino, A. I., Carvahlo, L. A., & Steptoe, A., (2014), Objectively assessed physical activity, adiposity and inflammatory markers in people with type 2 diabetes, *BMJ Open Diabetes Research and Care*, 2, e000030. [Impact factor not available]
 11. Hamer, M., Bostock, S., **Hackett, R.**, & Steptoe, A., (2013). Objectively assessed sedentary time and type 2 diabetes mellitus: a case control study, *Diabetologia*, 56, 2761-2762. [Impact factor 6.671]
 12. **Hackett, R.A.**, Hamer, M., Endrighi, R., Brydon, L. & Steptoe, A., (2012), Loneliness and stress-related inflammatory and neuroendocrine responses in older men and women, *Psychoneuroendocrinology*, 37, 1801-1809. [Impact factor 4.944]

10. Conference presentations

1. “Diurnal cortisol patterns, future diabetes and impaired glucose metabolism in the Whitehall II cohort”. Poster presentation at the APS conference, Denver, CO, USA (March 2016).

2. Hostility and physiological responses to acute stress in people with type 2 diabetes Poster presentation at the APS conference, Savannah, GA, USA (March 2015).

3. Dysregulated cardiovascular responses to laboratory stress in people with type 2 diabetes. Oral presentation as part of a symposium delivered at the International Congress of Behaviour Medicine, Groningen, the Netherlands (August 2014). The abstract can be found in the *International Journal of Behavioral Medicine* (2014), 21, Suppl 1, S60.

4. Loneliness and stress-related inflammatory and neuroendocrine responses in older men and women. Oral presentation at the UK Society of Behavioural Medicine conference, Stirling, UK (Dec 2011).

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12. Appendices

12.1. Diabetes Study consent form

Study Number:

Patient Identification Number for this trial:

CONSENT FORM (Confidential)

Title of project: **The Psychobiology of Social Position: The Diabetes Study**

Name of Study Researchers: Dr Mark Hamer, Dr Roberto La Marca, Dr Antonio Lazzarino, Ms Ruth Hackett, Ms Sophie Bostock, Ms Bev Murray and Ms Livia Urbanova.

Any questions to: Psychobiology group, Department of Epidemiology and Public Health, University College London, 1-19 Torrington Place, London, WC1E 6BT. Telephone 020 7679 1804, Email: ruth.hackett.09@ucl.ac.uk

**Please initial
box**

1. I confirm that I have read and understood the information sheet (Version 2.0, 19/01/2011) for the above study and have had the opportunity to ask questions.

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

3. I understand that responsible individuals may look at sections of my medical notes where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

4. I give my permission for my GP to be informed that I am taking part in this research

5. I agree to take part in the above study.

Name of patient

Date

Signature

Researcher

Date

Signature

12.2. Diabetes Study information sheet

The Psychobiology of Social Position: The Diabetes Study

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.

Thank you for reading this.

What is the purpose of the study?

People with type 2 diabetes are at a higher risk of developing cardiovascular disease, for example, heart attacks, angina and stroke. These diseases develop over many years, and are thought to be influenced by a range of different factors. However, less is known about the role of social and psychological factors in this process. We are interested in investigating the way in which the body responds to psychosocial stress, and whether this is associated with increased risk of cardiovascular disease.

Who is organising and funding the research?

This research study is funded by the British Heart Foundation, and its purpose is to learn more about how our emotions and behaviour influence the cardiovascular system in health and disease. The results will help advance our knowledge of the links between the mind and the body, and develop new methods of improving patient care. The study is being carried out by Professor Andrew Steptoe from the Department of Epidemiology and Public Health at University College London. The research team who will carry out the work includes Dr Mark Hamer, Dr Roberto La Marca, Dr Antonio Lazzarino, Ms Ruth Hackett, Ms Sophie Bostock, Ms Bev Murray and Ms Livia Urbanova.

Why have I been chosen?

One hundred and twenty five people who have been diagnosed with type 2 diabetes mellitus, like yourself, will be invited to participate in the study over the next twelve months.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form to keep. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision not to take part or withdraw will not affect your medical treatment in any way.

What will happen to me if I take part?

The study consists of four parts: the assessment of physical activity over the course of seven days, filling in a questionnaire booklet, attending a session at our department and the measurement of saliva in everyday life.

If you agree to take part in the study, we will arrange an appointment for you to attend a testing session at the Department of Epidemiology and Public Health, University College London. The test session takes approximately 3 hours, starting at 9.15 a.m. or 1.30 p.m. We realise that this might seem like quite a long period of time, but we will ensure your comfort and a small snack will be available. During the session you will be asked to perform two 5-minute tasks; a visual puzzle and a hand-eye co-ordination task. These tasks do not require any special skills and most people find them interesting or even fun to do.

Throughout the session we will be taking a number of measures, which include: blood pressure will be measured by a small cuff placed around the fingers of one hand; heart rate by placing two electrodes on the chest; a chemical that is produced during stress called cortisol that can be measured in the saliva by chewing gently on a cotton swab for 2 minutes; various chemicals in the blood that will be taken by inserting a small needle in your arm. We will also monitor your blood glucose at two time points using a finger prick technique.

Although we get a lot of information from the session in the laboratory, we will learn even more if we also know more about what happens to you in everyday life situations. Therefore, we would like you to provide saliva samples at various times during a

normal day so that we can assess the level of cortisol. For this we will provide you with salivettes containing a cotton swab and instructions on how to use them. In order for us to see how situations in everyday life influence the measurements we are taking, we ask you to keep a diary and to fill this in every time you provide a saliva sample. This should not take long, as you only need to tick the appropriate responses. To assess physical activity you will be asked to wear a tiny device fitted to your belt called an Actigraph, which will measure your activity levels over the course of seven days.

What else do I have to do?

There are no other requirements and you should carry on as normal.

What are the possible disadvantages of taking part?

We do not anticipate any disadvantages in participating in this study. If, however, any problems become apparent that may require ongoing medical management we will advise you to contact your GP so that you can seek medical treatment as early as possible. We will also have a medically trained member of staff on site during the laboratory session.

What are the possible benefits of taking part?

Although there may be no direct benefits to you personally, we hope that you find the research an interesting experience. The information we get from this study may also help us to treat future patients with cardiovascular disease better. Your participation to help further this research would be appreciated. We realise that you will be devoting a considerable amount of time to this study (~5 hrs), and we will therefore be offering an honorarium to all participants as a token of our thanks. This will consist of £50 worth of Marks and Spencer's gift vouchers.

Will my taking part in this study be kept confidential?

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.

What if something goes wrong?

We do not expect you to suffer any adverse effects from this study. There are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you. You will be able to contact the research team in the first instance.

What will happen to the results of the research study?

The study will recruit 125 participants over a 12-15 month period. The results will be statistically analysed and findings subsequently published in peer reviewed journals. You will not be identified in any publication.

Who has reviewed the study?

The joint UCL/UCLH Committees on the Ethics of Human Research have reviewed the study and given a favourable ethical opinion.

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 1804.

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

12.3. Sleep problems questionnaire

How often in the past month did you:

(Please tick one answer for each question)

	not at all	1-3 days	4-7 days	8-14 days	15-20 days	21-31 days
Have trouble falling asleep?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5	<input type="checkbox"/> 6
Wake up several times per night?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5	<input type="checkbox"/> 6
Have trouble staying asleep (including waking far too early)?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5	<input type="checkbox"/> 6
Wake up after your usual amount of sleep feeling tired and worn out?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5	<input type="checkbox"/> 6
Have disturbed or restless sleep?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5	<input type="checkbox"/> 6

12.4. Alcohol consumption

Thinking of the last 7 days, **how much of each of the following did you drink?** (If it helps, think back over each day to this time last week).

If none, please enter 0.

		Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
a. Beer, lager, cider b. Wine c. Martini, sherry, port d. Spirits Other alcoholic drinks	pints	1	2	3	4	5	6	7
	glasses	1	2	3	4	5	6	7
	glasses	1	2	3	4	5	6	7
	measures	1	2	3	4	5	6	7
	glasses	1	2	3	4	5	6	7
		1	2	3	4	5	6	7
		1	2	3	4	5	6	7

12.5. Cook-Medley Cynical Hostility scale

Below are some statements which describe people's beliefs and attitudes and the way they might react to some situations. If the statement applies to you or describes you in general, tick the True column. If the statement does not describe you, indicate False.

		True	False
a. ■	I think a great many people exaggerate their misfortunes to gain the sympathy and help of others.	<input type="checkbox"/> 1	<input type="checkbox"/> 2
b. ■	I think most people would lie to get ahead.	<input type="checkbox"/> 1	<input type="checkbox"/> 2
c. ■	When someone does me wrong I feel I should pay him back if I can, just for the principle of the thing.	<input type="checkbox"/> 1	<input type="checkbox"/> 2
d. ■	Most people are honest chiefly through fear of being caught.	<input type="checkbox"/> 1	<input type="checkbox"/> 2
e. ■	Most people will use somewhat unfair means to gain profit or an advantage rather than to lose it.	<input type="checkbox"/> 1	<input type="checkbox"/> 2
f. ■	It takes a lot of argument to convince most people of the truth.	<input type="checkbox"/> 1	<input type="checkbox"/> 2
g. ■	I don't blame anyone for trying to grab everything he/she can get in this world.	<input type="checkbox"/> 1	<input type="checkbox"/> 2
h. ■	No one cares much what happens to you.	<input type="checkbox"/> 1	<input type="checkbox"/> 2
i. ■	It is safer to trust nobody.	<input type="checkbox"/> 1	<input type="checkbox"/> 2

j.	Most people make friends because friends are likely to be useful to them.	1	2
k.	Most people inwardly dislike putting themselves out to help other people.	1	2
l.	I am often inclined to go out of my way to win a point with someone who has opposed me.	1	2
m.	I do not blame a person for taking advantage of someone who lays himself open to it.	1	2
	People generally demand more respect for their own rights than they are willing to allow for others.	1	2

12.6. Center for Epidemiological Studies-Depression (CES-D) scale

Below is a list of ways that you might have felt or behaved recently. Please tell us how often you have felt this way during the past week.

(Tick one box on each line)

		Rarely or none of the time	Some or a little of the time	A moderate amount of time	Most or all of the time
a.	I was bothered by things which don't usually bother me	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b.	I did not feel like eating; my appetite was poor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c.	I felt that I could not shake off the blues even with the help of family or friends	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d.	I felt that I was just as good as other people	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e.	I had trouble keeping my mind on what I was doing	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
f.	I felt depressed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

g. ■	I felt that everything I did was an effort	1	2	3	4
h. ■	I felt hopeful about the future	1	2	3	4
i. ■	I thought my life had been a failure	1	2	3	4
j. ■	I felt fearful	1	2	3	4
k. ■	My sleep was restless	1	2	3	4
l. ■	I was happy	1	2	3	4
m. ■	I talked less than usual	1	2	3	4
n. ■	I felt lonely	1	2	3	4
o. ■	People were unfriendly	1	2	3	4
p. ■	I enjoyed life	1	2	3	4
q. ■	I had crying spells	1	2	3	4
r. ■	I felt sad	1	2	3	4
s. ■	I felt that people disliked me	1	2	3	4
t. ■	I could not get going	1	2	3	4

12.7. Life Orientation Test -Revised (LOT-R) scale

The following statements concern your attitudes and opinions. Please indicate the extent you agree with each of the following statements. There are no right or wrong answers.

(Please tick one answer for each question)

		Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
a.	In uncertain times, I usually expect the best	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b.	It's easy for me to relax	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c.	If something can go wrong for me, it will	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d.	I'm always optimistic about my future	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e.	I enjoy my friends a lot	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

f. It's important for me to keep busy 1 2 3 4 5

g. I hardly ever expect things to go my way 1 2 3 4 5

h. I don't get upset too easily 1 2 3 4 5

i. I rarely count on good things happening to me 1 2 3 4 5

j. Overall, I expect more good things to happen to me than bad 1 2 3 4 5

12.8. Financial strain

(Please tick one box for each question)

1.	At the present time:	No difficulty	With some difficulty	Very great difficulty
a.	Are you able to afford furniture or household equipment that needs to be replaced?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
b.	Do you have enough money for the kind of food you and your family should have?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
c.	Do you have problems in paying your bills?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
d.	Do you have enough money for the kind of clothing you and your family should have?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
e.	Are you able to afford to replace major items (such as a car) when you need to?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
f.	Do you have enough money for the leisure activities you and your family want?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3

g.

Are you able to afford a home
suitable for you and your family?

 1 2 3

2.

At the end of the month, do you have:

(Please tick one)

Some money left over

 1

Just enough to make ends meet

 2

Not enough to make ends meet

 3

12.9. Task impact questionnaire (including subjective stress)

Please answer the following questions by circling the number that best describes the way you felt during the task

1. How difficult did you find the task?

Not at all
difficult

Very
difficult

1 2 3 4 5 6 7

2. How involved in the task did you feel?

Not at all
involved

Very
involved

1 2 3 4 5 6 7

3. How well do you think you performed the task?

Not at all
well

Very well

1 2 3 4 5 6 7

4. How stressed did you feel during the task?

Not at all
stressed

Very
stressed

1 2 3 4 5 6 7

5. How much in control of the task did you feel?

Not at all in
control

Very in
control

1 2 3 4 5 6 7

6. How relaxed did you feel during the task?

Not at all
relaxed

Very
relaxed

1 2 3 4 5 6 7