Demographic and psychological factors in type 2 diabetes risk and progression

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Thesis submitted for the degree of Doctor of Philosophy

Student Declaration

I, [redacted], 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.

Signature: [redacted] Date: 18/06/2020

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Abstract

Demographic and positive psychological factors are relevant to Type 2 Diabetes (T2D) risk and progression, but the biological underpinnings are unclear. This PhD consists of four studies aiming to better understand the role of demographic and positive psychological factors on T2D risk and progression, and the biological mechanisms involved.

Study 1 used data from a nationally representative cohort to examine a) the relationship between different types of subjective well-being and T2D incidence in initially healthy participants and b) the amount of association explained by sociodemographic, behavioural, and clinical characteristics. Hedonic but not eudaimonic well-being predicted lower T2D rate over 12 years. Sociodemographic, behavioural, and clinical factors together accounted for 36% of the association.

Studies 2 and 3 used data from a laboratory stress testing study. Study 2 tested sex differences in inflammatory stress responses in people with existing T2D. Results showed that women with T2D produced larger interleukin(IL)-6 stress responses compared with men. Study 3 examined the association between hedonic well-being and inflammatory stress responses in individuals with T2D. Hedonic well-being was associated with lower inflammatory markers pre- and post-stress. Inflammatory stress responses did not differ in people varying in hedonic well-being.

Study 4 is a 7.5-year follow-up of this laboratory study. Study 4 looked at the possible mediating role of IL-6 stress (re)activity in associations linking sex or hedonic well-being with health outcomes in participants with T2D. Results showed that women with

T2D experienced worse mental health-related quality of life at follow-up and greater IL-6 stress responses at baseline predicted diminished mental health-related quality of life. IL-6 stress responsivity did not mediate the link between sex and mental health at follow-up.

These findings highlight the role of hedonic well-being in T2D development. The relationship between sex or hedonic well-being with T2D outcomes may be mediated via increased inflammatory (re)activity.

Impact Statement

There are several ways in which the knowledge presented in this thesis could be impactful. These include: 1) Future research: Type 2 diabetes (T2D) is one of the fastest health challenges of our times, therefore it remains a key area of research. This PhD aimed to investigate the impact of demographic and psychological factors in T2D risk and progression, as well as the biological underpinnings of these relationships, with an overall purpose the development of more effective preventive and management strategies. Findings from this work fill critical gaps in the literature and open new avenues for future research. More precisely, results from this PhD encourage the investigation of a) well-being and lifestyle interventions for the prevention of T2D, b) anti-inflammatory interventions in individuals with established T2D who show increased inflammatory (re)activity for the prevention and/or management of mental health difficulties, and c) interventions to improve quality of life and mood comorbidities, especially in women with T2D. 2) Clinical practice: Counselling and psychological support delivered in the context of diabetes have been recognised as fundamental aspects of diabetes care. This PhD research highlights the role of subjective well-being as a novel target for interventions and public health policies seeking to reduce the risk of T2D in high-risk populations, such as those with pre-diabetes. Additionally, this research has introduced, for the first time, the predictive value of increased inflammatory stress responsivity in relation to long-term mental health disturbances in this patient group. These findings could support the implementation of immunomodulatory strategies in people with T2D for the prevention of mental health-related outcomes. These results also demonstrate that not only inflammatory levels but also inflammatory reactivity to stress should be on

the focus of preventive interventions. In light of this, increased inflammatory reactivity could be considered as a mental health risk factor in healthcare settings and therefore could be included in the assessment of the risk of future mental health problems in people with T2D. Such assessment would help identify those patients who may benefit from targeted preventative interventions. Furthermore, findings from this PhD highlight the role of sex as an individual factor that also needs to be considered in clinical settings. Specifically, women with T2D have been found to show elevated inflammatory stress reactivity as well as they are more likely to experience worse mental health-related quality of life in the long-term compared to men with diabetes. These results suggest that women with T2D should be at the forefront of the focus on preventive and management interventions. 3) Dissemination of findings: I have presented my work in two international conferences. Also, two of my projects have been published in peer-reviewed scientific journals and one study is under review for publication. I included the findings from my research into academic lectures that I gave to undergraduate students of Psychology at the University of Roehampton. Finally, this knowledge has been incorporated into non-academic presentations given within the Department of Behavioural Science and Health at University College London.

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List of abbreviations

ADA = American Diabetes Association

ANCOVA = Analysis of Covariance

ANOVA = Analysis of Variance

APA = American Psychiatric Association

BMI = Body mass index

CAD = Coronary artery disease

CAR = Cortisol awakening response

CASP = Control, Autonomy, Self-realization, Pleasure (scale)

CBT = Cognitive Behaviour Therapy

CES-D = Centre for Epidemiological Studies-Depression (scale)

CHD = Coronary heart disease

CI = Confidence interval

CRH = Corticotropin-releasing hormone

CRP = C-reactive protein

CVD = Cardiovascular disease

ELSA = English Longitudinal Study of Ageing

EMA = Ecological momentary assessments

ER = Endoplasmic reticulum

GC = Glucocorticoid

HbA1c = Glycated hemoglobin

HPA = Hypothalamus – pituitary – adrenal

HR = Hazard ratio

HRA = Health Research Authority

HSS = Heart Scan Study

IDF = International Diabetes Federation

IFN-γ = Interferon-gamma

IL = Interleukin

IL-1 beta = IL-1β

IL-1ra = IL-1 receptor antagonist

kg/m^2 = Kilograms per square metre

LC = Locus coeruleus

MCP-1 = Monocyte chemoattractant protein-1

MI = Myocardial infarction

mmHg = Millimetres of Mercury

mmol/l = Millimoles per mole

NHS = National Health System

OR = Odds ratio

pg/ml = Picograms per mililitre

PPAE = Percentage of protective association explained

RR = Relative risk

SAM = Sympathetic – adrenal – medullary

SD = Standard deviation

SE = Standard error

SES = Socioeconomic status

SF-36 = Short Form-36

SNS = Sympathetic nervous system

SPSS = Statistical Package for the Social Sciences

T1D = Type 1 diabetes

T2D = Type 2 diabetes

Th = T-helper

TNF-α = Tumor necrosis factor-alpha

TSST = Trier Social Stress Test

UCL = University College London

UK = United Kingdom

US = United States

WHO = World Health Organisation

WHR = Waist-to-hip ratio

β = beta (cells)

Δ = Delta (change score)

Chapter 1. Literature review

This chapter will describe the existing literature relating to this thesis. Firstly, the condition of type 2 diabetes (T2D) will be described and the role of subjective well-being in the onset of T2D will be outlined. Following this, evidence for sex differences in T2D health outcomes and the impact of subjective well-being in T2D progression will be presented. The biological stress response will then be described and the potential mediating role of biological stress responsivity linking sex or subjective well-being with health outcomes will be introduced, with a particular focus on inflammatory responsivity. The limitations of work to date will also be reported, highlighting the gaps that this PhD research sets out to address.

1.1 The concept of diabetes

1.1.1 Introduction to diabetes

Diabetes Mellitus is a progressive metabolic disorder characterised by markedly raised blood glucose levels that exceed normal physiological ranges (International Diabetes Federation [IDF], 2019). The prevalence of diabetes is steadily increasing worldwide (Hex, Bartlett, Wright, Taylor, & Varley, 2012). An estimated 463 million adults aged 20-79 years are currently living with diabetes, representing 9% of the world's population in this age group (IDF, 2019). The total number is projected to increase to 578 million (10% of the world's population) by 2030 and to 700 million (11% of the world's population) by 2045 (IDF, 2019). Future estimates are also demonstrating an increasing and spreading burden worldwide, including the United Kingdom (UK). Specifically, the number of adults with diabetes is projected to rise by 55% by 2035, with poorer nations being those most markedly affected. Indeed, although once

thought mainly to be a disease of the richest/high-income countries (as classified by the World Bank), low- and middle-income countries are expected to bear the brunt of diabetes in the future. In particular, the greatest proportional increase is expected in the African continent and in the Middle-East, as well as among Mexicans, Guatemalans, Bangladeshis, Cambodians, Philipinos, and Vietnamese (Guariguata et al., 2014). These region-related differences in trends might be inherent to socioeconomic differences, as well as to urbanisation that characterises these countries and the associated lifestyle changes[1] (Banks, Marmot, Oldfield, & Smith, 2006; Danaei et al., 2011; Tobias, 2011; Whiting, Guariguata, Weil, & Shaw, 2011). Figure 1.1 illustrates projections of diabetes prevalence globally, in adults 20-79 years old. Concluding, such is the growth of diabetes worldwide that it is one of four priority non-communicable diseases targeted for action by world leaders, along with cardiovascular diseases (CVDs), cancer, and chronic respiratory diseases (World Health Organization [WHO], 2016a). Today, there is a globally agreed target to halt the rise in diabetes by 2025 (WHO, 2020a).

[1] The role of lifestyle factors in the development of diabetes is described on page 24.

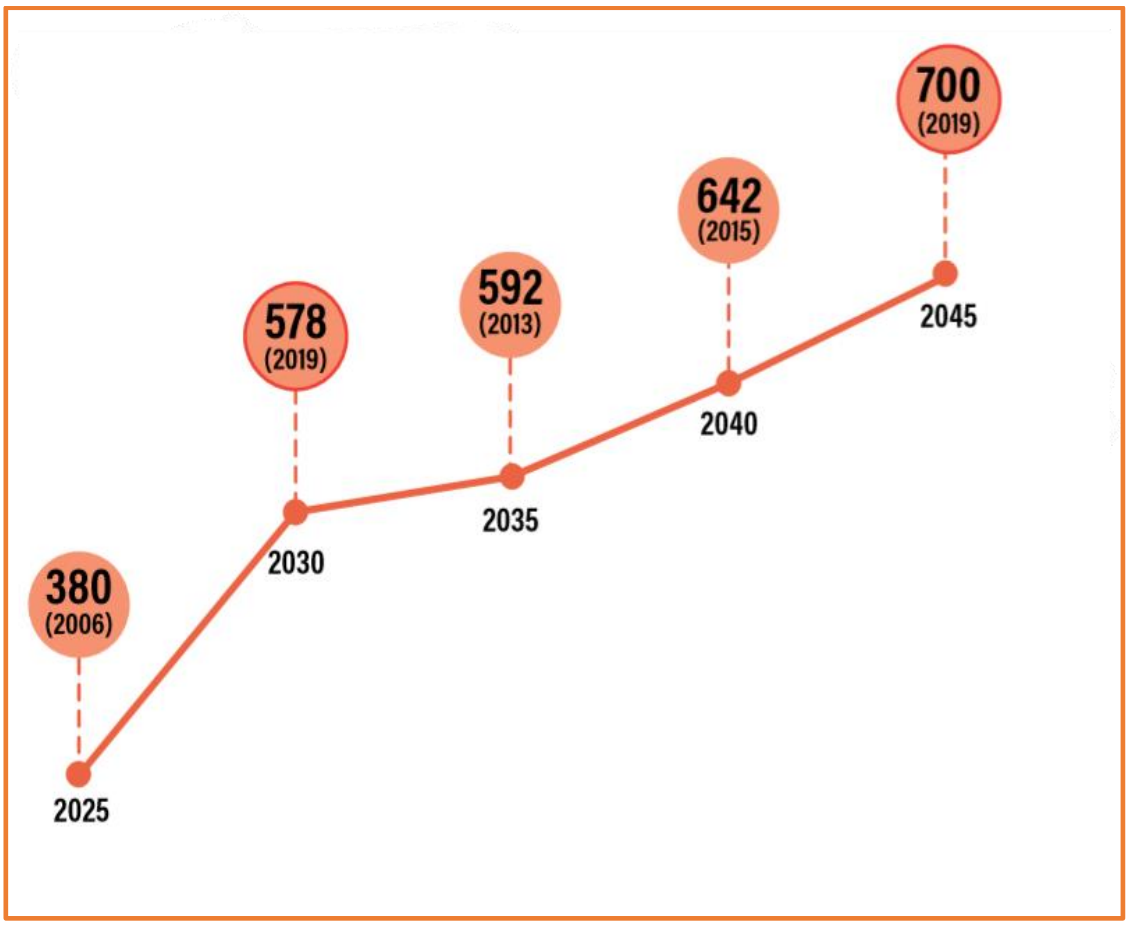

Figure 1.1. Estimates and projections of diabetes global prevalence in adults 20-79 years old. Numbers represent millions. Adjusted from "IDF Diabetes Atlas 9th edition" by International Diabetes Federation, 2019 (https://www.diabetesatlas.org/en/resources/). Copyright (02.06.2020) by the Atlas team. Reprinted with permission.

1.1.2 Classification of diabetes

The American Diabetes Association (ADA) currently recognises four diabetes diagnoses. These include type 1 diabetes (T1D), T2D, gestational diabetes, and diabetes due to other causes (American Diabetes Association [ADA], 2019). Briefly, T1D is characterised by β cell destruction usually leading to absolute insulin deficiency (ADA, 2019). In healthy people, pancreatic beta (β) cells produce the endocrine peptide hormone insulin which plays a central role in the metabolism of carbohydrates, allowing body tissues (mainly the liver, muscles, and adipose tissue) to ingest glucose from blood circulation in order to be used as an energy source

(Niswender & Schwartz, 2003). T2D accounts for more than 90% of all diabetes cases and is characterised by relative insufficient insulin secretion on the background of insulin resistance (ADA, 2019). Insulin resistance refers to the inhibition of the actions of insulin in peripheral tissues (Niswender & Schwartz, 2003). Gestational diabetes develops during pregnancy with blood glucose levels usually returning to normal ranges after childbirth (ADA, 2019). The fourth category of diabetes due to other causes includes the least prevalent cases among all four categories. Causes involve genetic defects in β cell function or insulin action, some diseases of the pancreas, and some endocrinopathies, among others (ADA, 2019). T2D is on the focus of this thesis, therefore it is described below more broadly.

1.1.3 T2D epidemiology

T2D accounts for the vast majority of diabetes cases globally, and it is this type of diabetes that is driving the rapid increase in prevalence (ADA, 2014). In 2018-2019 in the UK, 3.919,505 people aged ≥17 years old were diagnosed with diabetes, and approximately 90% of them have T2D (Diabetes UK, 2020). It has also been estimated that almost 1 million people with T2D in the UK are undiagnosed (Diabetes UK, 2020).

T2D is typically characterised by relative insulin deficiency on the background of insulin resistance. As mentioned earlier, insulin promotes glucose uptake into insulin-dependent body tissues. In the presence of insulin resistance and insulin deficiency, due to defects in peripheral tissues and β cells failure, respectively, insulin target tissues cells are unable to ingest glucose, eventually resulting in elevated glucose concentrations in the bloodstream (Petersen & Shulman, 2018). Consequently, the clinical manifestation of T2D is hyperglycemia and diagnostic criteria are based on

plasma glucose levels, either the eight-hour fasting plasma glucose or after a 75-gram oral glucose tolerance test or established glycated hemoglobin (HbA1c) cut-off points. The clinical cut-off points for fasting glucose and glucose tolerance are ≥7.0 millimoles per litre[2] (mmol/l) and ≥11.1mmol/l[3], respectively (ADA, 2019). HbA1c reflects the average blood glucose concentration over the previous two to three months (ADA, 2017). The clinical cut-off point for HbA1c is 48 mmol/mol[4] (ADA, 2019).

T2D is typically a condition of adults. Indeed, ageing is strongly associated with T2D and much of the increase in T2D prevalence is driven by population growth and extension of life expectancy (Peters, Huxley, Sattar, & Woodward, 2015). Nevertheless, children and adolescents may also be diagnosed with the condition. A study in the United States (US) showed that T2D diagnoses in children and adolescents aged between 10-19 years have been dramatically increased, especially in ethnic minority populations (Dabelea et al., 2014). Projections suggest a 4-fold increase in the number of youths <20 years old with T2D in 2050 (Imperatore et al., 2012).

T2D develops gradually and at earlier stages may not be severe enough for the individual to notice symptoms of marked hyperglycemia. These symptoms include polydipsia (frequent thirst), polyuria (increased volume of urination), weight loss, polyphagia (increased hunger), and blurred vision (ADA, 2014). When glucose levels do not meet the criteria for the formal diagnosis of T2D but are beyond normal levels, individuals face an increased risk of subsequent development of T2D. Therefore, this so-called pre-diabetes stage encompasses individuals who have impaired fasting

[2] Equals to 126 milligrams per deciliter.
[3] Equals to 200 milligrams per deciliter.
[4] Equals to 6.5%.

glucose and/or impaired glucose tolerance and/or HbA1c within a specific high-risk range (ADA 2014; WHO, 2016a). It is estimated that 70% of people with pre-diabetes will eventually develop overt T2D (Tabák, Herder, Rathmann, Brunner, & Kivimäki, 2012). Along with hyperglycemia and insulin resistance, both pre-diabetes and T2D are strongly associated with obesity (especially visceral obesity), dyslipidemia, hypertension[5], procoagulant state, pro-inflammatory state and endothelial dysfunction (the role of pro-inflammatory markers in T2D is described in detail on page 86). Increases in the incidence of lifestyle-related disorders such as obesity, mirror increases in the incidence of T2D (WHO, 2016a). Indeed, lifestyle modification has been suggested to reduce T2D risk by 40 - 70% (Tabák et al., 2012).

1.1.4 T2D risk factors

1.1.4.1 Traditional risk factors

The most widely recognised and dominant risk factor for the development of T2D is obesity. Obesity contributes to the pathophysiology of insulin resistance and T2D progression (Belkina & Denis, 2010). The mechanisms through which obesity may increase the risk of T2D are described in more detail on page 86. The majority of people with T2D are either overweight or obese[6] or have an increased percentage of visceral fat[7] (Guh et al., 2009). In the UK, obesity accounts for 80 - 85% of T2D cases

[5] The combination of glucose intolerance, obesity, hypertension, and dyslipidemia characterises the so-called metabolic syndrome (Eckel, Alberti, Grundy, & Zimmet, 2010).

[6] Overweight and obesity are defined as abnormal or excessive fat accumulation that may have negative health implications. A simple index that is commonly used to classify overweight and obesity in adults is body mass index (BMI). This is calculated as the person's weight in kilograms divided by the square of their height in metres (kg/m^2). For adults, overweight is a BMI ≥ 25 kg/m^2 and obesity is a BMI ≥ 30 kg/m^2 (WHO, 2020b).

[7] An assessment of visceral (abdominal) fat can be provided by measuring the waist-to-hip ratio (WHR). This is calculated by dividing the circumference of the waist by the circumference of the hips. Abdominal obesity is defined as a WHR > 0.90 inches for men and > 0.85 inches for women (WHO, 2008).

(Diabetes UK, 2020). The major contributor for overweight and obesity is an energy imbalance between calories consumed and calories expended resulting from dietary factors and physical inactivity (WHO, 2020b). Nevertheless, along with the spreading burden of T2D from richer to poorer countries and from older to younger age groups, T2D is increasingly represented by both obese and relatively lean individuals, indicating a growing heterogeneity in T2D pathophysiology and phenotype (Gregg, Sattar, & Ali, 2016; Tuomi et al., 2014).

Dietary factors, sedentariness, physical inactivity, smoking, and high alcohol intake are also established behavioural risk factors for T2D. Dietary factors known to increase the risk of T2D include consumption of low-fibre and high glycemic index foods, as well as high intake of saturated fats and frequent consumption of processed meat (Wu, Ding, Tanaka, & Zhang, 2014). Interestingly, the effect of diet on T2D risk has been found to be independent of BMI in some studies (Wu et al., 2014), suggesting that direct biological mechanisms may also be relevant. The role of physical activity on glucose metabolism and body weight has long been established. Physical activity may contribute to 30-50% reduction in the development of T2D (Bassuk & Manson, 2005) by helping achieve weight loss (Telford, 2007). With regards to smoking as a risk factor for T2D, a meta-analysis of 25 prospective cohort studies involving 1.200,000 participants demonstrated that the risk of T2D is greater for heavy smokers (≥ 20 cigarettes/day) compared to lighter smokers and greater for active smokers compared to former smokers (Willi, Bodenmann, Ghali, Faris, & Cornuz, 2007), consistent with a dose-response relationship. Additionally, smokers tend to engage in unhealthy behaviours (such as lack of physical activity, poor fruit and vegetable intake, and high

alcohol intake) that may increase T2D risk via weight gain (Chiolero, Wietlisbach, Ruffieux, Paccaud, & Cornuz, 2006; van Dam, Rimm, Willett, Stampfer, & Hu, 2002). Furthermore, smokers have higher risk of abdominal fat accumulation compared to non-smokers even with a normal BMI (Seidell, 1997; Shimokata, Muller, & Andres, 1989; Simon, Seeley, Lipschutz, Vittinghoff, & Browner, 1997; Wareham et al., 1996). The mechanism is not well-understood but because smoking has an anti-estrogenic effect (Tankó & Christiansen, 2004; Windham, Mitchell, Anderson, & Lasley, 2005) it could be related to a hormonal imbalance that might lead to central obesity. Nicotine or other components of cigarette smoking may also have a direct effect on pancreatic β cells contributing to inadequate compensatory insulin secretion in the background of insulin resistance (Attvall, Fowelin, Lager, Von Schenck, & Smith, 1993; Eliasson, Asplund, Evrin, & Lundblad, 1995; Facchini, Hollenbeck, Jeppesen, Chen, & Reaven, 1992; Frati, Iniestra, & Ariza, 1996; Janzon, Berntorp, Hanson, Lindell, & Trell, 1983; Schwartz, Il'yasova, & Ivanova, 2003). The latter is supported by associations linking cigarette smoking with pancreatic cancer or chronic pancreatitis (Jaffiol, Thomas, Bean, Jégo, & Danchin, 2013). With regards to alcohol as a risk factor for T2D, a U-shaped association has been reported. Specifically, light-to-moderate alcohol intake seem to decrease the incidence of T2D, whereas heavy drinkers and binge drinkers are at increased risk for developing diabetes (Polsky & Akturk, 2017). Heavy alcohol intake favours weight gain which in part explains the increased T2D risk (Wannamethee, Shaper, Perry, & Alberti, 2002). Also, heavy alcohol consumption may partially affect T2D risk via its effect on triglyceride metabolism (Scragg & Metcalf, 2004).

Genetic predisposition also plays a role in T2D risk as demonstrated in some twin studies (Poulsen et al., 2009; Sanghera & Blackett, 2012). For example, first-degree relatives of people with T2D show higher concordance rates compared to the incident rate in the general population (Kobberling, 1982). Genome-wide association studies have identified approximately 75 susceptibility loci related to T2D (Wu et al., 2014). However, not all twin studies have found evidence of a significant genetic component in T2D (Poulsen, Kyvik, Vaag, & Beck-Nielsen, 1999). Even in cases of monozygotic twins, concordance rates are not as high as 100%; thus, it is probable that genetic vulnerability interacts with environmental determinants to influence the risk of T2D.

Apart from traditional risk factors, other environmental factors are receiving growing attention in relation to T2D development. Sociodemographic characteristics, such as socioeconomic status (SES), seem to be associated with T2D onset, with findings supportive of a social gradient in health. For example, in a large study of > 48,000 French participants aged 35 to 80 years, it was found that the risk of T2D increased linearly with socioeconomic deprivation levels. More precisely, the prevalence of T2D was three to eight times higher in the most deprived participants. After accounting for a range of covariates, the risk of diabetes remained significantly higher among deprived versus non-deprived participants (Jaffiol et al., 2013). T2D behavioural and clinical risk factors are more frequently seen in deprived populations (Connolly & Kesson, 1996; Jaffiol, Thomas, Bean, Jego, & Danchin, 2013; Kavanagh et al., 2010; Phillips et al., 2012; Unwin et al., 1995). These individuals have limited access to healthcare and are less likely to visit their doctor (Jaffiol et al., 2013). Psychological risk factors, such as symptoms of depression and anxiety, are also more common in

deprived groups (Bell et al., 2005; Collins, Corcoran, & Perry, 2009; Egede & Zheng, 2003; Thomas, Jones, Scarinci, & Brantley, 2003). Indeed, an emerging body of literature suggests that psychological factors contribute to the risk of T2D. The role of psychological risk factors in the development of T2D is discussed in detail below.

1.1.4.2 Psychological risk factors

1.1.4.2.1 Negative emotional well-being and T2D

The role of psychological factors in the risk of T2D onset has received recent attention. A growing body of longitudinal studies indicates that psychological factors contribute to the risk of T2D in initially diabetes-free individuals. The vast majority of work so far has investigated associations between negative psychosocial factors and future T2D onset. More specifically, previous studies have mainly focused on negative psychological factors such as perceived stress, work-related stress (encompassing phenomena such as job strain[8], long working hours, burn-out, and effort-reward imbalance), psychological disorders including depression[9], anxiety[10], and post-traumatic stress disorder[11], negative personality traits such as anger and hostility, and adverse early-life events[12]. Overall, findings revealed that negative psychosocial

[8] Job strain is defined as increased demands coupled with decreased decision control at work (Nyberg et al., 2014).

[9] Depression (major depressive disorder) is a common and serious medical illness that negatively affects what the person feels, thinks, and acts. Depressive symptoms (e.g. sadness, hopeless, helpless) can vary from mild to severe and must last at least two weeks (for most of the time) for the diagnosis of clinical depression (American Psychiatric Association [APA], 2020a).

[10] Anxiety is an umbrella term that encompasses several anxiety disorders including generalised anxiety disorder, panic disorder, and phobias. Anxiety disorders differ from feelings of nervousness or anxiousness. They involve excessive (out of proportion to the situation or age-inappropriate) fear or anxiety relating to a future concern and are associated with muscle tension and avoidance behaviour ultimately hindering the person's ability to function normally (APA, 2020b).

[11] Post-traumatic stress disorder is a stress-related disorder that is related to a high-intensity, stressful/traumatic event (Shalev, Liberzon, & Marmar, 2017).

[12] Early-life events can be prospectively measured using a life-course methodology. This approach enables the investigation of the long-term health outcomes of physical and social exposures during

factors increase the risk of developing T2D in the long-term (Hackett & Steptoe, 2017). Adverse early-life events, particularly childhood neglect (Huang et al., 2015), depression (from depressive symptoms to clinical depression), and work-related stress, mainly job strain and long working hours (≥55 hours per week), are the most frequently examined factors in relation to T2D, where the most convincing, meta-analytic data exists (Hackett & Steptoe, 2017). These factors are not only risk factors for T2D but also, they have prospectively linked to adverse health outcomes in people with existing T2D. For example, clinical depression, high depressive symptoms, and poorer mental health-related quality of life have been associated with increased risk of T2D complications[13] (Gonzalez et al., 2010; Iversen et al., 2015; Novak et al., 2016; Rådholm, Wiréhn, Chalmers, & Östgren, 2016; Scherrer et al., 2011; Sieu et al., 2011; Ting et al., 2013; Williams et al., 2010), and cardiovascular and all-cause mortality (Dalsgaard et al., 2014; Hofmann, Köhler, Leichsenring, & Kruse, 2013; Park, Katon, & Wolf, 2013).

1.1.4.2.2 Subjective well-being and T2D

While the association between negative psychological factors and T2D risk has been well-documented, less is known about the effect of positive psychological factors, such as subjective well-being, on T2D risk. Subjective well-being is not simple to define because it is a multidimensional component that incorporates several inter-related constructs. Nevertheless, one influential taxonomy differentiates between three sub-

gestation, childhood, adolescence, young adulthood and later adult life. The aim of this approach, which is usually used by epidemiologists, is to better understand the psychosocial, behavioural, and biological processes that influence health and disease risk across the whole life span (Kuh, Ben-Shlomo, Lynch, Hallqvist, & Power, 2003).

[13] The complications of T2D are described on page 36.

components of subjective well-being: hedonic (or affective) well-being, eudaimonic well-being, and evaluative well-being (Stone & Mackie, 2013).

Hedonic well-being focuses on a person's emotional state, feelings or moods, such as enjoyment of life, pleasure, vitality, or happiness. It is typically assessed retrospectively by asking participants about their mood over the previous week or month. Eudaimonic well-being is a more complex construct that refers to judgments about a sense of purpose, meaningfulness, fulfilment in life, and self-realisation. Its assessment demands more extensive cognitive processing than ratings of mood and involves relatively complex introspection about attributes aggregated over time (Ryan & Deci, 2001; Steptoe, 2019a). The third sub-type of subjective well-being, evaluative well-being, refers to appraisals of how satisfied people are with their lives (Steptoe, Deaton, & Stone, 2015). Other positive psychological constructs that are related to subjective well-being include optimism and stress resilience. Optimism refers to the general expectation of positive outcomes in life (Steptoe, 2019a). Stress resilience is defined as the ability of the individual to withstand the experience of severe adversity and cope effectively with the challenges of life (Steptoe, 2016).

An extensive body of evidence from longitudinal studies suggests that subjective well-being is a protective factor for physical disability, cognitive decline, morbidity, and mortality among older adults (Chida & Steptoe, 2008; Martin-Maria et al., 2017; Steptoe, 2019a). The prospective association between positive psychological characteristics and T2D onset has been documented in a small number of previous studies. Two recent studies using epidemiological data from the English Longitudinal Study of Ageing (ELSA) found an independent relationship between an aggregate

measure of subjective well-being and T2D incidence (Hazard ratio [HR] = 0.82, 95% Confidence Interval [CI; 0.71, 0.95]) and HbA1c levels (*B* = -0.04, 95% CI [-0.06, -0.01]), respectively, but only in participants younger than 65 years old (Okely & Gale, 2016; Poole, Hackett, Panagi, & Steptoe, 2019). Analyses were adjusted for sociodemographic, behavioural, and clinical risk factors, as well as depressive symptoms (Okely & Gale, 2016; Poole et al., 2019). Additionally, in 97 diabetes-free elderly women, hedonic well-being (positive affect) but not eudaimonic well-being (purpose in life and personal growth) was inversely associated with HbA1c levels (*B* = -0.24, $p < 0.001$) two years later after adjusting for baseline HbA1c, sociodemographic, and health factors (Tsenkova, Love, Singer, & Ryff, 2007). Interestingly, in the study by Tsenkova et al. (Tsenkova et al., 2007), low positive affect, low purpose in life, and low personal growth amplified the detrimental effect of low income on HbA1c levels, contributing to ever higher levels of HbA1c in adjusted analyses. A more recent study including a national sample of 3907 older adults drawn from the Health and Retirement Study cohort tested the association between purpose in life and incident pre-diabetes or T2D over four years of follow-up. Results showed that participants with a higher sense of purpose in life at baseline had lower risk (Odds ratio [OR] = 0.78, $p = 0.037$, 95% CI [0.62, 0.98]) of developing objectively measured pre-diabetes or T2D (as indexed by HbA1c measurements) after adjusting for demographic variables, physical health, physical function, depression, and psychiatric diagnoses (Hafez et al., 2018). Another study that used data from 7800 participants who were drawn from the Whitehall II epidemiological cohort study found that emotional vitality (but not optimism) was associated with a 9-15% decrease in the odds of having self-reported doctor-diagnosed T2D after 13 years (Boehm, Trudel-Fitzgerald, Kivimaki, &

Kubzansky, 2015). Results remained significant after adjusting for demographic characteristics, health behaviours, and clinical risk factors, but were attenuated when depressive symptoms were included in the model. In the same study, emotional vitality was not associated with T2D as detected with a clinical assessment (using an oral glucose tolerance test). It is not clear why associations differed for self-reported doctor diagnosis and screen-detected diabetes. Considering that these analyses included a sub-sample of 288 individuals, these findings should be interpreted with caution. The authors also suggest that tests for screen-detected diabetes could have lower specificity than tests carried out by a physician. However, this hypothesis was not directly tested.

In another project using data from 754 participants from Midlife Development in the United States study, hedonic well-being, particularly positive emotions, was prospectively associated with a 7% reduced risk of incident cardiometabolic conditions, including T2D, over the 9-year of follow-up. However, this association was attenuated after adjusting for covariates including health behaviours and depressive symptoms (Boehm, Chen, Williams, Ryff, & Kubzansky, 2016). In another analysis of the Midlife Development in the United States study, positive emotions were associated with a lower relative risk (RR) for T2D (RR = 0.66, 95% CI [0.47, 0.93) in a high-risk group of participants with a parental history of diabetes (Tsenkova, Karlamangla, & Ryff, 2016). This pattern of results persisted after adjusting for sociodemographic and health measures, as well as negative and depressed affect.

Findings from large-scale prospective studies further suggest protective associations between life satisfaction and T2D risk (Boehm et al., 2015; Feller, Teucher, Kaaks,

Boeing, & Vigl, 2013; Piciu, Johar, Lukaschek, Thorand, & Ladwig, 2018; Shirom, Toker, Melamed, Berliner, & Shapira, 2012), and stress resilience and T2D risk (Crump, Sundquist, Winkleby, & Sundquist, 2016), after adjusting for a range of known risk factors. Hedonic and eudaimonic well-being are the focus of this thesis, and longitudinal associations between the two types of well-being and incident T2D have been examined and presented in Study 1 (see Chapter 3).

Despite the fact that previous studies demonstrated an independent association between increased well-being and reduced T2D risk, the extent to which this link can be explained by other factors remains unclear. Sociodemographic, behavioural, and clinical factors are plausible pathways through which subjective well-being reduces T2D risk. As mentioned previously, sociodemographic characteristics, including wealth and SES, are associated with diabetes onset, with findings supporting a social gradient in health (Jaffiol et al., 2013). Regular physical activity is consistently associated with subjective well-being, and its effect on glucose metabolism and body weight has been well-documented (Black et al., 2015; Kim, Kubzansky, Soo, & Boehm, 2017; Windle, Hughes, Linck, Russell, & Woods, 2010). Other health behaviours such as smoking and increased alcohol consumption are also relevant both to reduced well-being (Geiger & MacKerron, 2016; Shahab & West, 2012) and diabetes risk (Koppes, Dekker, Hendriks, Bouter, & Heine, 2005; Willi et al., 2007). Well-being is associated with a more favourable cardiovascular prolife in healthy and diseased populations (Boehm & Kubzansky, 2012), which in turn can protect against T2D development (Eckel et al., 2010).

The maintenance of significant relationships between subjective well-being and T2D after adjustment for negative or depressed affect in some previous studies (Okely & Gale, 2016; Poole et al., 2019; Shirom et al., 2012; Tsenkova et al., 2016) is a striking finding that indicates that the effect of positive psychological factors is not always secondary to the absence of negative factors (Brouwers et al., 2013; Steptoe, O'Donnell, Badrick, Kumari, & Marmot, 2008). Subjective well-being is not merely the mirror of depression, anxiety, or distress, but both positive and negative factors can co-exist (Larsen, Hershfield, Stastny, & Hester, 2017). Indeed, moods may fluctuate rapidly, and many life experiences involve both positive and negative components. In addition, the fact that someone is not depressed, anxious, or distressed does not imply that they are joyful or happy. Subjective well-being involves moving beyond the neutral state (neither happy not sad) to positive states of enjoyment, sense of purpose, and life satisfaction. In other words, reducing mental suffering does not imply that we have increased happiness or overall well-being. The recognition of the separate roles of positive, neutral, and negative psychological states has stimulated the development of Positive Psychology as a separate field. Positive Psychology studies do not just replicate the findings of negative factors by reversing the terms used, but instead they have identified new relationships with health and human functioning. For example, subjective well-being has distinct relationships with a range of health outcomes and potentially operates via different behavioural and/or biological pathways compared to negative factors.

The presence of positive factors could possibly explain the variability of findings seen in some studies of negative factors and T2D (Abraham et al., 2015; Atlantis,

Vogelzangs, Cashman, & Penninx, 2012; Demmer et al., 2015; Engum, 2007; Farvid et al., 2014; Golden et al., 2006; Huang et al., 2015; Kumari, Head, & Marmot, 2004; Vancampfort et al., 2016; Williams, Magliano, Tapp, Oldenburg, & Shaw, 2013). For example, the evidence between anxiety (symptoms or disorder) and increased T2D risk is currently mixed (Hackett & Steptoe, 2017). The effect of subjective well-being and related constructs (e.g. stress resilience) on T2D might be more robust than the effects of anxiety, hence abolish the negative impact of anxiety resulting in null finding between anxiety and T2D.

The three aspects of subjective well-being are moderately, positively inter-correlated (Kashdan, Biswas-Diener, & King, 2008). Some researchers have combined these concepts into broader constructs (Seligman, 2011; VanderWeele, 2017), such as the notion of flourishing (VanderWeele, 2017). Nevertheless, it is recognised that enjoyment of life may not be always accompanied by a sense that life is worthwhile, and *vice versa*. In many cases, people who have achieved purpose and fulfilment in their lives are not always joyful (Steptoe, 2019a). The strength of the associations with health outcomes may also vary across the different types of well-being (Boehm et al., 2016; Boehm & Kubzansky, 2012; Boehm et al., 2015; Martin-Maria et al., 2017). For instance, optimism has emerged as a robust predictor of cardiovascular health, although associations are less consistent for optimism and cancer (Hernandez et al., 2018). It has, therefore, been argued that the investigation of the individual contribution of the different types of well-being is worthwhile in health research (Hernandez et al., 2018; Steptoe, 2019a).

1.1.5 T2D complications and comorbid conditions

As well as being a disorder *per se*, the chronic hyperglycemia in T2D is linked with long-term damage, dysfunction and ultimately failure of various bodily organs and blood vessels. The harmful effects of T2D can be distinguished into two broad categories namely microvascular and macrovascular complications (Forbes & Cooper, 2013; Fowler, 2008; Shah et al., 2015). Microvascular complications reflect damage to the small blood vessels whereas macrovascular complications reflect damage to the large blood vessels. Microvascular complications of T2D include retinopathy, nephropathy, and neuropathy. Retinopathy refers to damage to the eyes and may lead to glaucoma, cataract, severe visual impairment, and blindness. Nephropathy refers to damage to the kidneys and may lead to renal failure or kidney disease. Neuropathy refers to damage to the nerves and may lead to impotence, foot pain, foot ulcers, infections, gangrene, and amputation (Forbes & Cooper, 2013; Fowler, 2008).

The macrovascular complications of T2D encompass various CVDs. The most common forms of CVDs are coronary artery disease (CAD), cerebrovascular disease, and peripheral arterial disease (Forbes & Cooper, 2013; Fowler, 2008). CAD is also known as coronary heart disease (CHD) or ischemic heart disease. CAD can be manifested as heart failure or angina (either stable or unstable), while in more severe cases myocardial infarction (MI) may occur. Cerebrovascular disease may lead to stroke (hemorrhagic or ischemic) and peripheral arterial disease may lead to gangrene and amputation (Forbes & Cooper, 2013; Fowler, 2008). As illustrated in Figure 1.2, many presentations of CVD including CAD, stroke, non-fatal MI, stable anginal, heart failure, and peripheral arterial disease are more common in people with T2D compared to

those without diabetes. Notably, in addition to microvascular and macrovascular complications, the condition of T2D is also known to directly or indirectly affect the musculoskeletal, hepatic, and digestive systems, and is increasingly linked with diverse cancers (Carstensen, Jørgensen, & Friis, 2014; Lu, Lin, & Kuo, 2009). A 2019 meta-analysis of 49 prospective cohort studies showed that T2D is associated with an elevated risk of all-cancer mortality, infectious disease mortality, and respiratory disease mortality (Wang et al., 2019).

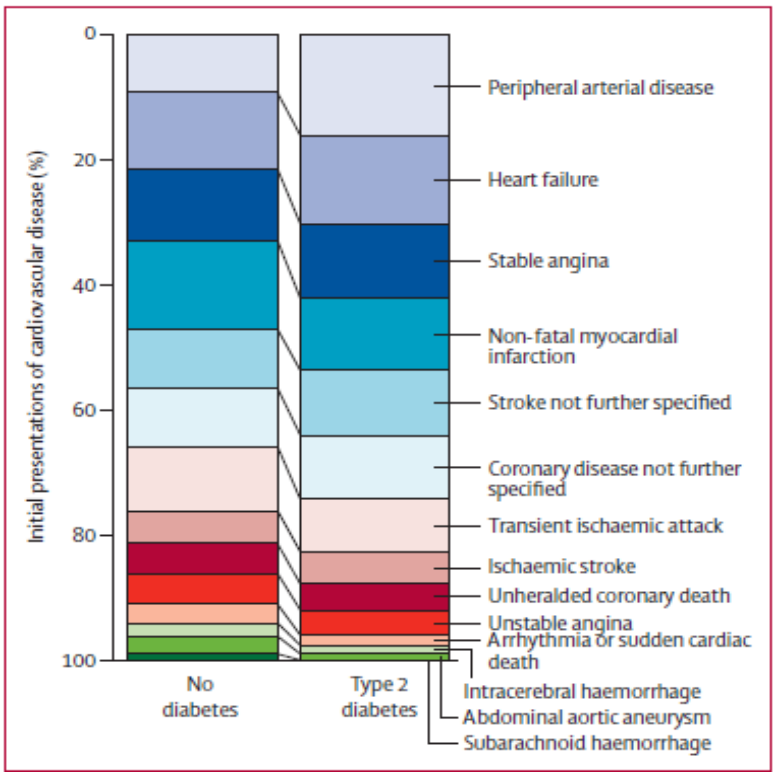

Figure 1.2. Cardiovascular diseases in participants with and without type 2 diabetes and no history of cardiovascular diseases. Reprinted from "Type 2 diabetes and incidence cardiovascular diseases: A cohort study in 1.9 million people" by A. D. Shah, C. Langenberg, E. Rapsomaniki, S. Denaxas, M. Pujades-rodriguez, C. P. Gale, J. Deanfield, I. Smeeth, A. Timmis and H. Hemingway, 2015, *The Lancet Diabetes & Endocrinology*, *3*, p. 108 Copyright (06.01.2020) by A. D. Shah. Reprinted with permission.

These complications and comorbid conditions are arguably the most devastating sequelae of diabetes, placing further strain on patients. Although the prevalence of diabetes complications varies between countries, diabetes remains one of the leading

causes of CVD, blindness, kidney failure, and lower-limb amputation worldwide (IDF, 2019). CVD, especially, is the leading cause of morbidity and mortality for individuals with diabetes. Specifically, CVD accounts for between one third and one half of all deaths in individuals with established diabetes (IDF, 2019), with MI and stroke being the leading causes of premature death (Emerging Risk Factors Collaboration, 2010). Compared to the general population, results from a meta-analysis of 102 studies showed that people with diabetes have a 2-fold excess risk of CVD (Emerging Risk Factors Collaboration, 2010). In 2012, diabetes or higher-than-optimal blood glucose levels and related complications caused 3.7 million deaths globally, 43% of which occurred before the age of 70 (WHO, 2016a). Individuals with diabetes die on average six years earlier than their counterparts without the condition, and approximately 58% of this survival difference is driven from excess CVD mortality, after adjustment for conventional CVD risk factors such as smoking, BMI, blood pressure, and lipids (Emerging Risk Factors Collaboration, 2010). Interestingly, a review of the literature indicated that the macrovascular complications of diabetes are not fully explained by the long-term elevation in blood glucose levels (Forbes & Cooper, 2013). And even though CVD rates have fallen over the past decades among people with T2D (Barengo, Katoh, Moltchanov, Tajima, & Tuomilehto, 2008), the reduction has been less than in the general population. Therefore, the increased risk of CVD in people with T2D still remains (Fox et al., 2015; Gore et al., 2012).

Not only is T2D related to cardiovascular events, such as MI and stroke (van Wijk et al., 2005), but also recovery following such events is attenuated in people with diabetes (Newman, Bang, Hussain, & Toole, 2007). Notably, people with diabetes are

twice as likely to be admitted to hospital compared to people without diabetes (Diabetes UK, 2020). In 2010, hospitalisation rates for stroke and MI in the US were 1.5 and 1.8 times higher, respectively, in individuals with T2D compared to those without diabetes (Centers for Disease Control and Prevention, 2014). In the UK, diabetes accounts for 25% of CVD-related hospitalisations (Diabetes UK, 2020). In individuals with diabetes and heart failure, diabetes is an independent predictor of repeated hospitalisation and MI-related mortality (MacDonald et al., 2008). Myocardial revascularisation procedures, such as coronary artery bypass graft or percutaneous coronary intervention, are also challenging in individuals with diabetes compared to those without diabetes. In particular, people with diabetes have substantially increased risk of death and adverse clinical outcomes following these procedures compared to those without diabetes (Hlatky et al., 2009; Kuchulakanti et al., 2006).

Apart from physical complications and comorbid conditions, people with established T2D face an elevated risk of developing neuropsychological and mood disorders including Alzheimer's disease (Biessels, Staekenborg, Brunner, Brayne, & Scheltens, 2006), and depression (Mezuk, Eaton, Albrecht, & Golden, 2008; Nouwen et al., 2010; Rotella & Mannucci, 2013). Also, rates of anxiety (symptoms and/or clinical disorders) and psychological stress are consistently higher among people with T2D compared with the general population (Grigsby, Anderson, Freedland, Clouse, & Lustman, 2002; Smith et al., 2013). Furthermore, people with T2D report lower physical and mental health-related quality of life (Arditi, Zanchi, & Peytremann-Bridevaux, 2019; Clouet, Excler-Cavailher, Christophe, Masson, & Fasquel, 2001) compared to those without

T2D. Indeed, living with diabetes involves various and high-demanding self-care behaviours and lifestyle changes that are essential to glucose control and the prevention of future complications. These include adherence to medication, checking blood glucose levels regularly, eating healthily, and exercising frequently (ADA, 2003). Daily self-care and lifestyle modifications along with the burden of existing complications or the threat of future complications and the lack of perceived social support (from family, friends, or healthcare professionals) have all been recognised to cause a considerable emotional impact in people with T2D. Almost 50% of people with T2D report this emotional strain (called diabetes distress), which was found to be more prevalent among women with T2D compared to men with the condition (Fisher et al., 2008; Nicolucci et al., 2013). Negative psychological factors in T2D, such as depression and diabetes distress, have been associated with lower physical and mental health-related quality of life (Jannoo, Wah, Lazim, & Hassali, 2017) and have been prospectively linked to sub-optimal medication adherence (Aikens, 2012), poorer glycemic control (Aikens, 2012; Fisher et al., 2010), and the progression of complications (Vedhara et al., 2010).

1.1.6 Management of T2D

The management of T2D can be achieved through lifestyle modifications and pharmacological therapy targeting hyperglycemia. Healthy lifestyle patterns and weight loss can improve glycemic control in overweight and obese individuals with T2D and reduce the need for pharmacological therapy (ADA, 2018). In obese patients who do not achieve durable weight loss and health improvements with reasonable non-surgical methods, surgical procedures for the management of obesity may also

be suggested (Davies et al., 2018). Pharmacological therapy mainly refers to the use of oral anti-hyperglycemic tablets (metformin is currently the first-line medication for the management of T2D), insulin therapy, or other injectable anti-hyperglycemic medications such as glucagon-like-peptide-1 receptor agonists (Davies et al., 2018). Methods of insulin administration include daily injections (the number of injections depends on the type of regimen provided) and continuous subcutaneous insulin infusion delivered using insulin pumps as an alternative of multiple daily injections in a full basal-bolus regimen (Landau, Raz, Wainstein, Bar-Dayan, & Cahn, 2017). Finally, in the Consensus Report by the ADA and the European Association for the Study of Diabetes published in 2018 (Davies et al., 2018), counselling and psychological support delivered in the context of diabetes are recognised as fundamental aspects of diabetes care.

The management of diabetes and its clinical ramifications have strong financial implications. Indeed, diabetes imposes a significant economic burden for countries and public health systems as well as for patients and their families. It has been estimated that the total health expenditure on diabetes in 2019 (regardless of whether this expenditure is born by patients themselves or by private or public payers or by government) was $760 billion, representing a 5% rise on the 2017 estimated costs (IDF, 2019). This economic impact of diabetes is expected to continue to grow. It is projected that the annual expenditure will reach $825 billion by 2030 and $845 billion by 2045. This reflects an increase of 9% and 11%, respectively, compared to 2017 figures (IDF, 2019). Notably, in 2019, UK was one of 10 top countries worldwide for total health expenditure due to diabetes in adults (20 - 79 years old), with the total

economic impact of diabetes being $14.1 billion (IDF, 2019). Almost 80% of these costs were spent for treating the complications of diabetes (Diabetes UK, 2020). Considering all these facts, there is an urgent need to better understand the development and progression of T2D, and the underlying mechanisms involved.

1.1.7 Sex differences in T2D complications and comorbid conditions

A plethora of studies have highlighted sex differences in the morbidity rate associated with T2D. For example, a 2013 review of cross-sectional studies concluded that men with

T2D experience less depression and anxiety, have more energy, and use more active, problem-oriented, and solving approach strategies than women with the condition (Siddiqui, Khan, & Carline, 2013). Additionally, men with T2D experience greater subjective well-being and health-related quality of life compared to women with T2D (Siddiqui et al., 2013). Women with diabetes seem to be less satisfied with their management of the disease and experience more social worry compared to men (Siddiqui et al., 2013). Two large studies with 755 and 1,353 participants with T2D, respectively, showed that women were more likely to experience worse mental health-related quality of life (Landman et al., 2010; Schunk et al., 2015) and diabetes distress than men (Fisher et al., 2008). Apart from self-reported mental health-related quality of life, clinical disorders like anxiety and eating disorders are more common in women with T2D (Kautzky-Willer, Harreiter, & Pacini, 2016). These differences between women and men with T2D may be related to a variety of underlying causes such as lower perceived family support (Kautzky-Willer et al., 2016), lower self-efficacy (Kautzky-Willer et al., 2016), increased anti-diabetic treatment concerns regarding

possible side effects (Inzucchi et al., 2012), and a sense of failure because of poorer disease control (Mohamed et al., 2013; Shibayama, Sato, Nishigaki, Ochiai, & Kazuma, 2011), observed in women with T2D.

In turn, the psychological impact of diabetes adversely affects treatment adherence ultimately leading to poorer lipid and glucose control in women with T2D and the development of complications (Billimek et al., 2015; Kautzky-Willer et al., 2016). Notably, older women with T2D face a greater risk of developing diabetes complications. Generally, more complications are observed in men up to the age of 60 years, whereas more complications are seen in women thereafter (Kautzky-Willer et al., 2016). Specifically, before the age of menopause, the incidence of T2D and atherosclerosis is higher in men compared with women, while after the menopause the incidence of T2D in women equals or exceeds the rates observed in same-aged men (Gubbels Bupp, 2015). Additionally, some diabetes complications and comorbid conditions are more prevalent in older women with T2D compared with their male counterparts. These include CHD, stroke, kidney disease, depression, and dementia (Anderson, Freedland, Clouse, & Lustman, 2001; Chatterjee et al., 2016; Kautzky-Willer et al., 2016; Peters, Huxley, & Woodward, 2014a, 2014b; Seghieri et al., 2017). The impact of CVD in women with diabetes was recently demonstrated in a meta-analysis of 49 prospective cohort studies involving 5,162,654 participants. It was found that diabetes confers an excess risk of CVD mortality and all-cause mortality in women compared to men with diabetes (Wang et al., 2019). Two other meta-analyses also showed that diabetes has a stronger association with gastric cancer risk and kidney cancer mortality in women with T2D compared with men with diabetes (Ge, Ben, Qian,

Wang, & Li, 2011; Larsson & Wolk, 2011). Moreover, longitudinal studies have indicated that the effect sizes for non-cancer and non-vascular mortality among participants with diabetes, as compared to controls without diabetes, were also significantly larger among female participants with diabetes (Emerging Risk Factors Collaboration, 2011).

On the other hand, men with T2D are more likely to develop microvascular complications including diabetes neuropathy and they face a more rapid progression of diabetic nephropathy than women with T2D (Kautzky-Willer et al., 2016; Maric-Bilkan, 2017). Other meta-analyses and literature reviews indicated that diabetes increases the risk of esophageal cancer and leukaemia in men, as well as cancer-related mortality (Castillo, Mull, Reagan, Nemr, & Mitri, 2012; Huang et al., 2012; Kautzky-Willer et al., 2019).

Figure 1.3 indicates mortality due to diabetes by sex and age in Europe Region[14] in 2019. As shown in Figure 1.3, mortality prevalence due to diabetes is slightly higher for men than women in ages < 70 years old (IDF, 2019; Kautzky-Willer et al., 2016), whereas is substantially higher in ageing women ≥ 70 years old. This pattern of results is consistent with UK and worldwide figures (IDF, 2019; WHO, 2016b).

[14] As defined by the IDF.

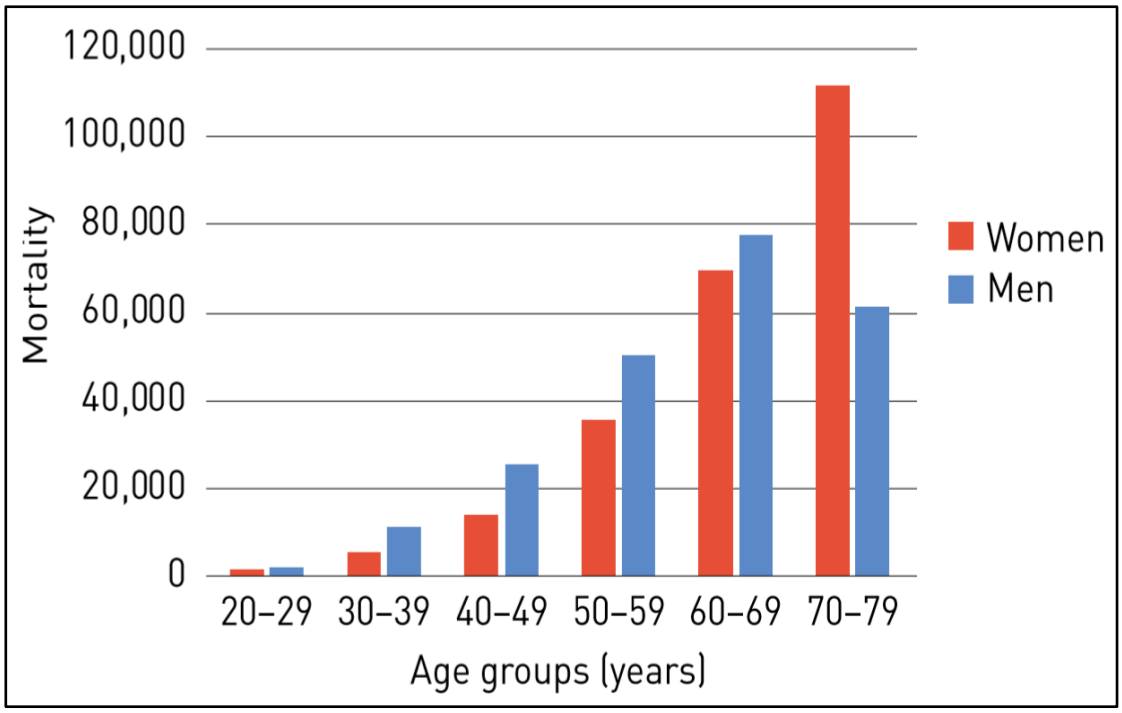

Figure 1.3. Mortality due to diabetes by age and sex in Europe, 2019. Reprinted from "IDF Diabetes Atlas 9th edition" by International Diabetes Federation, 2019 (https://www.diabetesatlas.org/en/resources/). Copyright (02.06.2020) by Atlas team. Reprinted with permission.

While such sex-specific differences are of increasing clinical interest, the underpinning mechanisms driving these differences are not entirely clear. Interestingly though, these sex differences in diabetes-related health outcomes are only partially explained by demographic, lifestyle, clinical, genetic, or hormonal factors (Espeland et al., 2018; Raparelli, Morano, Franconi, Lenzi, & Basili, 2017). A recent meta-analysis of individual data from 68 prospective studies showed that BMI, blood pressure, and total cholesterol each had continuous log-linear associations with heart disease and stroke mortality that were similar in strength among those with and those without diabetes, irrespective of sex (Prospective Studies Collaboration and Asia Pacific Cohort Studies Collaboration, 2018). These findings suggest that the stronger CVD effects of diabetes seen in older women compared to similarly-aged men cannot be entirely explained by the different levels of established CVD risk factors, including BMI, blood pressure, and

cholesterol levels. Additionally, a 2019 meta-analysis (Wang et al., 2019) found that women with the metabolic syndrome had 16% higher risk of heart disease compared with men with the condition, while no sex differences in heart disease risk were apparent in healthy controls. Understanding the underlying mechanisms of sex differences in T2D complications and comorbid conditions might contribute to how these are recognised, treated or prevented in either sex.

1.1.8 The role of subjective well-being in people with T2D

As mentioned earlier in this chapter, people who experience greater subjective well-being live healthier and longer lives (Chida & Steptoe, 2008; Martin-Maria et al., 2017). The links between well-being and health/longevity are particularly relevant to people with T2D because of their greater vulnerability to ill-health and premature mortality. However, there is a dearth of prospective studies testing the protective role of subjective well-being in T2D outcomes. Albeit limited, existing research has suggested that, among patients who have T2D, subjective well-being and related constructs are related to superior physical health outcomes. Stress resilience, for example, has been prospectively associated with better glucose control (as indexed by HbA1c) in people with T2D (Vitaliano, Smith, Yi, & Weinger, 2008). In addition, enjoyment of life, life satisfaction, and self-efficacy have been independently associated with fewer diabetes complications, lower risk of heart disease, and reduced risk of all-cause mortality in previous longitudinal studies (Celano, Beale, Moore, Wexler, & Huffman, 2013; Huffman, DuBois, Millstein, Celano, & Wexler, 2015; Moskowitz, Epel, & Acree, 2008).

It is conceivable that the link between subjective well-being and physical health outcomes in people with T2D may be partially explained by healthier behaviours. Maintaining a healthy lifestyle is particularly important for individuals with T2D since it is a key component of glucose control, and psychological states may affect lifestyle choices, as described previously. Indeed, older people with greater hedonic and eudaimonic well-being have more prudent and healthier lifestyles. For example, they are more physically active in their leisure time, enjoy better sleep, maintain healthier diets, and use preventive healthcare services more conscientiously (Kim, Strecher, & Ryff, 2014; Steptoe, 2019a). In patients with T2D, especially, greater positive affect and optimism have been prospectively linked to more frequent physical activity, healthier diet, and reduced smoking (Moskowitz et al., 2008). When experiencing positive mood, a person may have greater confidence in meeting diet and exercise goals as well as more energy and vitality to engage in self-management and control (Huffman et al., 2015). In addition, associations between subjective well-being and health outcomes are frequently somewhat attenuated when health behaviours are taken into account, implying that these factors possibly play an important mediating role.

Nevertheless, several prospective studies have shown that the significant relationship between subjective well-being and survival is sustained after adjusting for lifestyle risk factors such as physical activity, smoking, and BMI (Chida & Steptoe, 2008; Giltay, Kamphuis, Kalmijn, Zitman, & Kromhout, 2006; Kubzansky & Thurston, 2007; Moskowitz et al., 2008; Ostir, Markides, Black, & Goodwin, 2000), suggesting that direct biological pathways might also be involved in the well-being – health

relationship. Previous studies, mostly cross-sectional in nature, have indicated that subjective well-being is related to a more favourable function of multiple bodily systems, including the neuroendocrine, cardiovascular, and immune system (Steptoe, 2019a). Nonetheless, the biological mechanisms linking subjective well-being with health outcomes in people with T2D remain to be elucidated.

1.2 The concept of stress

1.2.1 The models of stress

One of the earliest and yet most dominant models of stress, the fight-or-flight model, was introduced by the American physiologist Walter Cannon (Cannon, 1929). Cannon supported the hypothesis that external threats can elicit direct biological arousal leading to one of two behavioural responses: either attack the threat (fight) or escape (flight). The model encompasses an evolutionary perspective as, during the hunter-gatherer era, the fight or flight response provided a protective and adaptive response/reaction to any acute threat to survival. During the last two decades, extensions and alterations of the initial model have been suggested (Bracha, 2004; Taylor et al., 2000), in line with the significant advances in the understanding of stress response biology.

The term 'stress'[15] was first used with its current meaning by the Hungarian-Canadian experimentalist Hans Selye who, in 1950, described the General Adaptation Syndrome

[15] The term 'stress' derives from the Indo-European root 'str' which has been historically linked to exertion of pressure. The Greek word 'strangalizein', its English synonym 'to strangle', as well as the Latin 'strigere' (to tighten) seem to have their origins in the very distant past (Chrousos, Loriaux, & Gold, 1988).

(GAS; Selye, 1950). Briefly, the GAS defines stress as a 3-stage process namely 1) alarm (stage of increased mobilisation when faced with an external stimuli, termed 'stressor'), 2) resistance (stage of coping by showing resistance to the stressor), and 3) exhaustion (when the person is repeatedly exposed to the stressor, hence unable to show further resistance). Both the fight-or-flight and the GAS models are based on animal studies and represent stimulus-response frameworks, emphasising a consistent, same-level, and non-specific *acute stress response* to any demand.

Subsequent studies of stress in humans resulted in the Social Readjustment Rating Scale (SRRS), developed by Thomas Holmes and Richard Rahe (Holmes & Rahe, 1967). The SRRS is a list of major life events with a common theme; They all demand a significant change from existing steady stages, therefore require life adjustment. Negative events such as death of a spouse or a close family member, marital separation, personal illness or injury, but also desirable events such as vacations, are listed in descending order, from the more demanding to the less demanding. Consequently, the magnitude of different stressors is highlighted in this list. The SRRS is used widely in epidemiological studies that examine the role of stressful life events in disease development and/or progression (further information about epidemiological studies is presented on page 60). The SRRS scale was revised by Hobson and colleagues (Hobson et al., 1998), and daily hassles and uplifts scales have also been developed (Kanner, Coyne, Schaefer, & Lazarus, 1981).

All the above-mentioned models of stress fail to acknowledge important aspects of the stress response including the subjective meaning or desirability of the stressor, perceived control and coping capacities, previous experiences, as well as individual

factors such as personality traits, sociodemographic, and psychological characteristics which could potentially have an effect on the subjective stress experience and the accompanying biological arousal.

The subjective nature of the stress response is reflected in the more-recently developed Transactional Model of Stress, by Richard Lazarus and Suzan Folkman (Lazarus & Folkman, 1984). This model describes a 2-step subjective stress appraisal: 1) the appraisal of the stimuli as being either irrelevant, or positive, or stressful and 2) the appraisal of the existing coping resources. These individual perceptions were hypothesised to play a key role in the stress experience. Therefore, a single stimulus may lead to stress for one person but not for another (stress is called 'perceived stress' in this concept). Albeit innovative, the Transactional Model of Stress could be criticised for its concrete appraisal process. For example, some people may still experience measurable biological stress arousal despite evaluating the stimuli as irrelevant or perceiving coping resources as adequate.

1.2.2 The acute stress response: biological and behavioural changes

Living organisms maintain a dynamic equilibrium, or *homeostasis*[16], which is threatened or perceived to be threatened by a broad spectrum of acute[17] stressors. In response to these stressors, a complex repertoire of biological and behavioural processes is activated. In humans, this neuroendocrine system is called 'the stress

[16] The word 'homeostasis', which means a steady state, was first coined by Walter Cannon in the 20th century (Cannon, 1939) and has opened the way for the definition of 'allostasis' (which means stability through change) by Bruce McEwen decades later (McEwen, 1998).

[17] Acute stressors are characterised by high intensity and short duration. Some examples of acute stressors include episodic life events, interpersonal conflicts, time pressure, and life-threatening conditions such as attacks. In contrast, chronic stressors are characterised by low intensity and long duration (no obvious end). Some examples of chronic stressors include socioeconomic deprivation, family conflicts or domestic violence, job insecurity, and being a caregiver.

system' and consists of two main elements: 1) the hypothalamus – pituitary – adrenal (HPA) axis and 2) the sympathetic – adrenal – medullary (SAM) axis, through which the central nervous system is connected with the periphery (Figure 1.4). The stress system has a basal level of circadian activity and is meant to react to stressors in a quantity- and time-limited fashion (Chrousos & Gold, 1992; McEwen, 1998).

On perception of a stressor, the HPA axis is activated by the hypothalamic corticotropin-releasing hormone *(*CRH). CRH stimulates the secretion of adrenocorticotropin hormone from the pituitary gland. In turn, adrenocorticotropin hormone triggers the release of glucocorticoids (GCs), primarily cortisol, from the adrenal cortex. Cortisol can influence up to 20% of expressed human genes and affects major biological systems including the metabolic, cardiovascular, and immune system. In particular, cortisol is involved in the mobilisation of energy stores (namely gluconeogenesis), stimulates the cardiovascular system, and suppresses the inflammatory response, a component of the innate immune system (Chrousos, 2009). The innate immune system, particularly the phenomenon of systemic low-grade inflammation, is distinguished from immune responses to infection or injury (where the adaptive immune system is activated). This former type of inflammation is systemic, and not limited to a local site of infection or injury. It is much lower in magnitude than the response to an acute infection or even in sepsis. Additionally, it is also a longer-term response, although transient responses may also occur after acute stress. Most importantly, there is usually no apparent external stimulus, such as injury or infection (Black, 2002; Charles, Travers, Walport, & Shlomchik, 2001). For the

purposes of this thesis, the stress system – immune system interactions are presented in more detail on page 56.

The sympathetic nervous system (SNS), which is part of the autonomic nervous system, is responsible for involuntary biological functions such as heart rate and blood pressure. On perception of a stressor, the SAM axis is also activated, triggering sympathetic nerve fibres originating from the locus coeruleus (LC) area of the brain to release noradrenaline which reaches the body's skeletal muscles and glands. In parallel, sympathetic nerve fibres stimulate the adrenal medulla to release adrenaline and noradrenaline (Chrousos, 1998). Increased adrenaline and noradrenaline (namely 'catecholamines') concentrations result in reduced blood flow to the gastrointestinal tract organs, the skin, and the kidneys, ensuring maximum blood flow to the heart, the brain, and the skeletal muscles. Acute changes resulting from sympathetic activation include increased heart rate and blood pressure, increased sweat production, pupil dilation, vasodilation, and dilation of the bronchioles in the lungs (Chrousos, 1998; Jansen, Van Nguyen, Karpitskiy, Mettenleiter, & Loewy, 1995).

Acute stress-induced changes are, therefore, the result of the activation of the stress system (HPA and SAM axes) and their interactions. Indeed, the stress system interacts with several bodily systems including the central nervous system, the cardiorespiratory system, the gastrointestinal system, the digestive system, the excretory system, the metabolic system, and the immune system, as well as affects growth, reproduction, and thyroid function (Vgontzas & Chrousos, 2002). These complex interactions can be reflected in behavioural adjustments as well, including increased alertness, improved perception, focused attention, vigilance, analgesia, and

suppression of feeding and reproductive function (Nicolaides, Kyratzi, Lamprokostopoulou, Chrousos, & Charmandari, 2015). Concluding, multiple interconnected biological systems are synchronised during the acute stress response ensuring a dynamic and balanced process of adaptation to environmental challenges.

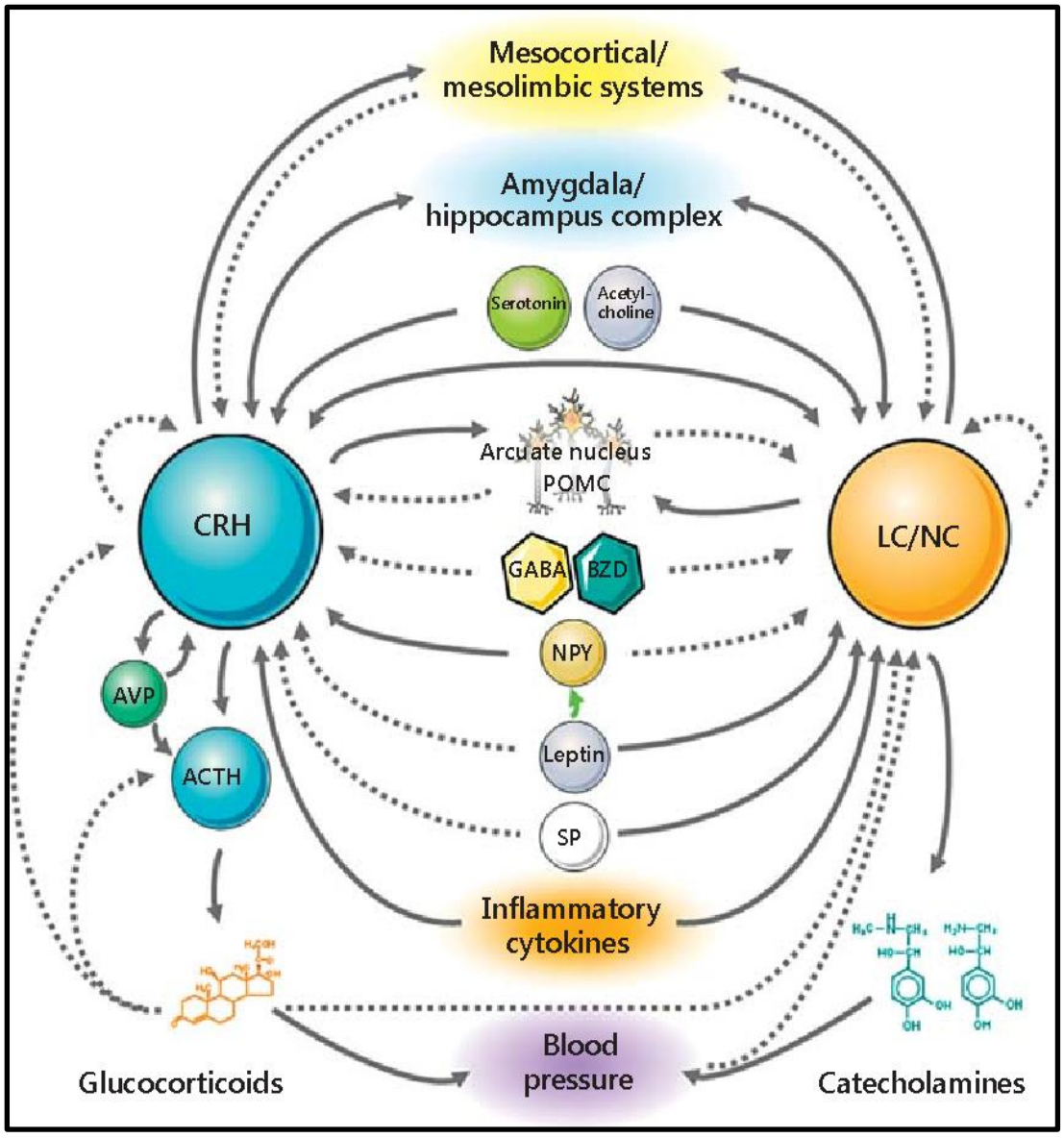

Figure 1.4. Schematic representation of the central and peripheral components of the stress system, their functional interrelations and their interactions with other homeostatic systems and molecules involved in the stress response. Activation is represented by solid lines and inhibition by dashed lines. ACTH = adrenocorticotropin hormone; AVP = = arginine vasopressin; BZD = benzodiazepine; CRH = corticotropin-releasing hormone; GABA = gamma-aminobutyric acid; LC = locus coeruleus; NPY = neuropeptide Y; POMC = pro-opiomelanocortin; SP = substance P. Reprinted from "Stress, the stress system and the role of glucocorticoids" by N. C. Nicolaides, E. Kyratzi, A. Lamprokostopoulou, G. P. Chrousos and E. Charmandari, 2015, *Neuroimmunomodulation*, *22*, p. 10. Copyright (16.04.2020) by N. C. Nicolaides. Reprinted with permission.

From an evolutionary perspective, these adaptive processes can protect individuals by increasing their chances of survival against life-threatening conditions, such as physical attacks. It is also acknowledged that these processes are central to maintaining pre-stress stability. Indeed, the role of the stress biomarkers (also called

'stress mediators'), including cortisol, catecholamines, and their interactions, is to actively restore pre-stress stability through compensatory mechanisms. Once the stressor has passed, these biomarkers are expected to return to appropriate physiological ranges (McEwen, 1998). However, repeated activation of the stress-related systems, as can happen in chronic stress, might result in biological dysregulation that can be reflected in either hyperactivity (an increased stress response and/or failure to shut off post-stress) or hypoactivity (an inadequate stress response) of the stress-related systems; a condition termed 'allostatic load' (McEwen, 1998). More precisely, when biological dysregulation exists, stress biomarkers, which typically boost adaptation, might become maladaptive operating in relatively extreme physiological ranges both under resting conditions (basal/circadian levels) and in response to stressful stimuli. Biological dysregulation might then become an internal stressor itself and non-adaptive behaviours, such as smoking or excessive eating, might also occur further adding to the existing load (Karatsoreos & McEwen, 2010; McEwen, 1998).

Examining systems in the body that are known to be stress-responsive and that have been found to play a key role in the development of several diseases, may help explain the pathways through which chronic stress adversely impacts physical and mental health. Altered processes such as neuroendocrine, autonomic, and inflammatory dysfunction, are thought to play an important role in the pathophysiology of various chronic conditions including T2D and CVD (Brotman, Golden, & Wittstein, 2007; Chrousos, 2009). Testing inflammatory processes, in particular, may provide useful insights on the underlying mechanisms linking chronic stress with physical diseases, as

low-grade inflammation is powerful in predicting morbidity and mortality. Low-grade inflammation has also been identified as a key player in age-related conditions such as T2D, CVD, cancer, Alzheimer's disease, and frailty (Brotman, Golden, & Wittstein, 2007; Chrousos, 2009; Emerging Risk Factors Collaboration, 2012; Couzin-Frankel, 2010; Esser, Paquot, & Scheen, 2015; Steptoe, Hamer, & Chida, 2007; Wang et al., 2013), and is linked with depression (Miller & Raison, 2016; Pariante, 2017) and poorer quality of life (Baturone et al., 2009; Dinh et al., 2019; Garvin, Nilsson, Ernerudh, & Kristenson, 2016; Li et al., 2019; Schoormans et al., 2012). The role of inflammation in T2D risk and progression is described on pages 86 and 88 of this chapter.

1.2.3 Stress system-immune system interactions during the acute stress response

Increasing evidence is providing insights on the interplay between the stress system and the immune system, showing that the stress system influences the immune system and *vice versa*. Cortisol and catecholamines are the two major mediators of the relationship between stress system and immune cells/responses. For example, cortisol influences leukocyte trafficking and/or function and suppresses the production of pro-inflammatory cytokines[18], including tumor necrosis factor-alpha (TNF-α) and the interleukins (IL-1, IL-6, IL-8, IL-12), and other inflammatory biomarkers, preventing their effects on target tissues. These inhibitory effects of

[18] Cytokines are signaling proteins secreted by multiple cell types such as macrophages, T lymphocytes (mainly helper T cells), vascular endothelial cells, adipose tissue, and neurons. They play a fundamental role in the communication within the immune system and in allowing the immune system to exchange information with host tissue cells (McInnes, 2017). They are responsible for most of the biological effects in the immune system including the regulation of inflammatory responses (Chung, 2009).

cortisol can be manifested both at basal/circadian state and in response to stress (Chrousos, 1998; Maier & Watkins, 1998; Padgett & Glaser, 2003).

The SNS also affects the immune system by transmitting humoral and nervous signals to both primary and secondary lymphoid organs, as well as by reaching all sites of inflammation through the postganglionic sympathetic neurons. Immune cells contain receptors and respond to products of these neurons including catecholamines and peripheral CRH (also known as immune CRH because of its actions on the immune cells). Catecholamines induce IL-6 secretion to the systemic circulation and the combination of GCs and catecholamines during stress induces a systemic switch from a T-helper-1 (Th-1) response (and a subsequent production of pro-inflammatory cytokines) to a Th-2 response (and a subsequent production of anti-inflammatory cytokines)[19]. This switch would be adaptive, if transient, but becomes dysfunctional when chronic, since the Th-1/Th-2 balance is essential to immunological health (Berger, 2000; Chrousos, 1998, 2009; Padgett & Glaser, 2003).

Conversely, pro-inflammatory cytokines participate actively in the stress response by stimulating both the HPA and the SAM axis, and are ultimately suppressed by cortisol secretion (Elenkov, Wilder, Chrousos, & Vizi, 2000). Interestingly, IL-6, despite its inherent pro-inflammatory activity, plays a key role in the control of inflammation by causing cortisol secretion and by directly suppressing TNF-α and IL-1 release. The stress system - immune system interaction forms a vital negative feedback loop that

[19] Helper T cells are divided into Th-1 and Th-2 and they produce Th-1-type responses and Th-2-type responses, respectively. Th-1-type responses tend to produce pro-inflammatory cytokines while Th-2-type responses tend to produce anti-inflammatory cytokines as a mechanism to counteract excessive production of pro-inflammatory cytokines (Berger, 2000).

protects the organism against an over-reaction of the immune system (Chrousos, 1998). However, in the context of a dysregulated stress system, immune system mechanisms may also become dysfunctional, potentially influencing the onset and/or course of inflammatory-related disorders (Elenkov et al., 2008). Figure 1.5 illustrates the interactions between the stress system and the immune system.

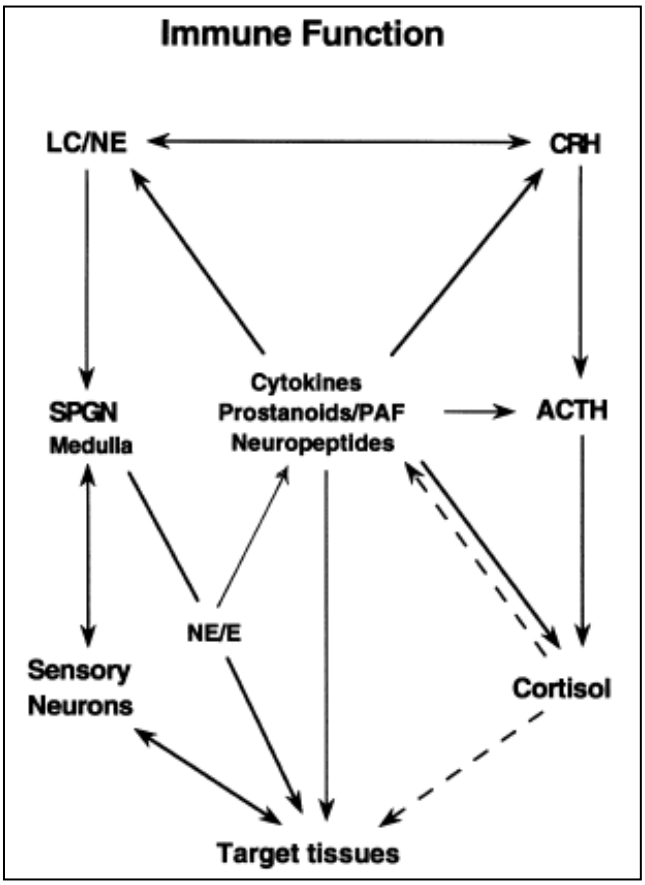

Figure 1.5. The stress system-immune system interactions. Stimulation is represented by solid lines and inhibition by dashed lines. ACTH = adrenocorticotropin hormone; CRH = corticotropin-releasing hormone*;* E = epinephrine (adrenaline); LC = locus coeruleus; NE = norepinephrine (noradrenaline); PAF = platelet activating factor; SPGN = sympathetic postganglionic neurons. Reprinted from "Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye memorial lecture" by G. P. Chrousos, 1998, *Annals of the New York Academy of Sciences, 851*, p. 235. Copyright (02.06.2020) by G. P. Chrousos. Reprinted with permission.

1.2.4 The measurement of stress

Multiple measurement tools have been developed and various measurement approaches are used in stress research to quantify an individual's stress levels. More

precisely, standardised questionnaires or scales measuring perceived/psychological stress are used. These contain items such as having inadequate control over external situations, feeling stressed, nervous, angry, tired, and frustrated, experiencing interpersonal conflicts and tension (Cohen, 1994; Levenstein et al., 1993). Additionally, lists or interviews on major life events or daily hassles are also used (Hanson et al., 2015; Hobson et al., 1998; Holmes & Rahe, 1967; Kanner et al., 1981). Furthermore, adverse early-life experiences (such as neglect, poor parental care, high parent-child conflicts, physical or emotional abuse), psychosocial and emotional factors (such as socioeconomic deprivation, carer burden, job stress, depressive or anxiety symptoms or disorders), and personality traits (e.g. pessimism, cynical hostility) are widely studied in stress - disease research in both cross-sectional and prospective studies (Kivimäki et al., 2015; Lazzarino, Hamer, Stamatakis, & Steptoe, 2013; Luby, Barch, Whalen, Tillman, & Belden, 2017; McLaughlin, Lane, & Bush, 2016; Nyberg et al., 2014; Pinquart, 2018; Pinquart & Sörensen, 2003; Poole et al., 2015; Tindle et al., 2018).

The above-mentioned measurement methods are usually based on self-reported information; therefore, responses may not be entirely accurate. For example, they may be biased by factors such as current emotional state, personality characteristics, and memory deficits. Memory deficits may especially bias studies with older participants and in cases when stress is measured retrospectively. Measuring stress biomarkers could provide more objective information on the stress experience. Epidemiological/population studies, naturalistic measurement studies, and laboratory stress testing studies can all incorporate the collection of biomarker data from humans (Steptoe & Poole, 2010).

In epidemiological/population studies, relatively large numbers of participants are followed over many years and biological data are provided at regular intervals, facilitating the tracking of changes in biomarkers prospectively. Basal/circadian levels of cortisol, IL-6, and other stress biomarkers such as C-reactive protein (CRP)[20] are usually assessed in epidemiological studies. These studies bear the advantages of large and often nationally representative samples, as well as the establishment of temporal precedence that allows the examination of exposure-outcome relationships (however, this is not the case with cross-sectional epidemiological studies). A further advantage of these studies is that a single investigation can examine various outcome variables. For example, cohort studies of smokers can simultaneously look at mortality from lung, heart disease, or cerebrovascular disease. Nonetheless, epidemiological studies are limited by the fact that many environmental confounders (e.g. noise and room temperature) are difficult to assess, and a detailed examination of the underlying biological mechanisms cannot be easily explored (Steptoe & Poole, 2010).

On the other hand, in naturalistic studies, measurements of biological data such as (salivary) cortisol, ambulatory blood pressure and heart rate are taken in real-life, everyday contexts (Grant, Hamer, & Steptoe, 2009; Steptoe, Hackett, et al., 2014; Steptoe et al., 2003). These studies can provide fruitful insights into how daily activities are linked to stress biology. They also capture basal/circadian rhythms and have improved ecological validity as measurements are taken under naturalistic conditions (Steptoe & Poole, 2010). Despite these assets, important limitations of naturalistic studies can also be raised. Firstly, sophisticated measurements (e.g. blood sampling)

[20] CRP is an acute-phase protein that is, at least partly, synthesised in the liver in response to increases in IL-6 and reflects vascular inflammation (Eapen et al., 2013).

are not practically easy due to technical restrictions. Secondly, the devices used in naturalistic studies (for example, for the measurement of ambulatory blood pressure) may be perceived as intrusive and therefore participants may not adhere to the study protocol regarding their use. In addition to this point, information on protocol adherence (e.g. timing of saliva sampling) and confounding factors (e.g. smoking, caffeine and alcohol consumption, food intake, sleep, physical activity, body posture) is self-reported and may therefore be inaccurate or incomplete (Steptoe, 2000; Steptoe & Poole, 2010).

1.2.4.1 Laboratory stress testing: capturing the acute stress response

As reported earlier in the thesis, dysfunctional activity of the stress-related systems might be health-damaging, accounting for several pathologic conditions. More detailed insights into the underlying biological function of multiple bodily systems can be provided using laboratory stress testing designs. Acute stress responses in the laboratory are hypothesised to index how people respond to everyday-life stressors. More specifically, in the laboratory, stress biomarkers can be measured before (pre-task), during, and after (post-task; immediately after and during a recovery period) acute stressful tasks. This experimental research strategy is typically focused on the dynamic *changes* in stress biomarkers from pre- to post-task and up to recovery. The changes cannot be detectable if single measures are taken. Both the immediate post-task and recovery stages are of importance as they can indicate either hyperactivity or hypoactivity to stress and/or a failure of the stress systems to shut off (Gerin, 2010).

A range of acute stress tasks known to elicit reliable biological responses are used in laboratory stress testing studies. The Trier Social Stress Test (TSST) is one of the most

frequently applied tasks in laboratory studies. Briefly, this task requires participants to make an interview-style presentation in front of an interview panel who do not provide feedback or encouragement, followed by a surprise mental arithmetic test (Allen et al., 2017). Other stress tasks (usually applied in combination) include mental tasks (e.g. colour-word tasks, mirror tracing, problem solving), mock job interviews, public speaking, exposure to upsetting movies or pictures, interpersonal conflict tasks, or stress and anger recall tasks. Another stress paradigm found to reliably increase biological stress responses is the multitasking framework (Purple Research Solutions, UK). This comprises of eight different performance-related and cognitive demanding tasks that can be applied either singularly or in combination. This strategy enables the experimental manipulation of participants' workload by increasing the number of tasks the participants have to complete or by changing the difficulty level of the tasks (Wetherell & Carter, 2014; Wetherell, Craw, Smith, & Smith, 2017). Biological measurements taken in laboratory stress testing studies include products of the stress system and its interactions: cortisol, catecholamines and α-amylase, heart rate and blood pressure, blood glucose, and also inflammatory markers (Gerin, 2010; Thoma, Kirschbaum, Wolf, & Rohleder, 2012).

Interestingly, different stress paradigms may elicit the activation of different biological systems. For example, the HPA axis has a higher threshold for activation compared to the SAM axis and acute elevations in cortisol are typically observed in conditions characterised by increased perceived uncontrollability involving performance tasks and social evaluative threats (e.g. threats to a valued aspect of self-identity or where the self is at risk of being negatively critisised by others; Dickerson and Kemeny, 2004).

Therefore, a situation of low perceived threat may involve just a brief increase in cardiovascular activity to activate the SAM axis response. However, the situation may not be threatening enough to activate the 'second wave', HPA axis response with is anti-inflammatory sequelae (Sapolsky, Romero, & Muncket, 2000). All of the conditions necessary for reliably inducing a cortisol response seem to be present in the TSST, which is associated with robust elevations in cortisol and has become a standard protocol for stress induction in healthy and clinical populations of all ages (Buske-Kirschbaum & Hellhammer, 2003; Jessop & TurnerCobb, 2008; Kirschbaum et al., 1995; Kirschbaum, Pirke, & Hellhammer, 1993; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004).

Also, the time course of different biomarker responses varies widely (Gerin, 2010), and different assessment methods can be used for a single marker. For example, cortisol and inflammatory stress responses can be measured both in circulating blood (plasma or serum) and in saliva (Hellhammer, Wüst, & Kudielka, 2009; Slavish, Graham-Engeland, Smyth, & Engeland, 2015). The use of salivary inflammatory markers is discussed more broadly in Chapter 9.

Laboratory conditions enable the control of environmental confounders, such as noise and room temperature. Loud noise and extreme temperature conditions can affect stress-related biological parametres, including the SAM axis responses (Andren, Lindstedt, Björkman, Borg, & Hansson, 1982; Morioka et al., 2005), therefore these factors are important to be controlled for. Moreover, blood sampling, which is technically hard to take in naturalistic studies, can be carried out. Laboratory studies also allow for multiple biomarkers to be assessed simultaneously. For instance, along

with blood sampling, heart rate and blood pressure measurements can be conducted using a Finometer device that allows for the continuous measurement of cardiovascular processes over the course of a laboratory session. Consequently, laboratory stress testing enables the investigation of multiple systems' function at the same time. Another advantage of laboratory studies is that the experimental manipulation of the sample (e.g. grouping healthy versus diseased samples) is feasible, something that is not attainable in epidemiological studies.

Despite the fact that the stress tasks are hypothesised to emulate real-life stressors, the artificial conditions of the laboratory raise the issue of decreased ecological validity. It is not known to what degree biological stress responses in the laboratory reflect real-world stress responses (Gerin, 2010). It is plausible that stressful tasks in the laboratory elicit different response patterns compared to real-life stressors due to factors such as habituation, experience, and adaptation. Nevertheless, a previous laboratory stress testing study has shown that plasma inflammatory responses to laboratory stress do not show any sign of habituation when up to three repeated (same) stress tasks are applied, especially in those participants with higher adiposity measures such as BMI, body fat %, and WHR (McInnis et al., 2014; McInnis et al., 2015; von Känel, Kudielka, Preckel, Hanebuth, & Fischer, 2006). Additionally, a meta-analysis of laboratory stress testing studies with a follow-up design revealed that enhanced cardiovascular responsivity/reactivity to, and slow recovery from acute stress in the laboratory, are associated with poorer cardiovascular risk status and CVD progression at follow-up, outside the laboratory (Chida & Steptoe, 2010). These studies support the ecological validity and predictive value of stress responses in the laboratory.

Concluding, the assessment of the dynamic biological responses to acute stress provides the opportunity to understand more fully how the body reacts to real-life stressors and hence elucidates the potential underlying mechanisms by which everyday stresses may influence the development and/or progression of several pathologies.

1.2.5 Inflammatory responses to laboratory stress

In humans, the underlying mechanisms linking stress with inflammation and disease can be explored using laboratory stress testing designs. Laboratory studies can add further evidence on the stress system - immune system interactions by demonstrating that various inflammatory markers respond acutely and reliably to acute laboratory stress. More precisely, a variety of pro- and anti-inflammatory cytokines have been measured in plasma (in most of the studies), serum, and saliva samples. These include IL-1 beta (IL-1β), IL-6, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-7, IL-8, IL-10, IL-12, TNF-α, interferon gamma (IFN-γ), fibrinogen, monocyte chemoattractant protein-1 (MCP-1), and CRP (Marsland, Walsh, Lockwood, & John-Henderson, 2017; Segerstrom & Miller, 2004; Slavish et al., 2015; Steptoe et al., 2007). Contrary to *in vivo* methods, *in vitro* studies assess *stimulated* cytokine production by using immunogens like lipopolysaccharides[21] (Marsland et al., 2017; Steptoe et al., 2007). It is conceivable though that in vitro methods may produce different results from in vivo methods, therefore the two strategies are usually analysed and examined separately (Marsland et al., 2017; Steptoe et al., 2007). Other pathophysiologically relevant

[21] Lipopolysaccharides are large molecules consisting of a lipid and a polysaccharide composed of O-antigen. It is a major constituent of the outer wall of almost all Gram-negative bacteria and it is highly immunogenic (Alexander & Rietschel, 2001).

processes that are currently gaining momentum in stress research include mitochondrial health (Picard & McEwen, 2018) and telomere shortening (Epel et al., 2004). For the purposes of this thesis, specific reference is made below to three inflammatory markers: IL-6, IL-1ra and MCP-1.

The circulating cytokine, IL-6, is one of the most frequently measured markers in laboratory stress testing studies. It is a pleiotropic cytokine produced from numerous cell types after stimulation by IL-1β. In turn, IL-6 stimulates the synthesis of CRP by hepatocytes in the liver (Steptoe et al., 2007). In studies including both healthy and diseased samples, mean plasma IL-6 absolute levels range between 0.07 - 6.34 picograms per mililitre (pg/ml; Marsland et al., 2017). Results from two meta-analyses of laboratory stress testing studies revealed that plasma IL-6 levels typically increase following stress tasks in the laboratory (Marsland et al., 2017; Steptoe et al., 2007). More specifically, IL-6 increases can be detectable in blood circulation as early as 10 minutes post-stress and reach a peak around 90 minutes following stress (Marsland et al., 2017). Findings from these two meta-analyses also showed stronger effects of the stress trial for healthy compared to diseased groups and for studies that employed relatively delayed sampling. For example, in the more recent meta-analysis of Marsland et al. (Marsland et al., 2017) larger effect sizes were observed at 90 and 120 minutes post-stress compared to 11-30 and 40-50 minutes post-stress. Similarly, Steptoe and colleagues (Steptoe et al., 2007) found stronger effects for studies that obtained samples at 30 - 120 minutes post-stress versus studies that carried out immediately post-stress sampling. Notably, in the meta-analysis of Marsland and colleagues (Marsland et al., 2017), IL-6 stress responses did not vary as a function of

task type (catergorised into social stressors versus other stressors) or assay methods, while differences in the magnitude of effects between healthy and diseased samples were observed only for IL-6 and not for the other cytokines studied (TNF-α and IL-1β). Interestingly, IL-6 responses have been found to be stable across time, even after repeated exposure to the same laboratory stressors, as previously noted (McInnis et al., 2014; McInnis et al., 2015).

Research investigating IL-1ra and MCP-1 stress responses is generally limited. IL-1ra is an anti-inflammatory agent involved in the inhibition of IL-1 action (Banerjee & Saxena, 2012). IL-1 and IL-6 administration can induce IL-1ra secretion (Bargetzi et al., 1993; Steensberg, Fischer, Keller, Møller, & Pedersen, 2003; Tilg, Trehu, Atkins, Dinarello, & Mier, 1994). MCP-1 is included in the chemoattractant cytokine family. It is produced after stimulation by other cytokines, including IL-6, and is involved in the regulation of migration and infiltration of macrophages (Deshmane, Kremlev, Amini, & Sawaya, 2009). Both IL-1ra and MCP-1 levels have been found to increase in response to stressful tasks in the laboratory (Hackett, Hamer, Endrighi, Brydon, & Steptoe, 2012; Marsland et al., 2017; Steptoe et al., 2007), although responses are more consistent for IL-6 than IL-1ra or MCP-1 (Marsland et al., 2017; Steptoe et al., 2007).

1.2.6 Individual differences in inflammatory responses to laboratory stress

A very prominent feature of biological stress responsivity is the large variability in the magnitude and/or duration of stress responses between individuals and across different situations. Animal and human studies have provided substantial knowledge of both internal (related to the individual) and external (related to the stressor itself)

variables that are relevant to shaping the stress response. Among individual factors, sex, age, physical condition[22], the perception of the stimulus, early life experiences, genetics, and epigenetic mechanisms are all investigated in relation to stress responsivity, as well as the effect of personality factors, psychosocial indicators such as social support and social hierarchy, and psychological characteristics. These internal characteristics interact with external factors such as the duration (as reported earlier, chronic stress is a key contributor of the acute stress response), intensity, unpredictability, and habituation of the stressor, as well as time of exposure, to modulate the stress response (Champagne et al., 2006; Chrousos & Gold, 1992; Chrousos & Kino, 2007; Kudielka et al., 2009; Levine, 1970; McEwen, 1998; Newport, Stowe, & Nemeroff, 2002; Novais, Monteiro, Roque, Correia-Neves, & Sousa, 2016; Szyf, Weaver, Champagne, Diorio, & Meaney, 2005). The identification of factors that determine the regulation and dysregulation of stress responsivity is of importance as these factors may reflect a different degree of vulnerability to several stress-related conditions and stress responsivity may provide an explanation of the links between these characteristics and disease risk and progression. During the last two decades, individual differences in the phenotyping of biological, particularly inflammatory, stress responses have been identified in several original studies, with the great majority of them published within the last 10 years. These studies have looked at associations between multiple individual characteristics and the magnitude of inflammatory responses in the laboratory. Individual characteristics included

[22] Including the presence of a clinical disease and medication intake, nicotine, coffee and alcohol consumption, dietary energy supplies, pregnancy, lactation, and breast-feeding (Kudielka, Hellhammer, & Wüst, 2009).

sociodemographic and job-related variables, behavioural/lifestyle factors, psychological factors, clinical and biological factors, and subjective responses to the tasks. Overall, findings from these laboratory studies demonstrated that negative factors (those that have been prospectively linked with ill-health) such as lower social status, childhood adversity, job stress, smoking history, loneliness, and hostility are cross-sectionally associated with enhanced inflammatory responses post-stress, measured as the difference in levels from pre- to post-stress and up to recovery (Bennett et al., 2013; Brydon et al., 2010; Carpenter et al., 2010; Derry et al., 2013; Girard, Tardif, Boisclair Demarble, & D'Antono, 2016; Hackett et al., 2012; Hackett, Lazzarino, Carvalho, Hamer, & Steptoe, 2015; Hamer et al., 2006). On the other hand, factors that are considered as health-protective, such as subjective well-being, physical fitness, stress management, and more time spent meditating are cross-sectionally linked with reduced inflammatory responses following laboratory stress (de Brouwer et al., 2013; Hamer & Steptoe, 2007; Pace et al., 2009; Steptoe, Wardle, & Marmot, 2005).

As mentioned previously, physical condition plays a role in biological stress responsivity. Of importance, only one previous study has looked at inflammatory stress responses in people with T2D compared to healthy controls (Steptoe, Hackett, et al., 2014). Interestingly, the two groups showed significant differences in IL-6 activity and reactivity. More precisely, people with T2D had higher IL-6 absolute concentrations pre-stress compared to the healthy control group coupled with diminished IL-6 stress responses. Despite smaller IL-6 increases, absolute levels

remained higher in the diabetes group throughout the laboratory session. A detailed description of this study is provided on page 79.

Sex differences in inflammatory stress responses in people with T2D and the role of subjective well-being in inflammatory stress responses in T2D are the focus of this thesis. Due to the lack of research in T2D samples, full details of previous studies (samples description, stress tasks, cytokines affected, effect sizes, statistical adjustments) that have examined these links in healthy samples are provided in Table 1.1 and Table 1.2, respectively (except one study that explored sex differences in IL-6 stress responses in patients with CAD).

Sex differences in circulating inflammatory stress responses have been described in four previous studies of healthy adults (Edwards, Burns, Ring, & Carroll, 2006; Endrighi, Hamer, & Steptoe, 2016; Lockwood, Marsland, Cohen, & Gianaros, 2016; Steptoe, Owen, Kunz-Ebrecht, & Mohamed-Ali, 2002). Three of these previous studies supported women to be more inflammatory-responsive than men following laboratory stress (Endrighi et al., 2016; Lockwood et al., 2016; Steptoe, Owen, et al., 2002), and one study reported a delayed response for female compared to male participants (Edwards et al., 2006). The smaller sample size of this latter study (N = 40), the younger age of participants, and the lack of adjustment for covariates (compared to the other studies) may have played a role in the inconsistency of the findings. Indeed, sex differences in inflammatory stress responses are more consistently observed in studies of older participants. It is not yet clear why sex differences are more frequently seen in older individuals, but it is plausible that the smaller levels of reproductive hormones associated with ageing in women might help

explain these findings. For example, in the study of Endrighi et al. (Endrighi et al., 2016), which found larger IL-6 stress responses for healthy women compared to men, all women participants were post-menopausal. The effect of menopause status on inflammatory stress responses was directly checked in one study that compared lipopolysaccharide-stimulated IL-6 responses to a speech task in 62 healthy men and women (Prather et al., 2009). Results confirmed that post-menopausal women show elevated IL-6 (as assessed immediately post-task and at 30 minutes post-task) and TNF-α (as measured immediately post-task) reactivity to stress compared to men, but no differences were observed between pre-menopausal women and men, suggesting that the observed sex differences in stress-induced cytokine production are largely attributable to differences between men and post-menopausal women. These findings further support the hypothesis that reproductive hormones might contribute to the observed sex differences in inflammatory stress responses and suggest that post-menopausal women may be particularly susceptible to stress-related inflammatory conditions. Indeed, oestrogens, which are thought to have anti-inflammatory effects, are reduced in ageing women (Winters, 2001), potentially having a permissive effect on cytokine production and gene expression. In the more recent study of Sullivan et al. (Sullivan et al., 2018), women with CAD exhibited greater IL-6 responses than similarly aged male patients. Interestingly though, differences between women and men with CAD decreased with increasing age. For example, at 40 years of age, the IL-6 response for women at 90 minutes post-stress was 83% higher compared with pre-task levels, whereas IL-6 increases for men were only 52% higher compared with pre-stress levels. At 50 years of age, the IL-6 response for women compared with men was 61% versus 42%, and at 60 years of age it was 42% versus

32%, respectively. Differences in the health condition of participants (they all had CAD) might explain discrepancies with previous studies. In the same study, the healthy control group, consisted of middle-aged participants, did not show any differences in IL-6 stress responses between women and men.

Table 1.1

Studies measuring sex differences in circulating inflammatory stress responses

Study	Participants	Stressor	Inflammatory markers/ time-points	Results (effect sizes)	Statistical adjustment
Sullivan et al. (2018).	819 patients with CAD and 98 healthy controls (age range 25-79 for CAD group and 18-60	Speech task.	IL-6, MCP-1, MMP-9, hs-CRP/ pre-task and 90 min post-task.	CAD group: IL-6 responses larger for women vs. men. Difference between women and men smaller with increasing age (see Appendix A for	Age, sex, time, age by sex, age by time, sex by time, age by sex by time, race, education, diabetes, hypertension, BMI, depression history, smoking,

	for healthy group).			effect sizes). No sex differences in MCP-1, MMP-9, or hs-CRP responses. Control group: No sex differences in IL-6 responses (no measurement of other factors in controls).	medication use[23], previous MI and heart failure, summed rest score, plate effect[24] and study source.
Endrighi et al. (2016).	506 healthy adults (mean age 62.9 ± 5.60).	Colour-word and mirror tracing.	IL-6/ pre-task, immediately post-task,45 min and75 min post-task.	Larger IL-6 responses at 45 and 75 min in women vs. men (effect sizes not reported).	Age, adiposity, SES, depressive symptoms, physical activity, alcohol (past week), smoking, rumination, task appraisals, subjective stress, statins use, hormone replacement, time of testing, pre-task IL-6.
Study	Participants	Stressor	Inflammatory markers/ time-points	Results (effect sizes)	Statistical adjustment
Lockwood et al. (2016).	57 healthy adults (age range 30-51).	Colour-word and multi-source interference tasks[25].	IL-6/ pre-task and 30 min post-task.	Greater IL-6 responses in women vs. men (η^2_p = 0.08, p = 0.038).	Age.
Edwards et al. (2006).	40 healthy students (mean age 21.3 ± 1.8).	Mental arithmetic.	IL-6/ pre-task, immediately post-task, 30 min and 60	Men showed an earlier peak in IL-6 (at 30 min) vs. women (at 60 min). Higher IL-6 levels for men at	No adjustments.

[23] Aspirin, beta blocker, statins, angiotensin-converting enzyme inhibitors, and antidepressants.
[24] To minimise any potential batch effect, the researchers deemed important to include biomarker plate as a random effect in their models to account for any correlation of values related to how the samples were run or prepared in the laboratory. Therefore, they included plate effect as a random intercept in the fully adjusted model.
[25] Modified versions of the tasks.

			min post-task.	30 min vs. women (η^2 = 0.10, p = 0.05).	
Steptoe, Owen, et al. (2002).	230 healthy adults (age range 45-59).	Colour-word and mirror tracing.	IL-6, IL-1ra, TNF-α/ pre-task and 45 min post-task.	Larger IL-6 responses in women vs. men. IL-1ra increased only in women. TNF-α increased only in men (effect sizes not reported).	For IL-6 model only: Hemoconcentration change, age, BMI, WHR, blood sampling method

Note. Effect sizes correspond to adjusted results. BMI = body mass index; CAD = coronary artery disease; hs-CRP = high-sensitivity-C-reactive protein; IL-1ra = interleukin-1 receptor antagonist; IL-6 = interleukin-6; MCP-1 = monocyte chemoattractant protein-1; MI = myocardial infarction; min = minutes; MMP-9 = and matrix metallopeptidase-9; SES = socioeconomic status; TNF-α = tumour necrosis factor-alpha; vs. = versus; WHR = waist-to-hip ratio.

The link between subjective well-being or related psychological constructs and circulating inflammatory stress responses appears consistent, albeit not widely explored. Specifically, in healthy participants, greater self-reported happiness over the day, self-compassion, and maintaining a positive outlook during the stress task in the laboratory have been cross-sectionally associated with smaller inflammatory increases post-stress after adjusting for covariates (Aschbacher et al., 2012; Breines et al., 2014; Steptoe et al., 2005). Table 1.2 presents full details of these studies.

Table 1.2

Studies measuring positive psychological characteristics and circulating inflammatory stress responses

Study	Participants	Moderator	Stressor	Inflammatory markers/ time points	Results (effect sizes)	Statistical adjustment
Breines et al. (2014).	41 healthy adults (age range 18-35)	Self-compassion.	TSST.	IL-6/ pre-task and 120 min post task.	Higher self-compassion predicted smaller IL-6 responses (*B* = -0.94, *p* = 0.003).	Age, sex, ethnicity, BMI, self-esteem, depressive symptoms, post-TSST distress, time of testing.

Aschbacher et al. (2012).	35 healthy post-menopausal women (age range 51-75).	Maintenance of positive outlook[26] from pre- to post-task.	TSST.	IL-1b, IL-6/ pre-task and 50 min post-task.	Decreased positive outlook predicted larger IL-1β response (*B* = -0.40, *p* = 0.017). No associations for IL-6 responses.	Age, BMI, caregiver status, antidepressant use, pre-task cytokine levels.
Steptoe et al. (2005).	216 healthy adults (age range 45-59).	Daily happiness.	Colour-word and mirror tracing.	Fibrinogen/ pre-task and immediately post-task.	Lower happiness predicted larger fibrinogen responses (OR = 3.72, 95% CI [1.16, 11.90]).	Age, sex, BMI, smoking, employment grade, pre-task fibrinogen, general health status.

Note. All effect sizes are adjusted results, except those presented for the study of Aschbacher et al. (Aschbacher et al., 2012). *B* = unstandardized beta coefficient; BMI = body mass index; CI = confidence interval; IL-1b = interleukin-1 beta; IL-6 = interleukin-6; min = minutes; OR = odds ratio; TSST = Trier Social Stress Test.

1.2.7 The clinical importance and predictive value of inflammatory stress responsivity in the laboratory: Evidence from follow-up studies

Increased inflammatory responsivity in terms of magnitude and/or duration, indicative of allostatic load, might be health-damaging, conferring elevated risk for inflammatory-related diseases. Only a few studies have examined the longitudinal association between inflammatory stress reactivity and future health outcomes. Albeit scarce, prospective evidence suggests that individuals who show increased inflammatory responses in the laboratory are prone to developing physical and mental conditions in the long-term. More precisely, Brydon and Steptoe (Brydon & Steptoe,

[26] Positive affect and cognition.

2005) examined inflammatory stress responses in 153 healthy, middle-aged civil servants who were exposed to two laboratory stress tasks (colour-word task and mirror tracing task) in relation to ambulatory systolic and diastolic blood pressure three years later. It was found that greater fibrinogen and IL-6 stress responses, as measured immediately post-task and at 45 minutes post-stress, respectively, predicted ambulatory systolic blood pressure at follow-up. Higher fibrinogen responses further predicted diastolic blood pressure at follow-up. Results were adjusted for ambulatory blood pressure levels at baseline, pre-stress blood pressure levels, acute blood pressure stress responses in the laboratory, pre-stress inflammatory levels, age, sex, BMI, and smoking[27]. Similarly, in a study of 636 healthy participants from the Whitehall II epidemiological cohort study, heightened fibrinogen responses to the same stress tasks (colour-word and mirror tracing) in the laboratory, as assessed immediately after stress, predicted higher incidence of clinical hypertension over an average 8-year follow-up in women but not in men (OR for high reactivity versus low reactivity group = 2.64, 95% CI [1.11, 6.30]), after adjusting for a range of covariates including sociodemographic factors, lifestyle variables, and pre-stress fibrinogen (Steptoe, Kivimaki, Lowe, Rumley, & Hamer, 2016). In another study of 155 healthy middle-aged participants from the Whitehall II study, larger fibrinogen and TNF-α responses at 45 minutes post-task predicted greater carotid artery stiffness at the 3-year follow-up (for fibrinogen: β = -5.87, 95% CI [-11.4, -0.36]; for TNF-α: β = -1.82, 95% CI [-3.52, -0.13]), adjusting for sociodemographic, lifestyle, and conventional risk factors for CVD including blood pressure, cholesterol levels, and

[27] Effect sizes were not reported in this study.

measures of adiposity (Ellins et al., 2008). TNF-α analyses were further adjusted for pre-stress CRP and changes in haematocrit and results were sustained. In the same study, pre-stress fibrinogen and TNF-α absolute levels were not associated with arterial stiffness at follow-up, adding value to examining inflammatory stress responsivity. In the same study, IL-6 stress responses did not significantly predict arterial stiffness. Nevertheless, carotid stiffness was not assessed during the baseline study, constituting an important limitation of the study. In a more recent study with participants from Whitehall II, Ellins and colleagues (Ellins, Rees, Deanfield, Steptoe, & Halcox, 2017) investigated the relationship between inflammatory responses to acute mental stress in the laboratory and endothelial dysfunction three years later in 158 volunteers. Findings from this study showed that elevated fibrinogen at 45 minutes post-stress predicted diminished flow-mediated dilation[28] following adjustment for pre-stress fibrinogen, baseline brachial artery diameter, reactive hyperemia, and other key cardiovascular risk factors (β = -0.047, p = 0.016). The association between the immediate fibrinogen response to stress and flow-mediated dilation was significant only in the presence of (mild) dyslipidemia. There was no significant association between change in IL-6 or TNF-α and flow-mediated dilation. Not only poorer CVD risk profile but also worse mental health at the follow-up has been found to be linked to increased inflammatory stress responsivity. To the best of my knowledge, only one longitudinal study to date has examined the relationship between inflammatory stress responses and mental health outcomes, particularly

[28] Flow-mediated dilation is a dynamic measure of arterial physiology and pathophysiology. It provides a measure of change in vasomotor tone which is caused by the local release of nitric oxide stimulated by the increase in shear stress during a standardised post-ischemic hyperemic response (Ellins et al., 2017).

depressive symptoms. Aschbacher et al. (Aschbacher et al., 2012) measured positive outlook before and after stress tasks (speech and math tasks), inflammatory responses 50 minutes after the onset of the tasks, and depressive symptoms one year later in a sample 35 older women. Findings showed that IL-1β stress responses predicted increases in depressive symptoms one year later after adjusting for covariates including pre-stress IL-1β levels, antidepressant use, and depressive symptoms at baseline (β = 0.501; p = 0.003). In the same study, IL-1β reactivity significantly mediated the relationship between maintenance of positive outlook from pre- to post-stress and depressive symptoms in the follow-up (indirect path *B* coefficient = -1.194, p = 0.030). IL-6 reactivity was not a significant predictor of depressive symptoms alone or in the adjusted model, although the direction of the effect was similar to IL-1β (β = 0.254, p = 0.137). Taken together, longitudinal associations between inflammatory stress reactivity and health outcomes predominantly show that greater inflammatory reactivity has negative implications for health in the long-term.

1.3 The psychobiological profile of T2D

As reported previously in this chapter, T2D is linked with an increased psychological burden. Epidemiological and laboratory studies further revealed that T2D is associated with dysregulation across multiple stress-related biological systems. One cross-sectional study of 1,000 participants showed that the condition of T2D is linked with heightened levels of various stress biomarkers including cortisol, dehydroepiandrosterone sulfate (along with cortisol, this is indicative of the HPA axis activity), catecholamines, blood pressure, cholesterol, HbA1c, and CRP, even after controlling for demographic and lifestyle factors (Mattei, Demissie, Falcon, Ordovas, & Tucker, 2010). Nevertheless, in this study by Mattei and colleagues (Mattei et al., 2010), biological measurements were taken only under resting conditions, hence capturing basal biological activity. Only one study to date has explored stress reactivity across multiple biological systems in people with T2D compared with healthy controls (Steptoe, Hackett, et al., 2014). In this study by Steptoe and colleagues, 140 individuals with doctor-verified T2D diagnosis and 280 healthy participants, aged 50 - 75 years old, underwent two laboratory stress testing tasks for 10 minutes in total (the Stroop colour-word task and a mirror tracing task). Multiple biological measurements were carried out before, during and up to 75 minutes post-stress. Results from this study indicated that the diabetes group had smaller neuroendocrine (salivary cortisol), autonomic (systolic blood pressure), inflammatory (IL-6), and metabolic (cholesterol) stress responses, coupled with delayed recovery in systolic and diastolic blood pressure, heart rate, and cholesterol compared to age, sex, and income-matched healthy controls. In addition, the diabetes group had lower diastolic blood pressure and higher heart rate compared to the healthy group throughout the stress session.

Notably, pre-stress IL-6 levels were higher in people with T2D so that despite smaller increases after stress, absolute levels remained higher compared to controls. Similarly, the diabetes group had higher pre-task cortisol levels than controls and elevated cortisol output over a single day.

Findings from the study by Steptoe et al. (Steptoe, Hackett, et al., 2014) highlight the role of blunted stress reactivity in this patient group and are in agreement with previous studies that suggested that blunted stress responses may also be related to ill-health. For example, evidence from previous laboratory stress testing studies (predominantly cross-sectional in nature) has shown that several adverse behavioural and health outcomes are associated with reduced cardiovascular (heart rate and/or blood pressure) and cortisol reactivity. These included depressive symptoms, disordered eating and bulimia, addictive behaviours such as substance dependencies and gambling, poorer self-rated health, poorer cognitive performance, and some personality characteristics (Carroll, Ginty, Whittaker, Lovallo, & de Rooij, 2017; Kelly-Hughes, Wetherell, & Smith, 2014). Additionally, attenuation in heart rate and cortisol stress responses has been related to a higher number of unfavourable childhood experiences in a dose-response manner (Lovallo, Farag, Sorocco, Cohoon, & Vincent, 2012), as well as to the duration of exposure (for cortisol) to these adverse events (Voellmin et al., 2015). Moreover, it has been demonstrated that smaller cardiovascular (heart rate and blood pressure) and cortisol stress responses in the laboratory are cross-sectionally related to obesity (Jones et al., 2012; Phillips, 2011) as well as increase the likelihood of becoming obese longitudinally after adjusting for covariates (Carroll, Phillips, & Der, 2008; de Rooij, 2013). Concluding, stress

hypoactivity may not be beneficial but instead may also reflect biological dysregulation in the body potentially predisposing individuals to negative health outcomes in the long-term.

In the laboratory study by Steptoe and colleagues (Steptoe, Hackett, et al., 2014), participants with T2D reported a more stressful psychosocial profile compared to healthy individuals. For example, they were more likely to report high depressive symptoms, hostility, financial strain, less neighbourhood social cohesion, less sense of control over their lives, and less optimism compared to controls, independently of education level and marital status. Participants with diabetes were also more likely to be separated, divorced, or widowed than participants without the condition.

Significant relationships between lifestyle and psychosocial variables and biological factors have also been identified in this study. For example, analysing the T2D group alone, it was found that hostility was positively associated with IL-6 increases post-stress and inversely associated with cortisol output following stress, suggesting that a psychosocial factor such as hostility further exaggerates disturbed biological processes in this patient group (Hackett, Lazzarino, Carvalho, Hamer, & Steptoe, 2015). Optimism on the other hand was associated with heightened systolic and diastolic blood pressure responses following acute stress and lower daily cortisol output (Puig-Perez, Hackett, Salvador, & Steptoe, 2017), indicating that people with T2D who are more optimistic respond to stress in a similar way as healthy individuals (Steptoe, Hackett, et al., 2014). In our more recent study (Hackett, Poole, Hunt, Panagi, & Steptoe, 2019), we observed that lonelier participants with T2D had greater MCP-1 concentrations throughout the laboratory session with values at 75 minutes

post-stress being significantly higher for lonelier individuals after adjusting for covariates. In the same study, loneliness was associated with lower cortisol levels throughout the session after controlling for covariates and greater reduction in cortisol levels post-task. With regards to lifestyle variables, evidence was found for an inverse association between moderate-to-vigorous physical activity, assessed objectively using accelerometers over one week, and IL-6, IL-1ra, and MCP-1 absolute levels, with associations being largely explained by higher BMI (Hamer et al., 2014).

Overall, the psychobiological profile of T2D is characterised by chronic psychosocial adversity and altered stress-related biological processes. It is plausible that dysfunctional stress biology as a result of chronic stress influences T2D pathogenesis over time· from visceral fat accumulation and reactive insulin hypersecretion to T2D and endothelial dysfunction with its cardiovascular and neurovascular sequelae (Chrousos, 2009). Indeed, stress-induced biomarkers are highly relevant to T2D, including the release of cortisol, glucose and lipids into the circulation, increased heart rate and blood pressure, and inflammatory cytokine expression. Animal models, human epidemiological studies, and laboratory stress testing studies have tried to define how these stress products may ultimately result in metabolic disturbances and therefore, T2D. Findings from these studies are presented below, with a specific focus given on the role of inflammatory cytokines in T2D development and progression to CVD[29].

[29] CVD is my first outcome of interest in the Diabetes Follow-up Study presented in Chapter 8. Therefore, specific mention is made here regarding diabetes progression to CVD.

1.3.1 Neuroendocrine dysfunction

A substantial number of studies have focused on neuroendocrine function in relation to metabolic abnormalities and T2D. Animal research has shown that chronic administration of corticosterone[30] in drinking water can result in hyperglycemia, insulin resistance, and dyslipidemia in rodents (Fransson et al., 2013; Karatsoreos et al., 2010). Conversely, the animal brain has been found to be more sensitised to insulin in the absence of the adrenal gland, where the GCs originate (Chavez et al., 1997). Similarly, patients with Cushing's syndrome, which is characterised by long-term hypercortisolism, and those taking GCs, face an increased risk of developing hyperglycemia and T2D (Clore & Thurby-Hay, 2009; Lacroix, Feelders, Stratakis, & Nieman, 2015). GC receptors are expressed on pancreatic β cells, and cortisol stimulation interferes with insulin sensitivity and decreases insulin secretion (Di Dalmazi, Pagotto, Pasquali, & Vicennati, 2012).

The epidemiological evidence for neuroendocrine dysfunction contributing to T2D derives mostly from cross-sectional studies that have investigated cortisol secretion in people with established T2D. Notably, cortisol shows marked circadian rhythms that are better captured with multiple samples across the day than a single sample (Adam & Kumari, 2009). Dysregulation of the HPA axis and, therefore, of cortisol secretion, can be manifested as alterations to the expected increase in cortisol levels in the morning (at 30 - 45 minutes post-waking; known as the cortisol awakening response [CAR]) and/or to the subsequent progressive decline (slope) across the day until bedtime (Adam & Kumari, 2009; Fries, Dettenborn, & Kirschbaum, 2009).

[30] Corticosterone is the primary GC involved in the stress response in rodents (Gong et al., 2015).

Epidemiological studies showed that cortisol secretion patterns differ significantly in people with T2D compared to healthy individuals. Specifically, two cross-sectional studies showed that the CAR is lower in patients with T2D compared to healthy controls (Bruehl, Wolf, & Convit, 2009; Champaneri et al., 2012), and women, but not men, with T2D have higher daily cortisol overall quantified as the area under the curve (Champaneri et al., 2012). The largest study to date involving 3,500 participants from the Whitehall II epidemiological cohort showed that people with T2D have a flatter slope (smaller decline) in cortisol levels over the day coupled with elevated cortisol levels in the evening compared with healthy controls (Hackett, Steptoe, & Kumari, 2014), in agreement with an earlier community cohort study (Lederbogen et al., 2011). The prospective relationship between the complete diurnal cortisol profile (morning and evening cortisol levels, the CAR, and the slope) and incident T2D has been examined only once, in a study of 3,270 initially healthy individuals from Whitehall II (Hackett, Kivimäki, Kumari, & Steptoe, 2016). A flatter diurnal cortisol slope and increased cortisol in the evening at phase 7 of the study (2002 - 2004) predicted impaired fasting glucose and/or T2D diagnosis at phase 11 (2012 - 2013). Raised evening cortisol levels have also been prospectively associated with cardiovascular mortality in 4,047 older men and women from the Whitehall II study (Kumari, Shipley, Stafford, & Kivimaki, 2011).

1.3.2 Autonomic dysfunction

Autonomic dysregulation has also been linked with T2D risk. Firstly, high blood pressure is a well-known risk factor for T2D (IDF, 2019), and a dose-response relationship between blood pressure and the risk of developing T2D has been

established. Specifically, in a UK study with 4.1 million adults free of T2D or CVD at baseline, objectively-measured systolic and diastolic blood pressure levels showed a dose-response association with the risk of developing T2D, independent of a range of covariates (Emdin, Anderson, Woodward, & Rahimi, 2015; Knowles & Reaven, 2016). A meta-analysis of 30 prospective studies showed that an increase of 20 millimetres of Mercury (mmHg) in systolic blood pressure is associated with a 1.77-fold higher risk of new onset T2D (Knowles & Reaven, 2016). Similarly, a dose-response relationship between resting heart rate and incident T2D has been reported in a meta-analysis of 10 cohort studies, with a 19% higher risk of incident T2D for every 10 beats/minute increment in heart rate (Aune, ó Hartaigh, & Vatten, 2015). Zhang and colleagues (Zhang et al., 2006) reported a cross-sectional association between psychological stress, hostility, and insulin resistance mediated by noradrenaline. As reported earlier in this chapter, noradrenaline is indicative of the SNS function, therefore this study showed that autonomic dysregulation is involved in the relationship between psychosocial stress and metabolic disturbances. In another cross-sectional study (Champaneri et al., 2012), men, but not women, with diabetes had higher urinary catecholamine levels compared with those without diabetes. Given the effects of catecholamines on inflammatory markers, as described earlier in this chapter, as well as the interaction between autonomic function and inflammation, that is especially observed in the context of the metabolic syndrome (Marvar et al., 2012; Thayer & Sternberg, 2006), inflammatory processes may be a mechanism through which autonomic dysfunction indirectly increases the risk of T2D. The links between inflammation and T2D risk and progression to CVD are presented in more detail below.

1.3.3. Inflammatory dysfunction

Inflammatory markers are elevated in obesity and obesity-related disorders such as metabolic syndrome and T2D (ADA, 2005; Bastard et al., 2000; Belalcazar et al., 2013; Bruun, Helge, Richelsen, & Stallknecht, 2006; Pickup, Mattock, Chusney, & Burt, 1997; Steptoe, Hackett, et al., 2014; Yudkin, Stehouwer, Emeis, & Coppack, 1999). Indeed, adipose tissue is a crucial site in the generation of pro-inflammatory factors including TNF-α, IL-1β, IL-6, and INF-γ (Chawla, Nguyen, & Goh, 2011; Esser, Legrand-Poels, Piette, Scheen, & Paquot, 2014; Hotamisligil, 2006; Kohlgruber & Lynch, 2015). Adipose tissue plays a central role in the induction of inflammation since chronic nutrient excess, such as excess glucose and lipids, leads to changes in its cellular composition and the production of inflammatory responses (Esser et al., 2014; Hotamisligil, 2006). An infiltration of macrophages into adipose tissue is seen in obesity in both animal and human models, characterised by the synthesis of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6, and INF-γ (Chawla et al., 2011; Kohlgruber & Lynch, 2015). Apart from adipose tissue, insulin-sensitive tissues, such as the liver and muscle cells, as well as pancreatic β cells, are also affected by circulatory inflammatory factors in the presence of obesity and T2D (Ehses, Ellingsgaard, Böni-Schnetzler, & Donath, 2009; Esser et al., 2014; Hotamisligil, 2006; Herbert Tilg & Moschen, 2008). Cytokines, which are over-expressed in these tissues, act predominantly in a paracrine manner (acting and affecting nearby cells[31]) to promote insulin resistance by inhibiting insulin receptor signalling in peripheral tissues through activation of the c-JUN N-terminal kinase and nuclear factor-kappa B

[31] Cytokines may also act in an autocrine manner (act on the same cells) or an endocrine/systemic fashion (act at distant cells by the secretion of soluble products into the circulation; Chung, 2009).

pathways or by inducing β cell dysfunction and subsequent insulin deficiency (Esser et al., 2014; Hotamisligil, 2006; Shoelson, Lee, & Goldfine, 2006; Wellen & Hotamisligil, 2005).

Interestingly, the production of pro-inflammatory cytokines observed in obesity is also a component of the acute stress response (Chrousos, 1998). Thus, it is conceivable that elevated inflammatory factors due to chronic stress may be implicated in the development of T2D even in the absence of obesity. Notably, a range of inflammatory mediators, including TNF-α, IL-6, and CRP, have been associated with insulin resistance and manifestations of the metabolic syndrome independently, in most cases, of the degree of obesity (Natali et al., 2006; Phillips & Perry, 2013; Pickup et al., 1997; Vozarova et al., 2002; Yudkin et al., 1999). Prospective cohort studies with diverse human samples have also shown that white blood cell count, inflammatory cytokines such as IL-1β and IL-6, chemokines, and CRP can predict new onset T2D regardless of the initial degree of adiposity measures and insulin resistance (Duncan et al., 2003; Spranger et al., 2003; Vozarova et al., 2002; Wang et al., 2013). Furthermore, animal models have shown that stress exposure precedes the development of chronic subclinical inflammation and promotes visceral obesity ultimately leading to insulin resistance, dyslipidemia, and hypertension (Black, 2003). In addition, stress-related psychosocial factors that are usually seen in people with T2D, such as lower SES, being part of an ethnic minority group, and depression are characterised by increased markers of inflammation (Miller & Raison, 2016; Tabassum et al., 2008; Wang et al., 2007), suggesting a common inflammatory pathway contributing to T2D development.

Since inflammatory factors work in synergy, the measurable contribution of individual molecules is difficult to determine, even in experimental models (Esser et al., 2014). Nevertheless, the inflammatory cytokine, IL-6, seems to be particularly important in T2D. In a 2013 meta-analysis, a dose-response relationship between IL-6 and risk of new onset T2D was observed (Wang et al., 2013), proposing that inflammatory dysregulation exists prior to diabetes development. Epidemiological evidence also indicates that people with T2D have elevated IL-6 absolute concentrations compared to healthy controls (Pickup, 2004). Experimental studies have shown that IL-6, specifically, inhibits adenosine monophosphate-activated protein kinase, an enzyme related in fatty acid oxidation, down-regulating the expression of genes involved in insulin-stimulated glucose transport and lipid uptake in adipose tissue (Pickup, 2004). A large study using Mendelian randomisation[32] further suggested a causal relationship between increased IL-6 and the risk of T2D. Specifically, it was found that a functional variant in the IL-6 receptor gene, which attenuates IL-6 signalling, is associated with a nearly significant reduced risk (OR = 0.97, p = 0.06) of developing T2D (Swerdlow et al., 2012), highlighting the negative contribution of IL-6 in metabolic function.

1.3.3.1 Inflammatory dysfunction in T2D and macrovascular outcomes

Elevated inflammatory levels are known to be health-damaging. In people with established T2D, increased inflammatory levels have been cross-sectionally and prospectively linked to elevated risk of both micro- and macro-vascular complications and premature mortality (Best et al., 2005; Gouliopoulos et al., 2018; Jager et al., 1999;

[32] Mendelian randomisation is a relatively new area of research that enables the estimation of the causal contribution of specific biological factors in disease outcomes by using the properties of common genetic variation (Davey Smith & Hemani, 2014).

Lowe et al., 2014; Matoba et al., 2019; Saito et al., 2000; Soinio, Marniemi, Laakso, Lehto, & Rönnemaa, 2006). IL-6, IL-1ra and MCP-1 are particularly important in this context as they seem to contribute to the risk of CVD, the major complication of T2D. More specifically, two large-scale genetic studies have provided evidence for a causal relationship between the IL-6 pathway and CHD. More precisely, it was found that the Asp358Ala variant in the IL-6 receptor gene, which attenuates IL-6 signalling on hepatocytes, monocytes, and macrophages, was associated with reduced production of CRP and fibrinogen, and subsequently, reduced risk of CHD (Emerging Risk Factors Collaboration et al., 2012; Swerdlow et al., 2012). Elevated IL-6 concentrations are predictive of future CVD (Danesh et al., 2008; Papanicolaou, Wilder, Manolagas, & Chrousos, 1998) and poorer outcomes in people with exiting CVD (Libby, Ridker, & Hansson, 2011). In the ADVANCE trial of 3,865 people with T2D and established CVD risk factors, IL-6 was associated with future fatal and non-fatal cardiovascular events, after adjusting for other inflammatory markers including fibrinogen and CRP (Lowe et al., 2014). In addition, heightened levels of IL-1ra and MCP-1 have been prospectively associated with newly-diagnosed CHD in initially healthy adults (Danesh et al., 2008; Herder et al., 2006; Tang et al., 2007; van Minkelen et al., 2009), and MCP-1 has been suggested to contribute to the pathogenesis of vascular diseases by enhancing recruitment of leukocytes to sites of inflammation in the vessel wall (Niu & Kolattukudy, 2009). It has also been found that MCP-1-induced protein, a novel zinc-finger protein, promotes oxidative and nitrosative stress that causes endoplasmic

reticulum stress[33] which leads to autophagy[34] and cardiac cell death involved in heart failure (Younce & Kolattukudy, 2010). Together these findings show that inflammatory processes are highly relevant to T2D progression by increasing the risk of CVD.

1.4 Summary of literature review: addressing the gap

In summary of what has been described in the introductory chapter of this thesis, acute stress triggers an orchestra of biological responses in the body, including neuroendocrine, autonomic, and inflammatory responses. Dysregulation of these stress-related systems is thought to play a key role in the pathophysiology of various chronic conditions, including obesity, T2D, and CVD, and is associated with poorer physical and mental health outcomes. T2D and its related complications and comorbid conditions account for a great percentage of disability and mortality rates globally, especially among older adults. Increased levels of inflammatory cytokines, indicative of inflammatory dysfunction, have been cross-sectionally and prospectively associated with T2D risk and progression to CVD. Therefore, it is conceivable that these altered processes may account for the future disease risk people with T2D face. People with T2D show blunted inflammatory responses to acute stress in the laboratory (also indicative of inflammatory dysregulation) and report experiencing chronic stress, such as increased depressive symptoms and financial strain, compared

[33] Endoplasmic reticulum (ER) stress describes the condition of accumulation of unfolded proteins in the ER. The ER is the subcellular entrance for a number of secretory and structural proteins as well as the site of biosynthesis for steroids, cholesterol, and other lipids. ER stress may lead to cells apoptosis. Previous reports have linked ER stress with various pathologies including diabetes (Kitamura, 2008).

[34] Autophagy is a self-degradative process. It plays a key role in removing aggregated proteins from the ER. Its deregulation has been linked to cell death (Glick, Barth, & Macleod, 2010).

to healthy adults. These facts support a direct link between chronic stress, inflammatory markers, and T2D risk and progression.

Although most of previous studies have linked chronic stress-related factors with T2D onset, less is known about the role of potentially protective factors, such as subjective well-being, in reducing the risk of future diabetes, and the extent to which this relationship can be explained by relevant factors.

The impact of subjective well-being on health would be even more important in populations at increased disease risk of ill-health and premature mortality, such as those with T2D. Subjective well-being has been associated with reduced inflammatory stress responses in healthy individuals. Indeed, a very prominent feature of inflammatory responsivity is the variation in the response magnitude between individuals, which can be observed by measuring inflammatory cytokines levels pre- and post-stress using a laboratory stress testing design. Apart from subjective well-being, sex differences in the inflammatory stress responses have also been identified in previous studies. Individual differences in the phenotype of inflammatory responses might explain differences in future disease vulnerability. Given the detrimental effects of stress and inflammation in T2D, and the greater vulnerability to future complications and comorbid conditions in people with T2D, translation of this research into people with this condition is warranted. Identifying the determinants of inflammatory responses in this patient group can be an important stand of future disease prevention, especially if the predictive value of inflammatory stress responsivity in this population can be established.

Results from laboratory stress testing studies suggested that increased inflammatory stress responses may mediate the relationship between environmental factors (e.g. demographic and psychological factors) and future disease risk. However, the cross-sectional nature of these previous laboratory studies does not allow longitudinal investigations and direct testing of the mediating role of increased inflammatory responses. The latter have been linked to faster progression of CVD risk factors in a small number of previous follow-up studies. Albeit informative, these studies are limited by the fact that they only included healthy individuals and they have mostly assessed disease risk factors (e.g. carotid artery stiffness) rather than disease endpoints (e.g. stroke). The mediating role of inflammatory stress responsivity has been directly tested in one small study of 35 healthy women with a 1-year follow-up. Examining the mediating role of inflammatory stress responsivity in linking individual characteristics (e.g. sex and well-being) with future physical and mental health outcomes in people with T2D will provide fruitful insights into how these factors influence health, which in turn could inform strategies for the prevention and treatment of T2D complications and comorbidities. Given the substantial burden of T2D for patients, their families, and nations, this understanding is essential.

Chapter 2. Thesis purpose, aims, and objectives

This chapter describes the purpose, aims, and objectives of the thesis, and maps the contribution of each subsequent chapter.

2.1 Purpose, aims, and objectives

The overall purpose of this thesis is to better understand the development and progression of T2D and to contribute to the development of preventive interventions. This thesis aims to explore the role of sex and subjective well-being in the development and progression of T2D and to shed light on the biological, particularly inflammatory, mechanisms that may link these individual factors with long-term physical and mental health outcomes in people with established T2D. Therefore, the objectives of this thesis are to:

1. Test the longitudinal association between subjective well-being and incident T2D in initially healthy individuals (Chapter 3);
2. Test the cross-sectional association between sex and inflammatory stress responses in people with T2D (Chapter 5);
3. Test the cross-sectional association between subjective well-being and inflammatory stress responses in people with T2D (Chapter 6);
4. Test the role of sex and subjective well-being on long-term physical and mental health outcomes in people with T2D, and the mediating role of inflammatory stress responses[35] (Chapter 8).

[35] Various individual factors would be important to examine in relation to inflammatory processes and future health outcomes. Sex and subjective well-being were chosen on the basis of previous research justifying their selection, especially in the context of T2D, as described earlier in this chapter.

2.2 My contribution to the research in this thesis

I developed the purpose, aims, and objectives of this thesis as well as the design of the studies with input from my supervisors, Professor Andrew Steptoe, Dr Lydia Poole, and Dr Ruth A. Hackett. I was responsible for all statistical analyses presented in this thesis. More specifically, I have analysed existing data from two datasets, the ELSA and The Psychobiology of Social Position: The Diabetes Study (the latter is reported as the 'Diabetes Study' or the original/baseline/initial study throughout this thesis). I have also collected and analysed original data by successfully carrying out the Psychosocial and Biological Aspects of T2D Complications and Comorbid Conditions: The Diabetes Follow-up Study (this study is reported as the 'Diabetes Follow-up Study' or the follow-up study throughout this thesis). Detailed information on my contribution to the Diabetes Follow-up Study is presented in Chapter 7.

Chapter 3. Study 1: Enjoyment of life predicts T2D incidence over 12 years: Findings from ELSA

Study 1 examined the prospective association between two sub-components of subjective well-being and T2D incidence using data from ELSA. This chapter provides a brief overview of the ELSA cohort, followed by an introduction to the topic of study, the methods used, the study findings, and a discussion of the study findings. The study has been submitted for publication to the *Journal of Epidemiology and Community Health*.

3.1 The ELSA study

The ELSA study was established in 2002 by a group of researchers based at University College London (UCL), the Institute for Fiscal Studies, the University of Manchester, and the National Centre for Social Research. This is a panel study of men and women aged 50 years and older who live in England. Originally, the study was set up to explore the dynamic relationships between socioeconomic factors, health, and well-being in an ageing population in England in the 21st century. The first sample was drawn from households that had taken part in the Health Survey for England (HSE)[36] in 1998, 1999, and 2001. These three years were chosen because they were the most recent at that time and also they could provide a sufficiently large size of the sample. ELSA used the core samples for these years, which totally involved more than 23,000 nationally representative responding households. The sample design in 1999 also included a boost sample that represented ethnic minorities. Nevertheless, because of funding

[36] The HSE is an annual cross-sectional study that is established to monitor the health of the general population in England (Mindell et al., 2012).

constraints it was impossible to follow-up the boost sample; thus, it was finally discarded (English Longitudinal Study of Ageing, 2019).

Since the initial data collection phase of ELSA in 2002-2003, the same group of respondents had participated at 2-yearly assessments, known as waves. At every subsequent wave, the sample is replaced or refreshed with more HSE participants in order to maintain the age profile requirement of the cohort. Each new sample added then becomes part of the cohort issued again at the following waves. Wave 1 sample included 11,391 core members with an age range from 50 to 100 years (mean age = 65 years). Entry criteria included being a member of a participating HSE household and having agreed to be re-contacted, being ≥ 50 years old, and living in a private household in England at the time of the first wave of fieldwork. The ELSA sample was designed to represent people aged 50 and over who live in England. Indeed, comparisons of the sociodemographic characteristics of participants with national census data showed that the ELSA participants are representative of the English population aged 50 and over. ELSA was approved by the London Multicentre Research and Ethics Committee (MREC/01/02/91) and all participants provided informed consent (Steptoe, Breeze, Banks, & Nazroo, 2013).

ELSA is a rich resource of high-quality data and is multidisciplinary in nature, involving the collection of various data. Data are collected using self-completion questionnaires and computer-assisted personal interviews at each wave (every two years). These include topics related to family and work, economic issues[37], social and psychological

[37] ELSA was the first study in England to assess detailed information on individual and family finances including 75 separate elements of wealth and income (Steptoe et al., 2013).

factors, physical and mental health, behaviour and cognition. Additionally, blood sampling (for the extraction of biological markers and genetic data) and a more detailed physical examination and performance are carried out during nurse visits at alternate waves (every four years). Changes over time are captured by either repeating the same key measurements at each wave, by asking participants directly about changes, and/or by adopting questions that allow participants to update previous answers. New topics may be introduced at distinct waves, other modules may be rotated on and off, while one-off topics/questions are included in specific waves. The dataset is openly available to researchers and analysts as soon as cleaning and checking are completed. The National Institute of Aging and a consortium of UK government departments coordinated by the Office for National Statistics currently provide the funds for the ELSA study (Steptoe et al., 2013).

3.2 Introduction

Subjective well-being is a multidimensional concept that is often divided into two sub-components, hedonic and eudaimonic well-being. As reported in the introductory chapter of this thesis, hedonic well-being encompasses positive feelings such as enjoyment of life, pleasure, and happiness, and is typically assessed retrospectively by asking participants about their mood over the previous week or month. Eudaimonic well-being, on the other hand, refers to judgments about a sense of purpose in life and self-realisation. Its assessment demands more extensive cognitive processing than mood ratings and involves relatively complex introspection about attributes aggregated over time (Ryan & Deci, 2001; Steptoe, 2019a).

There is compelling evidence that both hedonic and eudaimonic well-being are relevant to health and longevity in older adults. Nationally representative prospective cohort studies in the UK and the US have shown that hedonic well-being, such as positive affect, is associated with reduced risk of death after adjustment for initial health status and other confounders (Chida & Steptoe, 2008; Steptoe & Wardle, 2011), including demographic and socioeconomic factors (Lawrence, Rogers, & Wadsworth, 2015; Steptoe & Wardle, 2011), health behaviours, and the adverse effects of depression (Steptoe & Wardle, 2011). Associations have also been found between greater hedonic well-being and reduced 10-year incident CHD, reduced functional decline and decreased incidence of disability in older adults (Davidson, Mostofsky, & Whang, 2010; Hirosaki et al., 2013; Ostir et al., 2000; Park-Lee, Fredman, Hochberg, & Faulkner, 2009). Three meta-analyses dealing with subjective well-being and longevity (Chida & Steptoe, 2008; Lamers, Bolier, Westerhof, Smit, & Bohlmeijer, 2012; Rasmussen, Scheier, & Greenhouse, 2009) provided convergent results for the role of positive affect and other related forms (e.g. optimism) in survival in both healthy and diseased populations.

Enjoyment of life, in particular, has been associated with reduced premature mortality in a dose-response manner in two analyses of the ELSA (Steptoe & Wardle, 2012; Zaninotto, Wardle, & Steptoe, 2016). Furthermore, in a study of 88,175 middle-aged Japanese participants, enjoyment of life was significantly associated with reduced incidence of CVD and lower mortality rate over a 12-year of follow-up, but only in men (Shirai et al., 2009). In another analysis using ELSA data, greater subjective well-being (enjoyment of life coupled with low depressive symptoms) was related not only to

longer but also healthier life, free of disability and chronic disease (Zaninotto & Steptoe, 2019). Moreover, an analysis of the National Health and Nutrition Epidemiologic Follow-Up Study revealed that enjoyment of life was associated with reduced all-cause mortality among individuals with existing diabetes (Moskowitz et al., 2008).

Research has also linked eudaimonic well-being with health outcomes. A recent meta-analysis of 66 predominantly cross-sectional studies found small-to-moderate effects of purpose in life in physical health indicators including better self-rated health and lower mortality (Czekierda, Banik, Park, & Luszczynska, 2017). Additionally, a stronger sense of purpose in life was prospectively linked to more prolonged survival in a pooled analysis of 10 longitudinal studies (Cohen, Bavishi, & Rozanski, 2016). Possessing a high sense of purpose in life has also been prospectively associated with slower development of age-related disability and reduced incidence of ageing-related conditions such as CVD and Alzheimer's disease (Boyle, Buchman, Barnes, & Bennett, 2010; Boyle, Buchman, & Bennett, 2010; Cohen et al., 2016; Kim, Delaney, & Kubzansky, 2019; Kim, Sun, Park, & Peterson, 2013; Steptoe et al., 2015). Moreover, a recent longitudinal analysis of the ELSA cohort demonstrated associations between lower sense of purpose in life and a range of negative health variables such as poorer self-rated health, chronic pain, obesity, and physician-diagnosed chronic diseases (Steptoe & Fancourt, 2019).

As mentioned in the introductory chapter of this thesis, there is growing evidence for the role of psychological factors in predicting the onset of T2D. While the association between negative emotional factors, such as depressive symptoms, with T2D risk is

well-investigated (Hackett & Steptoe, 2017), less is known about the relationship between positive factors and T2D risk. The relationship between subjective well-being and T2D or T2D trajectories has been described in detail in Chapter 1. Overall, results from previous studies manifested a significant protective association between subjective well-being and T2D risk independent of covariates, including depression or negative affect. Age and sex differences in the links between well-being and T2D have also been reported in previous studies (Feller et al., 2013; Okely & Gale, 2016; Poole et al., 2019). Nevertheless, the majority of studies to date have tested a single dimension of well-being or overall well-being, and none of the previous studies directly compared hedonic and eudaimonic aspects of well-being and T2D risk. Nevertheless, it has been suggested that consideration of the individual contribution of the different types of well-being is worthwhile (Hernandez et al., 2018; Steptoe, 2019a). Additionally, as reported in Chapter 1, although previous studies demonstrated an independent association between well-being and T2D, the extent to which this relationship can be explained by other relevant factors, such as sociodemographic, behavioural, and clinical characteristics, remains unclear.

The aim of the current study was to explore the role of subjective well-being on T2D incidence. The objective of the study was to test the separate effect of hedonic well-being (as indexed by enjoyment of life) and eudaimonic well-being (as indexed by purpose in life) on T2D rate, and to estimate the amount of the association explained by sociodemographic, lifestyle, and clinical factors. It was hypothesised that higher enjoyment of life and a stronger sense of purpose in life would be associated with a

reduced rate of T2D onset and that these effects would be, at least in part, be explained by sociodemographic, behavioural and clinical risk factors.

3.3 Method

3.3.1 Participants

The current study used longitudinal data from the ELSA cohort. As reported previously in this chapter, this is a nationally representative study of people 50 years and older living in England. In the present study, I tested the association between two different domains of subjective well-being measured at Wave 2 (2004/5) and incident T2D using data from Wave 3 (2006/7) through to Wave 8 (2016/7). The first nurse data (which included HbA1c measurement), were collected at Wave 2 (2004/5); thus, Wave 2 was selected as the baseline. A total of 8780 core members participated at baseline. At the time of writing this thesis, Wave 8 (2016/7) is the most recently completed phase of data collection that is available for analysis. People in the current study were followed-up for 11.6 years on average.

In 2004/5, all participants were free from self-reported diabetes diagnosis or high blood sugar diagnosis. Participants were excluded from the current analysis if they had incomplete data (one or more missing) on exposure measures (*n* = 1343) or any of the covariates (*n* = 2597). Participants were included in analyses if they provided follow-up data on diabetes incidence in at least one wave. Therefore, 706 participants with missing outcome data were also excluded from analysis. These exclusion criteria resulted in an analytical sample of 4134 participants. A flow diagram of the sample size is depicted in Figure 3.1.

Sociodemographic, behavioural, and clinical variables were compared between the analytical sample (N = 4134) and those excluded due to missing data (N = 4646). Significant differences between the two groups were checked using independent samples *t*-tests for continuous variables and Chi-square tests for categorical variables. Significant differences were observed in age, financial wealth, ethnicity, marital/cohabitation status, BMI, physical activity, smoking, alcohol consumption (p = 0.002), hypertension, coronary heart disease (CHD), and HbA1c (all other ps < 0.001)[38]. Compared to those excluded due to missing data, participants of this study were younger and wealthier on average. They were more likely to be of white ethnicity and to be married or cohabiting. Participants of this study also had better behavioural and health profile; They were more likely to be non-smokers and physically active, they had lower BMI and HbA1c levels, and were less likely to have hypertension or CHD at baseline. Participants of the study were also more likely to consume alcohol more often that those excluded from the analysis.

[38] More information on these variables is given on page 105.

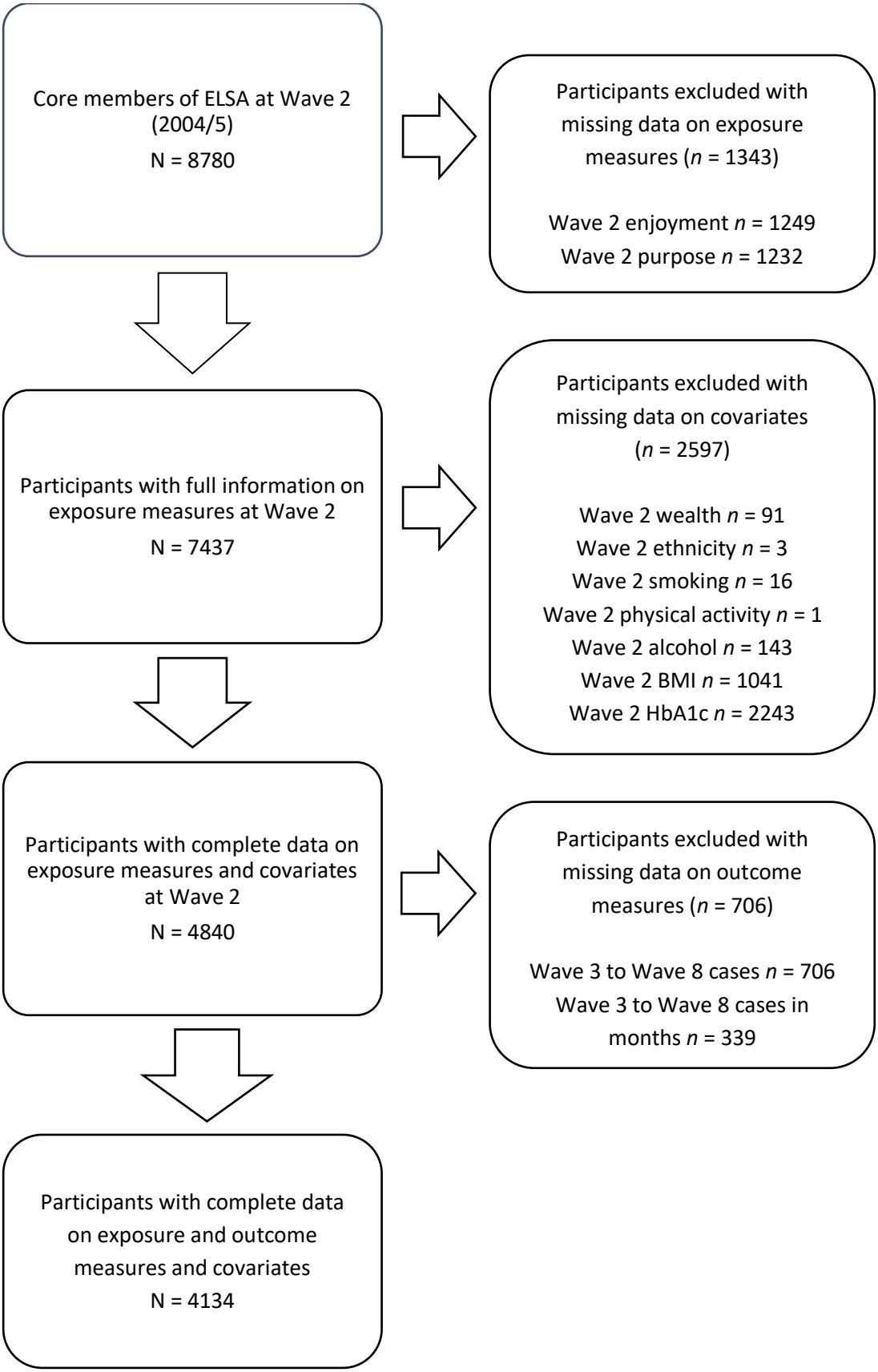

Figure 3.1. Flow diagram of those included and excluded from the analysis. BMI = body mass index; ELSA = English Longitudinal Study of Ageing; HbA1c = glycated hemoglobin; N = number; *n* = number.

3.3.2 Study measures

3.3.2.1 Exposure variables: Enjoyment of life, purpose in life

Enjoyment of life and purpose in life at Wave 2 (2004/5) were assessed using items from the Control, Autonomy, Self-realization, and Pleasure (CASP)-19 as measures of hedonic and eudaimonic well-being, respectively. The CASP-19 is a self-reported questionnaire that was developed and validated to measure quality of life in old age (Hyde, Wiggins, Higgs, & Blane, 2003). Enjoyment of life was indexed with four items from the CASP-19 ("I enjoy the things that I do", "I enjoy being in the company of others", "On balance, I look back on my life with a sense of happiness", "I feel full of energy these days"). Participants responded to each of the items on a 4-point Likert-type scale (from 1 = never to 4 = often; scores can range from 4 to 16). Items were assessed using the continuous range of scores, with higher scores indicating greater enjoyment of life. A 3-level categorical variable was also created for graph purposes (low score ≤12, middle score = 13 - 14, and high score ≥15). The CASP-19 enjoyment of life subscale has been used in previous studies of physical capability and all-cause mortality (Steptoe, de Oliveira, Demakakos, & Zaninotto, 2014; Steptoe & Wardle, 2012). Internal consistency (Cronbach's α) of this subscale was 0.69 in this sample.

Purpose in life was measured using one item from the CASP-19. Participants were asked to rate how often they feel that their life has meaning (from 1 = never to 4 = often; scores can range from 1 to 4). The continuous score was used with higher scores indicating a higher sense of purpose in life. In sensitivity analysis, main analyses were re-run using a binary measure (low score = 1 – 2 and high score = 3 – 4).

3.3.2.2 Outcome variable: Time to T2D

Time to T2D diagnosis was based on self-reported information taken between Wave 3 (2006/7) and Wave 8 (2016/7). Specifically, participants were asked at each wave whether a physician had given them a diagnosis of diabetes or high blood sugar since their last interview. The time of diagnosis was indexed as the wave at which the participant first reported a diagnosis of diabetes or high blood sugar (the duration, in months [continuous variable], based on when diabetes/high blood sugar were first reported).

3.3.2.3 Covariates

Analyses were adjusted for a range of sociodemographic, behavioural, and health variables. All covariates were measured at baseline (2004/5). Self-reported socioeconomic position was indexed as quintiles of total financial wealth including participants' gross financial wealth net of debt (savings and investments, the value of any home and other property, the value of any business assets, and physical wealth such as artwork and jewellery). Financial wealth was found to be the most relevant indicator of SES in relation to health in ELSA (Demakakos, Biddulph, Bobak, & Marmot, 2016). Self-reported ethnicity (White, non-White) and relationship status (married or cohabiting, neither married or cohabiting) were also measured. Participants reported frequency of physical activity[39] (light or none weekly, moderate or vigorous once a week, moderate or vigorous more than once a week), their smoking status (smoker, non-smoker), and frequency of alcohol consumption (≥ 5 times/week, < 5

[39] The objective measurement of physical activity using accelerometry was not available at baseline (Wave 2), as it was firstly introduced at Wave 6. Therefore, self-reported information on the frequency of physical activity at Wave 2 was used in this study.

times/week). Height (in centimetres [cm]) and weight (in kilograms [kg]) were objectively measured by a nurse to calculate BMI. The latter was computed as body mass in kgs divided by the square of the height in metres (kg/m^2). Also, self-reported doctor diagnosis of hypertension was combined with objective blood pressure assessments carried out during the nurse visit. Objective hypertension was defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg (National Institute for Health and Care Excellence guidelines, 2019). A combined binary variable of self-reported diagnosis and/or objective assessment of hypertension was generated (no, yes). Similarly, I generated a measure of prevalent CHD at baseline, which included angina and/or MI self-reported diagnosis by 2004/5 (no, yes). For HbA1c, blood samples were drawn during the nurse visit and analysed at the Royal Victoria Infirmary Laboratory in Newcastle upon Tyne, UK (Sproston, & Mindell, 2004). The Diabetes Control and Complication Trial units, measured in %, are reported throughout this chapter. The International Federation of Clinical Chemistry units, mmol/mol, are also provided.

Analyses were also adjusted for depression status in secondary models. Depressive symptoms were measured using the Centre for Epidemiological Studies-Depression (CES-D) scale (Radloff, 1977). This 20-item standardised questionnaire is widely used in population studies to measure depressive symptoms in the general population over the previous week (Brunner et al., 2014; Demakakos, Zaninotto, & Nouwen, 2014). The 20 items primarily measure affective and somatic dimensions of depression, especially reflected in complaints such as depressed mood, feelings of guilt and worthlessness, helplessness and hopelessness, psychomotor retardation, loss of

appetite, and sleep disturbance. The 8-item version was used for the purposes of this study. The psychometric properties of this shorter version have been found to be comparable to the original 20-item version (Steffick, 2000). Items included statements such as 'I felt depressed', 'I felt everything I did was an effort', 'my sleep was restless'. A dichotomous (yes, no) response to each item resulted in a total score ranging between 0 (no symptoms) to 8 (all eight symptoms). A cut-off point of ≥ 4 was used to define significant depressive symptoms (Demakakos, Pierce, & Hardy, 2010) and a combined variable of a self-reported doctor diagnosis of depression and/or a positive CES-D score were used to produce a binary depression variable (no, yes). Internal consistency was good in this sample (Cronbach's $\alpha = 0.78$).

3.3.3 Statistical analysis

I first examined univariate associations between the two exposure variables (enjoyment of life and purpose in life) and sample characteristics. Univariate associations were carried out using Pearson's *r* correlations for continuous variables and independent samples *t*-tests and Kruskal-Wallis H tests for categorical variables, as appropriate.

It was ascertained that the proportional hazards assumption was not violated by using log (-log (survival)) versus. log (time) graphs. Two Cox proportional hazards regression models[40] were then used to examine associations between the two different domains of well-being at baseline (enjoyment of life and purpose in life) and T2D incidence over the 12-year follow-up after adjustment for covariates: age, sex, financial wealth,

[40] Enjoyment of life and purpose in life were highly correlated with each other, therefore they were inserted to two separate regression models to test for their effects on diabetes incidence.

ethnicity, marital/cohabitation status, physical activity, smoking status, alcohol consumption, BMI, hypertension, CHD, and HbA1c. In order to estimate the proportion of the association explained by sociodemographic, behavioural, and clinical covariates, my models were built sequentially, calculating the percentage of protective association explained (PPAE) by the inclusion of different groups of covariates (Lin, Fleming, & De Gruttola, 1997). PPAE = 1 – ((1 – HR of E + X) * 100) / ((1- HR of E) * 100) * 100 where HR = hazard ratio, E = exposure, and X = explanatory variables being tested. This approach makes no assumptions about the order in which covariates are entered, as with a hierarchical model. Therefore, five separate models were tested for each exposure variable: 1) unadjusted model, 2) adjusted for age, sex, financial wealth, ethnicity, and marital/cohabitation status, 3) adjusted for physical activity, smoking status, alcohol consumption, and BMI, 4) adjusted for hypertension, CHD, and HbA1c, and 5) fully adjusted model. Enjoyment of life and purpose in life were treated as continuous scores where HRs and 95% CIs represent a 1-unit increase. Time to event was measured in months from Wave 2 (2004/5) to the time of the follow-up wave at which the participant first self-reported doctor diagnosis of T2D or high blood sugar.

Depression status was added in a secondary analysis to test for the independent effect of well-being on T2D. Furthermore, I examined whether there was a moderating effect of age by entering a mean-centred interaction term in the fully adjusted models. An interaction term was also inserted in the fully adjusted models to test for a moderating effect of sex in the link between well-being and T2D.

In sensitivity analyses, I re-run my primary analysis after excluding participants who developed T2D within two years from baseline (by Wave 3, 2006/7). Secondly, the main analysis was repeated after excluding participants with an HbA1c value of ≥ 6.5% (equals to 48 mmol/mol) at baseline (WHO, 2011). As reported in the introductory chapter of this thesis, this clinical cut-off point is applied for the diagnosis of diabetes (ADA, 2019), therefore it was used in the current study to reflect an objective measure of baseline diabetes. These analyses were carried out in order to rule out the possibility of reverse causality (underlying diabetes or undiagnosed diabetes at baseline influencing well-being levels). Finally, since the purpose in life measure was found to be negatively skewed (skewness = -1.80, kurtosis = 2.89), main analysis was re-run using a binary measure.

T2D incident cases are plotted on a graph to reflect the time to diagnosis for low, middle, and high enjoyment categories at baseline. The level of significance was set at $p < 0.05$, though exact significance levels are reported throughout. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 25 (SPSS, Chicago, IL).

3.4 Results

A total of 4134 participants (56% women) free of T2D at baseline took part in the study. Participants were 64.97 years old on average (SD = 8.99) and the majority of them were married or cohabiting (73%), and of white ethnicity (99%). The average BMI was within the overweight range (27.56 kg/m^2, SD = 4.63) and the mean HbA1c was 5.46% (SD = 0.44; equals to 36.2 mmol/mol, SD = 2.46). The mean enjoyment of

life score was 14.23 (SD = 1.75), and the mean purpose in life score was 3.59 (SD = 0.71) in this sample.

Univariate associations between exposure variables and sample characteristics are shown in Table 3.1. On average, people who scored higher in enjoyment of life and purpose in life were younger, married or cohabiting, and wealthier. Moreover, they were more likely to drink more, to be non-smokers, and to engage in physical activity more frequently. Enjoyment of life but not purpose in life was associated with female gender. Higher enjoyment of life score was also associated with lower BMI and lower risk of hypertension. Inverse relationships were also observed between enjoyment of life and purpose in life and the risk of CHD and depression, and HbA1c levels. Ethnicity was not related to either enjoyment of life nor purpose in life (see Table 3.1).

Table 3.1

Sample characteristics and associations with subjective well-being at baseline (N = 4134)

Characteristic	n (%) or M ± SD	Associations with enjoyment of life: Effect size, *p* value	Associations with purpose in life: Effect size, *p* value
CASP enjoyment of life [score]	14.23 ± 1.75	-	$r = 0.62$, $p < 0.001$
CASP purpose in life [score]	3.59 ± 0.71	$r = 0.62$, $p < 0.001$	-
Age [years]	64.97 ± 8.99	$r = -0.05$, $p = 0.003$	$r = -0.03$, $p = 0.040$
Sex [women]	2305 (55.8)	$d = 0.07$[41], $p = 0.026$	$d = 0.04$, $p = 0.231$
Total net financial wealth [£]		$\varepsilon^2 = 0.04$[42], $p < 0.001$	$\varepsilon^2 = 0.01$, $p < 0.001$
Quintile 1	603 (14.6)		
Quintile 2	638 (15.4)		
Quintile 3	861 (20.8)		
Quintile 4	977 (23.6)		
Quintile 5	1055 (25.5)		
Ethnicity [White]	4094 (99.0)	$d = 0.08$, $p = 0.642$	$d = 0.01$, $p = 0.894$
Marital/cohabitation status [married or cohabiting]	3023 (73.1)	$d = 0.29$, $p < 0.001$	$d = 0.29$, $p < 0.001$
Physical activity per week		$\varepsilon^2 = 0.03$, $p < 0.001$	$\varepsilon^2 = 0.01$, $p < 0.001$
Light or none	664 (16.1)		
Moderate or vigorous 1 day	1007 (24.4)		
Moderate or vigorous >1 day	2463 (59.6)		
Smoking [smokers]	564 (13.6)	$d = 0.33$, $p < 0.001$	$d = 0.21$, $p < 0.001$
Alcohol consumption per week [< 5 days]	3097 (74.9)	$d = 0.13$, $p < 0.001$	$d = 0.09$, $p = 0.029$
BMI [kg/m^2]	27.56 ± 4.63	$r = -0.03$, $p = 0.043$	$r = 0.01$, $p = 0.409$
Hypertension case [yes]	1698 (41.1)	$d = 0.11$, $p < 0.001$	$d = 0.01$ $p = 0.735$
CHD case [yes]	400 (9.7)	$d = 0.21$, $p < 0.001$	$d = 0.11$, $p = 0.035$
[a]HbA1c [%]	5.46 ± 0.44	$r = -0.05$, $p = 0.004$	$r = -0.04$, $p = 0.007$
[b]Depression case [yes]	526 (12.7)	$d = 0.93$, $p < 0.001$	$d = 0.77$, $p < 0.001$

Note. Associations between exposure variables and sample characteristics were checked with Pearson's *r* correlations for continuous measures and independent samples *t*-tests and Kruskal-Wallis H tests for categorical measures. BMI = body mass index; CASP = Control Autonomy Self-realisation Pleasure; CHD = coronary heart disease; HbA1c = glycated hemoglobin; kg/m^2 = kilograms per square metre; M = mean; N = number; n = number; SD = standard deviation. [a]HbA1c levels equal to 36.2 ± 2.46 millimoles per mole. [b]*n* = 4104.

[41] *d* stands for Cohen's *d*. It is used as an effect size indicator for *t*-tests. Effect sizes of 0.2, 0.5 and 0.8 are considered as small, medium, and large, respectively (Cohen, 1988).

[42] ε^2 stands for epsilon squared and is an effect size indicator for the non-parametric Kruskal-Wallis H test (Kelley, 1935). ε^2 coefficient estimates the value from 0 = no relationship to 1 = perfect relationship (Tomczak & Tomczak, 2014).

Three hundred and twelve (8%) incident cases of T2D were reported over the average 11.6-year follow-up period. Univariate analysis showed that those who developed T2D during the follow-up were more likely to have lower enjoyment of life scores at baseline compared to those who did not develop diabetes ($t(4132) = 3.35$, $p = 0.001$, $d = 0.19$, 95% CI [0.14, 0.54]). However, purpose in life scores did not differ between those who did and did not develop T2D over the follow-up in unadjusted analysis ($p = 0.143$).

Cox regression analyses confirmed a significant inverse association between enjoyment of life and incident T2D in all five models. As shown in Table 3.2, enjoyment of life was associated with an 11% reduction in the rate of T2D onset in unadjusted analysis (HR = 0.89, $p < 0.001$, 95% CI [0.84, 0.94]). Sociodemographic factors (age, sex, financial wealth, ethnicity, and marital/cohabitation status) accounted for 27% of the association between enjoyment of life and T2D. Behavioural factors (physical activity, smoking status, alcohol consumption, and BMI) accounted for 27% reduction in the HR for enjoyment of life compared with the unadjusted model. Clinical variables (hypertension, CHD, and HbA1c) accounted for 18% of the association between enjoyment of life and T2D. In the final, fully adjusted model including sociodemographic, behavioural, and clinical covariates together, the significant association between enjoyment of life and T2D was maintained. Specifically, for every 1-unit increase in reported enjoyment of life, there was a 7% reduction in the hazard of T2D (HR = 0.93, $p = 0.020$, 95% CI [0.87, 0.99]), with sociodemographic, behavioural, and clinical factors combined accounting for a 36% reduction in the HR for enjoyment of life compared with the basic, unadjusted model.

Table 3.2

Cox proportional hazards regression on enjoyment of life in 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 - Potential mediators and confounders (N = 4134)

	HR	95% CI	*p* value	PPAE
Unadjusted model	0.89	0.84 to 0.94	< 0.001	-
+ sociodemographic variables	0.92	0.87 to 0.97	0.003	27%
+ behavioural variables	0.92	0.86 to 0.97	0.004	27%
+ clinical variables	0.91	0.86 to 0.97	0.003	18%
+ sociodemographic, behavioural, and clinical variables	0.93	0.87 to 0.99	0.020	36%

Note. CI = confidence interval; HR = hazard ratio; N = number; PPAE = percentage of protective association explained.

The fully adjusted model is presented in Table 3.3. In this model, enjoyment of life (HR = 0.93, $p = 0.020$, 95% CI [0.87 to 0.99]), sex (HR = 0.64, $p < 0.001$, 95% CI [0.50 to 0.81]), BMI (HR = 1.10, $p < 0.001$, 95% CI [1.08, 1.13]), hypertension (HR = 1.35, $p = 0.013$, 95% CI [1.07, 1.71]), and HbA1c (HR = 2.58, $p < 0.001$, 95% CI [2.33, 2.85]) were all significant predictors of T2D rate over the follow-up. Significant findings from the Cox regression analysis are also illustrated in Figure 3.2. The full individual models of enjoyment of life and T2D are also presented in Appendix B.

Table 3.3

Cox proportional hazards regression on enjoyment of life in 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 after adjusting for sociodemographic, behavioural, and clinical variables (N = 4134)

	HR	95% CI	*p* value
CASP enjoyment of life [score]	0.93	0.87 to 0.99	0.020
Age [years]	1.01	1.00 to 1.03	0.070
Sex [ref. cat.: men]	0.64	0.50 to 0.81	< 0.001
Financial wealth [£]			
Quintile 1 [ref. cat.]	1		
Quintile 2	1.01	0.67 to 1.52	0.962
Quintile 3	1.14	0.79 to 1.65	0.472
Quintile 4	0.97	0.67 to 1.42	0.891
Quintile 5	0.69	0.46 to 1.04	0.076
Ethnicity [ref. cat.: White]	1.87	0.83 to 4.23	0.131
Marital/cohabiting status [ref. cat.: married or cohabiting]	0.75	0.57 to 1.00	0.051
Physical activity per week			
Light or none [ref. cat.]	1		
Moderate or vigorous 1 day	0.94	0.66 to 1.34	0.737
Moderate or vigorous >1 day	0.96	0.71 to 1.31	0.802
Smoking status [ref. cat.: smokers]	0.94	0.68 to 1.30	0.708
Alcohol consumption per week [ref. cat.: < 5 days]	0.86	0.64 to 1.16	0.332
BMI [kg/m^2]	1.10	1.08 to 1.13	< 0.001
Hypertension [ref. cat.: no]	1.35	1.07 to 1.71	0.013
CHD [ref. cat.: no]	1.12	0.79 to 1.59	0.526
HbA1c [%]	2.58	2.33 to 2.85	< 0.001

Note. BMI = body mass index; CASP = Control Autonomy Self-realisation Pleasure scale; CHD = coronary heart disease; CI = confidence interval; HbA1c = glycated hemoglobin; HR = hazard ratio; kg/m^2 = kilograms per square metre; N = number; ref. cat. = reference category.

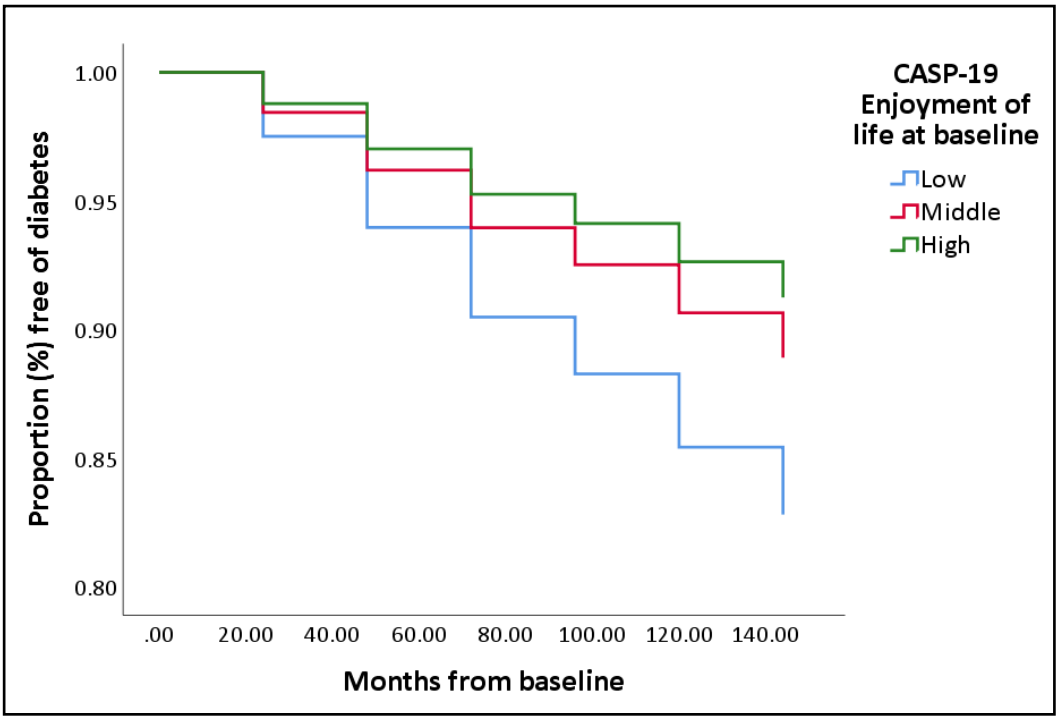

Figure 3.2. Kaplan-Meier survival curves for incident type 2 diabetes in low, middle and high enjoyment of life groups in 4134 participants from the English Longitudinal Study of Ageing. Results are unadjusted. Horizontal axis = time in months since baseline (2004/2005). CASP = Control Autonomy Self-realisation Pleasure scale.

Purpose in life showed a significant inverse association with T2D in unadjusted analysis. For every unit increase in reported purpose in life, there was a 15% reduction in the hazard of T2D (HR = 0.85, p = 0.032, 95% CI [0.74, 0.99]). As shown in Table 3.4, the association between purpose in life and T2D was attenuated in models adjusted for sociodemographic (HR = 0.90, p = 0.154, 95% CI [0.77, 1.04]), behavioural (HR = 0.87, p = 0.066, 95% CI [0.75, 1.01]), and clinical variables (HR = 0.93, p = 0.389, 95% CI [0.80, 1.09]), and in the fully adjusted model (HR = 0.92, p = 0.322 95% CI [0.79, 1.08]). Compared with the unadjusted model, sociodemographic variables together accounted for 33% of the association between purpose in life and T2D, behavioural factors accounted for 13% of the association, and clinical factors for 53%. Sociodemographic, behavioural, and clinical factors combined accounted for a 47%

reduction in the HR for purpose in life compared with the basic model. The full individual models of purpose in life and T2D are presented in Appendix B.

Table 3.4

Cox proportional hazards regression on purpose in life in 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 - Potential mediators and confounders (N = 4134)

	HR	95% CI	*p* value	PPAE
Unadjusted model	0.85	0.74 to 0.99	0.032	-
+ sociodemographic variables	0.90	0.77 to 1.04	0.154	33%
+ behavioural variables	0.87	0.75 to 1.01	0.066	13%
+ clinical variables	0.93	0.80 to 1.09	0.389	53%
+ sociodemographic, behavioural, and clinical variables	0.92	0.79 to 1.08	0.322	47%

Note. CI = confidence interval; HR = hazard ratio; N = number; PPAE = percentage of protective association explained.

Depression was included in a secondary model along with enjoyment of life, age, and sex to test for an independent effect of enjoyment of life on T2D rate. Results revealed a significant effect of enjoyment of life independently of depression (HR = 0.91, *p* = 0.002, 95% CI [0.86, 0.97]). The association between purpose in life and diabetes adjusted for age, sex, and depression was not significant (HR = 0.91, *p* = 0.222, 95% CI [0.78, 1.06]). Secondary analyses also indicated that the relationship between the two independent variables, enjoyment of life or purpose in life, and diabetes incidence did not differ according to age (age by enjoyment of life: fully adjusted HR = 1.00, *p* = 0.287, 95% CI [1.00 to 1.01]; age by purpose in life: fully adjusted HR = 1.01, *p* = 0.556, 95% CI [0.99, 1.02]) or sex (sex by enjoyment of life: fully adjusted HR = 0.96, *p* = 0.477,

95% CI [0.85, 1.08]; sex by purpose in life: fully adjusted HR = 0.88, *p* = 0.435, 95% CI [0.65, 1.21]).

Table 3.5

Cox proportional hazards regression on enjoyment of life in 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 after adjusting for covariates including depression (N = 4134)

	HR	95% CI	*p* value
CASP enjoyment of life [score]	0.91	0.86 to 0.97	0.002
Age [years]	1.01	1.00 to 1.02	0.163
Sex [ref. cat.: men]	0.60	0.48 to 0.75	< 0.001
Depression [ref. cat.: no]	1.42	1.03 to 1.96	0.033

Note. CASP = Control Autonomy Self-realisation Pleasure scale; CI = confidence interval; HR = hazard ratio; N = number; ref. cat. = reference category.

In sensitivity analysis, 70 participants who developed T2D within two years from baseline (by Wave 3; 2006/7) were excluded (resulting in N = 4064), and the association between enjoyment of life and T2D onset remained significant (fully adjusted HR = 0.92, *p* = 0.021, 95% CI [0.86, 0.99]). Secondly, the main analysis was repeated after excluding possible undiagnosed cases of T2D at baseline. Sixty-six participants with HbA1c value ≥ 6.5% (equals to 48 mmol/mol) at baseline were excluded (resulting in N = 4068) and the association between enjoyment of life and T2D onset remained significant (fully adjusted HR = 0.93, *p* = 0.029, 95% CI [0.87, 0.99]). Finally, purpose in life models were repeated in sensitivity analyses using a binary measure instead of a continuous variable and the relationship between purpose in life and incident diabetes was not significant in either unadjusted (HR = 0.74, *p* = 0.111, 95% CI [0.51, 1.07]) nor fully adjusted analysis (HR = 0.75, *p* = 0.135, 95% CI [0.51, 1.09]).

3. 5 Discussion

This study looked at the longitudinal association between two different aspects of subjective well-being and incident T2D over a period of 12 years using data from ELSA, a nationally representative study of adults who live in England. Results of the study were supportive of the hypothesis that subjective well-being, specifically enjoyment of life, plays an important role in diabetes pathology. After adjusting for covariates, 1-unit increase in the enjoyment of life score at baseline was associated with a 7% reduction in the hazard of T2D at follow-up. Moreover, results revealed that the relationship between enjoyment of life and T2D could be partly attributed to sociodemographic (27%), behavioural (27%), and clinical (18%) characteristics. Together these factors explained 36% of the association between enjoyment of life and T2D. The significant inverse relationship between enjoyment of life and T2D was upheld after the exclusion of participants who developed diabetes within two years from baseline or after excluding participants with undiagnosed, objectively measured T2D (using HbA1c cut-offs) at baseline.

In unadjusted analysis, purpose in life was associated with reduced T2D rate. However, this association was reduced in analyses adjusted for sociodemographic, behavioural, or clinical factors, and in the fully adjusted model. This finding might suggest an indirect link between purpose in life and diabetes that warrants investigation in future research. However, the use of a single purpose in life rating may have also played a role in the null results. A previous meta-analysis showed that the association between purpose in life and physical health outcomes is more robust when measures combine items referring to meaning in life and meaning-related sense of harmony, peace, and

well-being compared with items focusing solely on meaning in life (Czekierda et al., 2017). Nevertheless, a single question has been applied in older adults in at least five previous studies showing significant associations with physical activity (Takkinen, Suutama, & Ruoppila, 2001), poorer self-rated health (Steptoe & Fancourt, 2019), chronic pain (Steptoe & Fancourt, 2019), obesity (Steptoe & Fancourt, 2019), mobility status (Lampinen, Heikkinen, Kauppinen, & Heikkinen, 2006), number of chronic illnesses (Lampinen et al., 2006; Steptoe & Fancourt, 2019), and all-cause mortality (Koizumi, Ito, Kaneko, & Motohashi, 2008). It is also possible that T2D is associated with particular types of well-being, as has been found in a previous study where enjoyment of life, but not optimism, was a significant predictor of T2D in the long-term (Boehm et al., 2015). Additionally, the two aspects of well-being examined here may differ in the level of emotional experiences they encompass. For example, enjoying life may require stronger emotional experiences than having a sense of purpose in life, and findings from Study 1 might suggest a role for emotions exerting a more powerful influence on glucose metabolism. Interestingly, in previous work involving participants with heart failure, distinct positive affect dimensions were associated with different markers of inflammation (Brouwers et al., 2013), suggesting separate biological correlates of different well-being measures. Concluding, these results support the idea that T2D is differentially related to hedonic and eudaimonic well-being, adding value to testing these different dimensions separately.

The protective association between enjoyment of life and T2D rate was significant in secondary analysis after adjusting for depressive symptoms. This finding, which is in line with previous studies (Okely & Gale, 2016; Poole et al., 2019), provides further

evidence of the independent association between subjective well-being and T2D, as described earlier in the introductory chapter of this thesis. Secondary analysis also revealed that the pattern of results is similar across younger and older adults and for both sexes. These findings contradict the age- and sex-dependent effect of well-being on T2D as described in previous studies (Feller et al., 2013; Okely & Gale, 2016; Poole et al., 2019) and provide evidence of a more general protective role for enjoyment of life. Inconsistent findings might be influenced by the precise measures of well-being, different sample sizes (Feller et al., 2013; Okely & Gale, 2016), or age and cultural differences between studies (Feller et al., 2013). For example, two previous studies found a protective effect of well-being only in younger (<65 years old) participants, but both studies assessed overall well-being instead of specific aspects of well-being (Okely & Gale, 2016; Poole et al., 2019). The analysis of Feller et al. (Feller et al., 2013), who found a protective association between life satisfaction and T2D rate in women but not in men, included more than 50,000 German participants aged 35 – 65 years old. For this reason, these previous studies are not directly comparable to the present findings.

Sociodemographic, behavioural, and clinical factors combined did not fully explain the protective effect of hedonic well-being on health, in line with previous studies on different health outcomes (Chida & Steptoe, 2008; Giltay et al., 2006; Kubzansky & Thurston, 2007; Moskowitz et al., 2008; Ostir et al., 2000). In the study of Okely & Gale (Okely & Gale, 2016), the association between overall well-being (as measured using the CASP-19) and incident T2D was only partially mediated by health behaviours and BMI. Future studies need to investigate the role of additional mechanisms linking

hedonic well-being with T2D. For example, enjoyment of life may have an impact on biological processes relevant to T2D, modulated via corticolimbic pathways. We have previously found that hedonic well-being is associated with reduced cortisol output over the day (Steptoe et al., 2008; Steptoe et al., 2005). Indeed, the most consistent evidence relates hedonic well-being with cortisol output, showing lower cortisol levels and a steeper cortisol decline over the day in people reporting greater hedonic well-being, after depression is included as a covariate (Steptoe, 2019a). However, most studies on well-being and biology have been cross-sectional. In contrast, one previous report showed that positive affect, measured using Ecological Momentary Assessments (EMA)[43], predicted lower cortisol output three years later (Steptoe & Wardle, 2005). Lower inflammatory levels have also been reported in people higher in daily happiness, even after daily sadness was taken into account (Panagi, Poole, Hackett, & Steptoe, 2019a; Stellar et al., 2015). In turn, dysregulated diurnal cortisol profile and elevated inflammatory factors have been prospectively linked to T2D risk (Hackett, Kivimaki, Kumari, & Steptoe, 2016; Hu, Meigs, Li, Rifai, & Manson, 2004; Wang et al., 2013). Cortisol and inflammatory factors seem to play a part in the pathophysiology of T2D, as described earlier in Chapter 1. Laboratory stress testing studies have also shown that hedonic well-being is associated with reduced inflammatory and cardiovascular reactivity to stress, establishing a dynamic relationship between well-being and stress-related biological processes (Steptoe et al., 2005).

[43] EMA involves repeated measurement (often in random time sampling) of participants' behaviour, experiences, or feelings. These are taken in real-life environments using methods such as written or electronic diaries or even biological sensors for biological assessments (Shiffman, Stone, & Hufford, 2008).

This study has several strengths. A large sample of participants were included, derived from a nationally representative cohort study. The longitudinal design of the cohort allowed the examination of T2D incidence in people free of T2D at baseline, with a relatively long follow-up period. Analyses were differentiated between types of well-being and adjusted for a wide range of covariates. Separate models were used to allow for the estimation of the percentage of association explained by the different risk factors. BMI and blood pressure levels at baseline, which constitute major T2D risk factors (Eckel, Alberti, Grundy, & Zimmet, 2010), were assessed objectively during the nurse visit. Additionally, the reverse causality argument was tested by excluding individuals who developed T2D within two years from baseline and those with objective T2D at baseline. Nevertheless, this study is not without limitations. Subjective well-being was measured at a single occasion, therefore changes in well-being over time were not considered. Nevertheless, the temporal stability of well-being has been documented in previous studies (Charles, Reynolds, & Gatz, 2001; Lucas, & Gohm, 2000). Patient reports of T2D diagnoses were used instead of objective clinical records, but other studies have reported high agreement between self-reported and clinically-derived diagnosis of diabetes (Haapanen, Miilunpalo, Pasanen, Oja, & Vuori, 1997). Additionally, diabetes diagnoses did not discriminate between Type 1 and Type 2, which may lead to diabetes misclassification, although T1D is diagnosed in individuals younger than the typical ELSA participant (T1D is also called 'juvenile diabetes' as it is diagnosed in childhood). Although multiple covariates were taken into account, I did not consider other potential mediators including the use of preventive healthcare services and self-care behaviours, such as adherence to medical advice (Kim, Strecher, & Ryff, 2014) and dietary factors (Mujcic & Oswald, 2016).

Finally, the proportion of ethnic minority participants in ELSA is small; thus, our results may not generalise to non-white ethnic groups.

This study provides evidence for the health-protective relationship between enjoyment of life and incident T2D. This association is only partially explained by sociodemographic, behavioural, and clinical risk factors. One implication is that efforts to increase enjoyment of life in middle- and older-aged adults might help delay the onset of T2D. This implication is discussed in more detail in the Discussion chapter (Chapter 9) of this thesis.

Chapter 4. The Diabetes Study: Data and methods

This chapter describes the data and methods of the Diabetes Study. The Diabetes Study provided existing data for the analyses presented in Chapter 5 and Chapter 6.

5.3 The Diabetes Study

The primary, overarching aim of the Diabetes Study was to assess biological stress responses across multiple biological systems in people with T2D compared to healthy controls. The secondary aim of the Diabetes Study was to test the role of individual characteristics (e.g. psychological and personality factors) in biological stress responses in the diabetes group. For the purposes of my PhD, I carried out two research projects using existing data from the Diabetes Study[44]. Briefly, the two projects examine the role of sex and subjective well-being, respectively, in inflammatory stress responses in people with T2D. The data and methods of the Diabetes Study are presented below, though more specific information about Study 2 and Study 3 (e.g. exposure and outcome measures, covariates, statistical analyses) are provided in subsequent chapters (see Chapter 5 and Chapter 6, respectively).

5.3 Participants

Between March 2011 and July 2012, 140 individuals (88 men, 52 women) aged 50 – 75 years old with a doctor-verified T2D diagnosis took part in a laboratory stress testing study. As mentioned above, the aim of the Diabetes Study was primarily to compare biological stress responses between healthy participants and people with

[44] I have also benefitted from contributing to the Diabetes Study by co-authoring two scientific papers using existing data from this study. Of the two papers, which are not part of this thesis, one has been published (Hackett et al., 2019) and the other is under review for publication (Bawa et al., under review).

T2D (Steptoe, Hackett, et al., 2014). Healthy participants were drawn from the Heart Scan Study (HSS), an earlier investigation which was also carried out by the Psychobiology Group of UCL. The HSS participants were recruited from the Whitehall II epidemiological cohort study between 2006 and 2008. The principal aim of the HSS was to investigate the complex relationships between socioeconomic and psychosocial factors, biological stress responses, and the development of CAD (Hamer, Endrighi, Venuraju, Lahiri, & Steptoe, 2012).

For the purposes of the Diabetes Study, participants with T2D were recruited from primary care practices and diabetic outpatient clinics in London. Inclusion was limited to patients without a history or a doctor diagnosis of CHD, inflammatory diseases, allergies, or mood disorders. These criteria were selected in order to reduce potential interference with biological processes and stress responsivity. It was not possible to exclude obese individuals from this study as obesity is one of the main risk factors for T2D (Guh et al., 2009), and the majority of eligible participants were obese. Potentially eligible participants were screened by telephone interviews carried out by members of the study team[45]. Also, on the day of stress testing participants were asked to report again any comorbidities they might have that would have excluded them from taking part. All participants were judged to be fluent in English language.

The Diabetes Study was powered to detect small to medium effect sizes ($\delta = 0.32$, $p < 0.05$), and at least 125 people needed to be recruited. The majority of participants (90%) were recruited from primary care clinics in the Camden area of London, since most of the people with T2D attending outpatient clinics had many comorbidities and

[45] The study team consisted of Dr Ruth A. Hackett, two research nurses, and a medical doctor.

therefore did not meet the stringent inclusion criteria of the study. Recruitment strategy is discussed more broadly in Chapter 9 of the thesis. All participants gave fully informed written consent to take part in the study and ethical approval was obtained by the UK National Research Ethics Service.

After recruitment, participants with T2D were matched to healthy controls (for the purposes of the larger trial aiming to compare biological stress responses in people with diabetes and healthy controls). Specifically, every participant with T2D was matched with two healthy controls as closely as possible by age, sex, and income category. Indeed, the two groups did not significantly differ in age, sex, or income (Hackett, 2016). More than one control per case is usually applied in case control studies in order to increase statistical efficiency and power (Rose & Laan, 2009). In the Diabetes Study, a ratio of two controls to one case was used due to a physical limit on the number of suitable controls available from the HSS, resulting in a final sample size of 140 participants with T2D and 280 matched controls.

5.3 Procedure

Before the laboratory stress testing, participants were asked to complete a 7-day diary. This included questions on feelings of happiness and sadness over the day. Detailed information about these measures is presented on page 132. After this 7-day diary period, participants were invited for individual stress testing in a light- and temperature-controlled laboratory at UCL.

Testing was performed in either the morning or the afternoon and was based on a standard protocol previously used in the same laboratory (Hamer, O'Donnell, Lahiri, & Steptoe, 2010; Steptoe, Owen, et al., 2002). Pre-testing instructions were to avoid

taking any anti-inflammatory or antihistamine medication up to seven days before testing, to refrain from performing vigorous physical activity and consuming alcohol from the evening before testing, and to avoid caffeinated beverages or smoking for at least two hours prior to the stress session. People who reported having colds or other infections on the day of testing were re-scheduled.

On the testing day, participants' anthropometric characteristics were firstly assessed by members of the study team and self-reported information on sociodemographic and behavioural factors as well as physical and mental health was collected before stress testing. Following this, a venous cannula was inserted into participants' forearm for blood sample collection. The participant rested for 30 minutes and within the last five minutes of the resting period a subjective stress rating was obtained, and a blood sample was drawn to detect pre-task inflammatory levels. Two 5-minute mental stress tasks were then administered, and blood was sampled again immediately after the tasks, at 45 minutes, and at 75 minutes after the completion of the tasks. Task perception ratings (e.g. task difficulty) were taken immediately after the tasks. Subjective stress ratings were also taken up to 75 minutes after the end of the tasks. Detailed information about the two mental stress tasks and study measures is presented in the sub-sections below.

Blood samples were collected using ethylenediaminetetraacetic acid tubes which were centrifuged immediately after collection at 2500 rpm for 10 minutes at room temperature. Ten minutes later, plasma was removed from the tubes, aliquoted into 0.5 ml portions and stored at -80^{o} Celsius degrees at UCL until batch analysis at a later

date. Figure 4.1 shows a volunteer undertaking biological measurements at the UCL stress laboratory where the Diabetes Study participants underwent their testing.

Figure 4.1. A volunteer undertaking biological measurements at the UCL stress laboratory.

5.3 Mental stress tasks

Two 5-minute mental stress tasks were administered in random order. These were a paper version of the Stroop colour – word interference task and a mirror tracing task. The Stroop task requires successive reporting of target colour words (e.g. blue and red) printed in an incongruous colour. The mirror tracing task requires participants to move a metal stylus to trace a star while looking only at the mirror image. When the stylus comes off the star's outer line, a loud noise is emitted by the device indicating that a mistake is counted (Lafayette Instruments Company, Lafayette, Indianapolis, US). Participants were informed that the average person achieves five tracings with a minimum number of mistakes in the 5-minute time. The two tasks are used widely in

laboratory stress testing studies as they present important advantages. For example, they have been shown to induce robust biological responses and they seem to stimulate similar appraisals of involvement and engagement from participants across the social gradient (Hamer et al., 2010; Steptoe, Feldman, et al., 2002). The two tasks have been used in previous studies carried out by the Psychobiology Group at UCL (Hamer et al., 2010; Steptoe, Feldman, et al., 2002). The mirror tracing task is illustrated in Figure 4.2.

Figure 4.2. The mirror tracing task. This was one of the two stress tasks administered during the laboratory session of the Diabetes Study. Reprinted from "Biological Psychology" by S.M. Breedlove and N.V. Watson, 2013, Sunderland, Massachusetts, MA: Sinauer associates. Copyright (02.06.2020) by Oxford Publishing Limited. Reprinted with permission.

4.5 The Diabetes Study measures

4.5.1 Biological variables

Three inflammatory markers were assayed from all four blood samples. These included IL-6 (pg/ml), IL-1ra (pg/ml), and MCP-1 (pg/ml). Plasma IL-6 was assayed using a Quantikine high sensitivity two-site enzyme-linked immunosorbent assay from R & D Systems (Oxford, UK). The minimum limit of detection for IL-6 was between 0.016 pg/ml and 0.110 pg/ml. IL-1ra and MCP-1 were analysed in duplicate using fluorescent-labelled capture antibody beads from Millipore (Milliplex Human Cytokine/Chemokine kit, Millipore Corporation, US), and concentrations were measured using Luminex flow cytometer technology from Bio – Rad (Bio – Plex, Hercules, California, US). The minimum limit of detection for IL-1ra was 2.3 pg/ml and for MCP-1 was 1.2 pg/ml. The mean intra-assay coefficients of variation for IL-6, IL-1ra, and MCP-1 were 7.3%, 4.6% and 6.1%, respectively. The mean inter-assay coefficients of variation for the three markers were 7.7%, 6% and 12%, respectively.

5.3.1 Sociodemographic variables

Sociodemographic variables, collected by self-report, included age (years), sex (man, woman), educational level (no qualifications, up to O-levels, A-levels or Ordinary National Certificate, degree or above[46]), household income (<£20,000, £20,000 – 40,000, £40,000 – 60,000, >£60,000), ethnicity (White, Asian, Afro-Caribbean, other), and marital status (married, single, divorced or separated or widowed). Household

[46] These four educational level categories are equivalent to the US elementary school diploma, middle or junior high school diploma, high school or senior high school diploma, and university undergraduate certificate or above, respectively.

income was used as it is thought to be a better indicator of SES than personal income in older ages (Banks, Karlsen, & Oldfield, 2003).

5.3.1 Behavioural and physical health-related variables

Behavioural and physical health-related measures included current smoker status (no, yes), moderate or vigorous physical activity per week (hours), height (cm), weight (kg), waist (cm), hip (cm), body fat (%), HbA1c (%), medication use at the time of testing, and physical health-related quality of life. Smoking status and physical activity were recorded by self-report. Anthropometric measurements were carried out using standardised techniques with participants wearing light clothing. More specifically, height was measured to the nearest 0.1 cm using a stadiometer, weight was assessed using a Tanita scale (Tanita Corporation of America, Inc., Arlington Heights, IL), and BMI was calculated (kg/m2). Body fat % was estimated by bioelectrical impedance body composition analysis. Waist and hip measurements were carried out (using a tape measure) for the calculation of WHR (the waist circumference divided by hip circumference). HbA1c was assessed from the pre-task blood draw using standardised techniques and the sample was sent to The Doctor's Laboratory for testing.

Medication categories were oral anti-diabetic medication (yes, no), insulin or other injectable anti-diabetic medication (yes, no), aspirin (yes, no), beta blockers (yes, no), anti-hypertensive medication (yes, no), and cholesterol-lowering drugs (yes, no). Medication use was self-reported but also confirmed by inspection of the medication packaging on the testing day. Physical health-related quality of life was assessed by self-report using the physical health component of the Short Form (SF)-36 Health Survey (Ware & Sherbourne, 1992). The SF-36 is a self-reported 36-item survey of

patient health. The physical health dimension includes subscales relating to 1) physical functioning, 2) bodily pain, 3) role limitations due to physical health problems, and 4) general health perceptions. All the four subscales were used to calculate an average physical health-related quality of life score for each participant, with higher scores reflecting better physical health (scores can range from 0 to 100).

5.3.1 Mental health-related variables

Mental health-related variables included daily happiness and daily sadness, depressive symptoms, and mental health-related quality of life. Daily happiness was measured with ratings made at the end of each day for seven consecutive days prior to stress testing, using daily diaries. More specifically, participants were asked to report the extent to which they had been feeling happy on that particular day using a 5-point Likert-type scale (from 0 = not at all to 4 = a lot). An average happinesss score over the week was computed, with higher scores indicating greater daily happiness. The mean coefficient of variation over the week was 0.28, proposing a relatively small mean variability of data. Similarly, an average sadness score was calculated by aggregating ratings made at the end of each day for seven consecutive days before the laboratory testing. Participants were asked to report the extent to which they had been feeling sad on that day using a 5-point scale ranging from 0 (not at all) to 4 (a lot). Higher scores indicated greater daily sadness. The mean coefficient of variation over the seven days was 1.18.

For depressive symptoms, participants completed the revised version of the 20-item CES-D scale (Radloff, 1977). As described previously in Chapter 3, this is a validated questionnaire used to measure depressive symptomatology in the general population.

Items include statements such as 'I felt that everything I did was an effort' and 'I felt that I could not shake off the blues'. Responses can range from 0 (rarely or none of the time [less than 1 day]) to 3 (most of all the time [5-7 days]). Responses were summed and higher scores indicated greater depressive symptoms over the previous week.

Mental health-related quality of life was assessed using the mental health component of the SF-36 Health Survey (Ware & Sherbourne, 1992). The mental health dimension of the SF-36 includes subscales relating to 1) role limitations due to personal or emotional problems, 2) general mental health, 3) social functioning, and 4) energy, fatigue, or vitality. All the four subscales were used to calculate an average mental health-related quality of life score and higher scores were indicative of better mental health (scores can range from 0 to 100).

5.3.1 Task-related variables

Task-related variables included subjective stress throughout the session, task perceptions, and time of testing (morning, afternoon). Subjective stress was measured throughout the laboratory session: before the tasks, immediately after each of the tasks, and at 45 and 75 minutes post-task. Participants were asked 'how stressed do you feel at the moment' (pre-task), 'how stressed did you feel during the (Stroop, mirror tracing) task' (immediately post-task; responses for the two different tasks were averaged to compute an overall score), and again the question 'how stressed do you feel at the moment' (at 45 and 75 minutes post-task). Participants answered on a 7-point Likert-type scale (from 1 = not at all stressed to 7 = very stressed). Higher scores indicated greater subjective stress at the different time points.

Perceptions of task stressfulness, involvement, control, difficulty, and performance were also taken after each of the tasks, with responses taken on a 7-point Likert-type scale (from 1 = not at all stressful, involved, in control, difficult, well to 7 = very stressful, involved, in control, difficult, well). Responses relating to the two different tasks were averaged to create overall scores for task stressfulness, involvement, control, difficulty, and performance.

Finally, further variables were measured during the Diabetes Study including financial strain, neighborhood social cohesion, social support, medication adherence, loneliness, self-esteem, cynical hostility, optimism, as well as heart rate, cortisol, and cholesterol levels. These data were not used for the purposes of this thesis therefore they are not presented here.

Chapter 5. Study 2: Sex differences in IL-6 stress responses in people with T2D

Study 2 explored sex differences in inflammatory stress responses in people with T2D using data from the Diabetes Study. This chapter provides a brief overview of the study background followed by the methods and results of the study and a discussion of the findings. The results presented here have been the subject of a peer-reviewed publication in *Psychophysiology* (Panagi, Poole, Hackett, & Steptoe, 2019b).

5.1 Introduction

Increasing evidence from basic and clinical research shows that sex, as a fundamental biological factor and an intrinsic individual characteristic, is relevant to the modulation of the acute stress response. A 2017 review of animal studies demonstrated consistent differences in biological stress responses between males and females, highlighting brain anatomical differences that are relevant to the acute stress response as well as differences at the level of the reproductive axis/hormones across the lifespan which interact with the HPA axis at different levels (Novais, Monteiro, Roque, Correia-Neves, & Sousa, 2017). For example, females have bigger LC, the brain area that produces noradrenaline during the acute stress response (Pinos et al., 2001). Furthermore, in response to acute stress, oestrogens display an exacerbation effect on the HPA axis (Novais et al., 2017; Viau & Meaney, 1996). In contrast, testosterone was found to decrease adrenocorticotropin hormone and GC levels after stress in males (Viau & Meaney, 1996), revealing an inhibitory effect over the anterior pituitary that is reverted by gonadectomy of male rats (Seale, Wood, Atkinson, Harbuz, & Lightman, 2004). Indeed, several studies showed that exogenous replacement of estrogens leads

to an enhanced response of the HPA axis while testosterone leads to an inhibition (Lund, Munson, Haldy, & Handa, 2004; Viau, Bingham, Davis, Lee, & Wong, 2005).

As described in the introductory chapter of this thesis, several studies have found sex differences in the morbidity and mortality rate associated with T2D. For example, some inflammatory-mediated conditions, including CVD, are more frequently seen in ageing women with T2D compared with their male counterparts (Peters et al., 2014a, 2014b). Even though these sex-specific differences are of increasing clinical interest, the underlying mechanisms driving these differences are not entirely clear yet. Previous research has demonstrated that sex differences in disease and mortality risk are only partially explained by demographic, lifestyle, clinical, genetic, or hormonal factors (Espeland et al., 2018; Raparelli et al., 2017).

Laboratory studies in humans, involving middle-age and older participants, have suggested differences in inflammatory stress responses between women and men. These studies are described in more detail in Chapter 1. Overall, evidence to date derives mostly from healthy participants (four studies involved healthy participants and only one study involved patients with CAD) and supports that women show greater IL-6 stress responses compared with similarly-aged men (Edwards et al., 2006; Endrighi et al., 2016; Lockwood et al., 2016; Steptoe, Owen, et al., 2002; Sullivan et al., 2018). Interestingly, evidence on sex differences in inflammatory stress responsivity appears to be more consistent for older compared to younger participants, as described in Chapter 1.

The mechanisms that underpin difference morbidity rates between men and women with T2D are likely, to some extent, the result of sex differences in stress-related

inflammatory pathways. However, no previous studies have explored sex differences in inflammatory stress responsivity in this patient group. This study aimed to better understand the biological pathways explaining sex differences in T2D progression. The objective of this study was to investigate potential differences in IL-6 stress responses between older men and women with T2D. I hypothesised that women with T2D will exhibit larger IL-6 stress responses than men with diabetes.

5.2 Method

5.2.1 Participants

Participants from the Diabetes Study took part in this project of sex differences in IL-6 stress responses. Detailed information regarding the Diabetes Study participants and the procedure (such as information on sample calculation, exclusion criteria, recruitment procedures, pre-testing instructions, testing day procedures and the mental stress tasks) was reported in Chapter 4. For this project, data were analysed for 121 participants (76 men, 45 women) who provided full data on predictor variable and all covariates.

Out of 121 participants of the current study, 29 participants had missing data on at least one IL-6 measurement[47]. Sensitivity analysis was carried out to check for significant differences in participants' characteristics between those with full data on all four IL-6 measurements and those with missing data on at least one IL-6 measurement. Analysis was carried out using independent samples *t*-tests for

[47] Missing inflammatory data owned to technical issues in blood sampling. More precisely, as the majority of participants were obese, maintaining a functioning cannula for the whole duration of the laboratory session presented technical challenges. For example, the cannula failed part-way through the session for some participants. The research team had a protocol to not re-attempt blood draw in these participants so as to avoid causing psychological stress.

continuous variables and Chi-square tests for categorical variables. Results revealed marginally significant difference for BMI such as those participants with higher BMI were more likely to have missing data on IL-6 ($t(36.93) = -1.98$, $p = 0.055$, $d = 0.46$, 95% CI [-5.70, 0.07]). There were no other significant differences between those with and without missing IL-6 data (ps ≥ 0.067).

5.2.2 Study measures

5.2.2.1 Predictor variable: Sex

Self-reported information on sex (man, woman) was used as predictor variable in this study.

5.2.2.2 Outcome variable: IL-6 stress responses

Plasma IL-6 levels were measured at four time points throughout the laboratory session: pre-task, immediately post-task, at 45 minutes post-task, and at 75 minutes post-task. Three IL-6 change/delta (Δ) scores were calculated to reflect the mean difference/change in IL-6 levels from pre-task to the three post-task measurements: immediately post-task minus pre-task levels (Δ immediately post-task), 45 minutes post-task minus pre-task levels (Δ 45 minutes), and 75 minutes post-task minus pre-task levels (Δ 75 minutes). The three change scores were used as outcome variables in this study, with higher positive change scores indicating greater increases in IL-6 from pre- to post-task measurements. Chapter 4 describes the blood sample collection and IL-6 assay methods and presents information on the minimum limit of detection and intra-assay coefficients of variation for IL-6.

5.2.2.3 Covariates

Covariates were chosen based on previous studies which have indicated their influence on inflammatory (re)activity. These included age (Steptoe, Owen, et al., 2002), household income as an indicator of SES (Steptoe, Owen, et al., 2002; Stringhini et al., 2013), smoking status (Marsland et al., 2017), BMI (McInnis et al., 2014), and depressive symptoms (Howren, Lamkin, & Suls, 2009). Glucose control (as indexed by HbA1c), oral anti-diabetic medication, and insulin or other injectable anti-diabetic medication at the time of testing were also chosen to be included in the model as these could reflect different levels of diabetes severity. Additionally, as IL-6 shows some diurnal variation with increasing levels over the course of the day (Vgontzas et al., 2005), time of testing (morning, afternoon) was also included as a covariate. Detailed information on these measures is reported in Chapter 4.

Other variables such as ethnicity, marital status, educational level, moderate or vigorous physical activity per week, body fat %, WHR, and cardiovascular medication (anti-hypertensive medication, cholesterol-lowering drugs, aspirin, and beta blockers) use at the time of testing were also considered in the analyses. Subjective stress at baseline and task perception variables including task stressfulness, involvement, control, difficulty, and performance were also included in analyses. These further variables were used in univariate analyses in order to give a broader description of the characteristics of men and women in this sample. Some of these extra variables were considered in secondary analyses. Full details on these measures are provided in Chapter 4.

5.2.3 Statistical analysis

The distribution of IL-6 concentrations was positively skewed (skewness > 1 for all four IL-6 measurements [values between 1.27 and 1.63] and kurtosis values between 1.53 and 2.98). Therefore, log-n transformation was applied in all analyses. Log-n transformation corrected the skewness of the data (skewness between -0.30 and 0.27 for all four values after log-n transformation). Characteristics between men and women were compared using Chi-square tests for categorical variables and independent samples *t*-tests for continuous variables.

Significant main effects and interactions were initially examined using mixed model Analysis of Variance (ANOVA), with sex as the between-subjects factor and IL-6 levels at the four time points (pre-task, immediately post-task, 45 minutes post-task, and 75 minutes post-task) as the within-subjects factor. Significant interactions were further explored using mixed model Analysis of Covariance (ANCOVA). In this second (and main) model, the three IL-6 change scores were used as the within-subjects factor, reflecting the mean difference in IL-6 levels from pre-stress to immediately post-task (Δ immediately post-task), 45 minutes post-task (Δ 45 minutes), and 75 minutes post-task (Δ 75 minutes). To avoid overadjustment, only nine covariates were included: age, household income, smoking status, BMI, HbA1c, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, depressive symptoms, and time of testing. Since change scores account for initial, pre-stress levels, the three change scores rather than the four absolute values were used as outcome variables in the main analysis.

Secondary analyses were carried out to further control for potential confounders using mixed model ANCOVA. Moreover, secondary analyses were conducted to test for the potential effect of age in the magnitude of IL-6 stress responsivity in female participants using mixed model and repeated measures ANOVA.

Results are presented as means and standard deviations (SDs) for continuous variables or numbers and percentages for categorical variables. The level of significance was set at $p < 0.05$, though exact p values are reported throughout. Statistical analyses were carried out using SPSS version 24 (SPSS, Chicago, IL).

5.3 Results

5.3.1 Sample characteristics by sex

Data were analysed for 121 participants (37% women) who provided full data on sex and all covariates (age, household income, smoking status, BMI, HbA1c, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, depressive symptoms, and time of testing). Table 5.1 shows sample characteristics by sex and comparisons between sexes. Men and women in this study did not differ in socioeconomic characteristics including age ($p = 0.506$), household income ($p = 0.060$), marital status ($p = 0.070$), or educational level ($p = 0.726$). There was a significant difference in ethnicity, with more men than women being of Asian origin; $\chi^2(3) = 9.77$, $p = 0.021$, $V = 0.28$[48]. Participants were obese on average and women had slightly (but not significantly) higher BMI than men; $t(119) = -1.92$, $p = 0.057$, $d = 0.35$, 95% CI [-4.11, 0.06]. Additionally, women had higher body fat % ($t(66.98) = -11.51$, $p < 0.001$, d

[48] *V* stands for Cramer's *V*. It is used as an effect size indicator in r x c frequency tables. Effect sizes of 0.1, 0.3, and 0.5 are considered as small, medium, and large, respectively (Cohen, 1988).

= 2.27, 95% CI [-15.67, -11.04])[49]. On the other hand, men had marginally higher WHR than women ($t(118) = 1.98$, $p = 0.050$, $d = 0.36$, 95% CI [0.00, 0.06]). Men and women did not differ in other lifestyle-related variables such as smoking status ($p = 0.803$) or frequency of physical activity ($p = 0.889$). Moreover, similar diabetes-related characteristics were observed between sexes including no differences in HbA1c levels ($p = 0.700$) or anti-diabetic medication (for oral medication: $p = 0.878$; for insulin or other injectable medication: $p = 1.000$). Men, however, were more likely than women to take aspirin at the time of testing; $\chi^2(1) = 5.85$, $p = 0.016$, $\phi = -0.25$[50]. Depressive symptoms ($p = 0.875$), pre-stress subjective stress ($p = 0.441$), and time of testing ($p = 0.765$) did not differ between sexes.

[49] Body fat % is another measure of fitness level calculated as the total mass of fat (essential fat and storage fat) divided by total body mass, multiplied by 100. The percentage of essential body fat is greater for women compared to men, due to the demands of pregnancy and hormonal functions. Therefore, physiological ranges vary according to sex. Based on the American Council on Exercise, the average body fat % is 25 - 32% for women and 18 - 25% for men. Obesity is defined as body fat % > 32% in women and > 25% in men (Muth, 2009).

[50] ϕ stands for Phi. It is used as an effect size indicator in 2 x 2 frequency tables. Effect sizes of 0.1, 0.3, and 0.5 are considered as small, medium, and large, respectively (Cohen, 1988).

Table 5.1

Sample characteristics by sex (N = 121)

Characteristic	Men (*n* = 76) n (%) or M ± SD	Women (*n* = 45) n (%) or M ± SD	Sex differences: Effect size, *p value*
Age [years]	64.09 ± 7.35	63.20 ± 6.70	*d* = 0.13, *p* = 0.506
Ethnicity White Asian Afro-Caribbean Other	 61 (80.3) 10 (13.2) 4 (5.3) 1 (1.3)	 35 (77.8) 1 (2.2) 4 (8.9) 5 (11.1)	*V* = 0.28, *p* = 0.021
Household income [£] <£20,000 £20,000-40,000 £40,000-60,000 >£60,000	 31 (40.8) 17 (22.4) 8 (10.5) 20 (26.3)	 21 (46.7) 17 (37.8) 3 (6.7) 4 (8.9)	*V* = 0.25, *p* = 0.060
Marital status Married Single Divorced, separated or widowed	 45 (59.2) 13 (17.1) 18 (23.7)	 17 (37.8) 13 (28.9) 15 (33.3)	*V* = 0.21, *p* = 0.070
[a]Educational level No qualifications Up to O-levels A-levels or ONC Degree or above	 5 (6.8) 16 (21.6) 7 (9.5) 46 (62.2)	 3 (6.7) 6 (13.3) 5 (11.1) 31 (68.9)	*V* = 0.11, *p* = 0.726
Smoking status [smoker]	11 (14.5)	5 (11.1)	ϕ = -0.05, *p* = 0.803
[b]Moderate or vigorous physical activity per week [hours]	4.08 ± 8.19	3.89 ± 4.18	*d* = 0.03, *p* = 0.889
BMI [kg/m^2]	30.11 ± 5.13	32.14 ± 6.33	*d* = 0.35, *p* = 0.057
Body fat [%]	30.96 ± 4.58	44.32 ± 6.94	*d* = 2.27, *p* = <0.001
[c]WHR	1.01 ± 0.07	0.97 ± 0.11	*d* = 0.36, *p* = 0.050
HbA1c [%]	7.28 ± 1.36	7.38 ± 1.64	*d* = 0.07, *p* = 0.700
Oral anti-diabetic medication [yes]	62 (81.6)	38 (84.4)	ϕ = -0.04, *p* = 0.878
Insulin or other injectable anti-diabetic medication [yes]	9 (11.8)	6 (13.3)	ϕ = 0.02, *p* = 1.000

Characteristic	Men (*n* = 76) n (%) or M ± SD	Women (*n* = 45) n (%) or M ± SD	Sex differences: Effect size, *p value*
Anti-hypertensive medication [yes]	55 (72.4)	30 (66.7)	ϕ = -0.06, *p* = 0.647
Cholesterol-lowering drugs [yes]	57 (75.0)	37 (82.2)	ϕ = 0.08, *p* = 0.486
Aspirin [yes]	33 (43.4)	9 (20.0)	ϕ = -0.24, *p* = 0.016
Beta blockers [yes]	7 (9.2)	4 (8.9)	ϕ = -0.01, *p* = 1.000
Depressive symptoms [score]	11.60 ± 8.19	11.84 ± 8.28	*d* = 0.03, *p* = 0.875
Baseline subjective stress [score]	1.45 ± 0.82	1.58 ± 1.01	*d* = 0.14, *p* = 0.441
Time of testing [morning]	32 (42.1)	21 (46.7)	ϕ = -0.04, *p* = 0.765

Note. Differences between sexes were tested using independent samples *t*-tests for continuous variables and Chi-square tests for categorical variables. AM = after midnight; BMI = body mass index; HbA1c = glycated hemoglobin; kg/m^2 = kilogram per square metre; ln = log-n; M = mean; Min = minutes; N = number; n = number, ONC = ordinary national certificate; pg/ml = picogram per millilitre; SD = standard deviation; WHR = waist-to-hip ratio. [a]*n* = 119, [b]*n* = 111, [c]*n* = 120.

5.3.2 Sex differences in task perceptions

Task perception ratings showed that female participants perceived the tasks as more stressful than male participants; *t*(188) = -2.78, *p* = 0.006, *d* = 0.53, 95% CI [-1.29, -0.22]. There were no significant sex differences in perceived task involvement (*p* = 0.153), control (*p* = 0.798), difficulty (*p* = 0.074), or performance (*p* = 0.674).

Table 5.2

Perceived task appraisals by sex (N = 121)

Task-related variables	Men (*n* = 76) M ± SD	Women (*n* = 45) M ± SD	Sex differences: Effect size, *p* value
Stress [score]	4.25 ± 1.50	5.00 ± 1.33	*d* = 0.53, *p* = 0.006
Involvement [score]	5.24 ± 1.52	5.66 ± 1.59	*d* = 0.27, *p* = 0.153
Control [score]	2.50 ± 1.21	2.43 ± 1.47	*d* = 0.05, *p* = 0.798
Difficulty [score]	5.63 ± 1.07	5.99 ± 1.00	*d* = 0.34, *p* = 0.074
Performance [score]	2.23 ± 1.17	2.13 ± 1.31	*d* = 0.08, *p* = 0.674

Note. Sex differences in perceived task appraisals were tested using independent samples *t*-tests. M = mean; N = number; n = number; SD = standard deviation.

5.3.3 Sex differences in IL-6 stress responses

Plasma IL-6 concentrations increased significantly (time effect $F(2.44, 219.28) = 13.18$, $p < 0.001$, $\eta^2_p = 0.128$[51]) from pre-task ($\bar{x} = 0.53 \pm 0.06$) to 75 minutes ($\bar{x} = 0.69 \pm 0.06$) after stress. There was also evidence for a significant sex by time interaction effect ($F(2.44, 219.28) = 5.39$, $p = 0.003$, $\eta^2_p = 0.06$; see Figure 5.1), providing insight of sex differences in the magnitude of IL-6 responses to stress.

Pairwise comparisons showed significant differences in women at 45 and 75 minutes post-stress compared to pre-stress levels (45 minutes compared to pre-stress: $p < 0.001$, 95% CI [0.09, 0.25]; 75 minutes compared to pre-stress: $p < 0.001$, 95% CI [0.14, 0.36]) and compared to immediately post-task values (45 minutes compared to immediately-post task: $p = 0.002$, 95% CI [0.05, 0.22]; 75 minutes compared to immediately-post task: $p < 0.001$, 95% CI [0.11, 0.31]). In men, IL-6 increases were not significant ($ps > 0.087$). There were no significant sex differences in pre-task ($p = 0.258$)

[51] η^2_p stands for partial eta-squared and is a measure of effect size used in ANOVA and ANCOVA models. Values for small, medium, and large effect sizes are 0.01, 0.06, and 0.14, respectively (Stevens, 2002).

or post-task (*p*s ≥ 0.405) IL-6 values between women and men (checked with independent samples *t* tests).

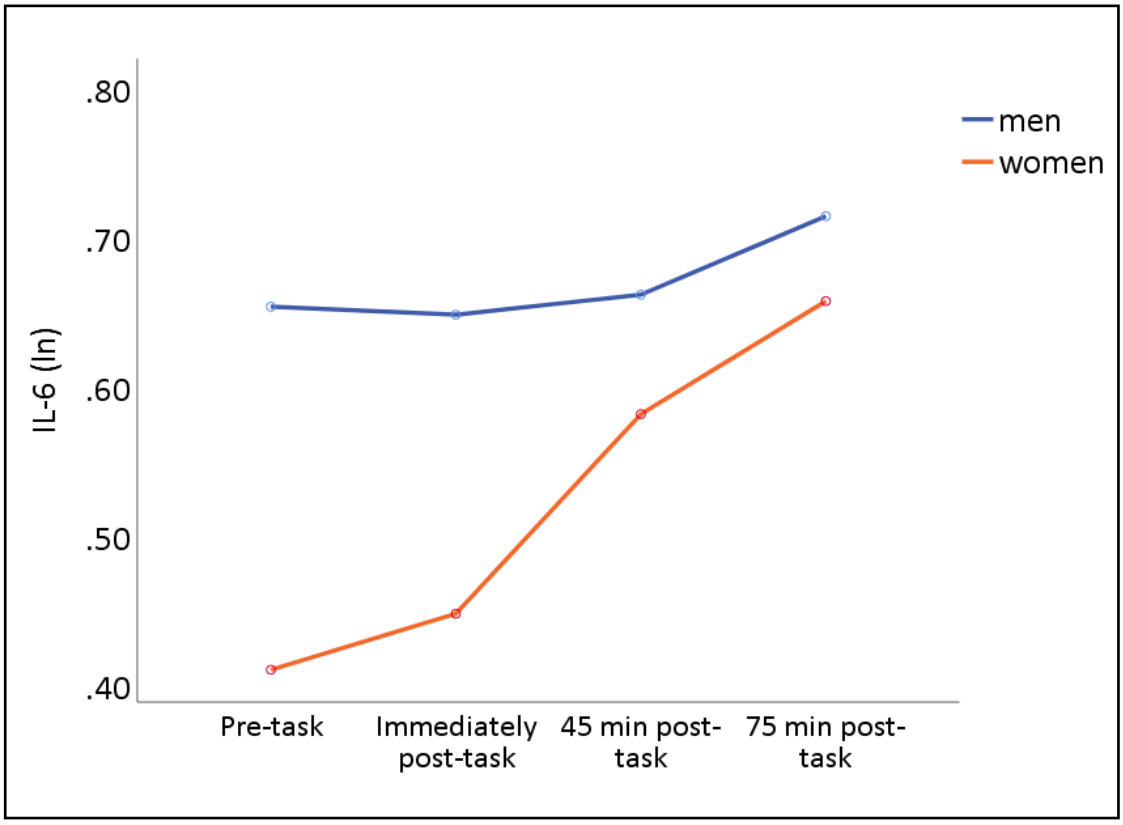

Figure 5.1. Mean plasma interleukin-6 values (log-n) at four time points in men and women with type 2 diabetes (*n* = 92). Results are unadjusted. IL-6 = interleukin-6; ln = log-n; min = minutes; *n* = number.

The ANCOVA model including sex as the between-subjects factor and the three IL-6 change scores (Δ immediately post-task, Δ 45 minutes, Δ 75 minutes) as the within-subjects factor showed a significant sex by time interaction after adjusting for covariates. Specifically, IL-6 responses differed significantly between men and women after controlling for age, household income, smoking, BMI, HbA1c, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, depressive symptoms, and time of testing ($F(2, 162) = 4.19$, $p = 0.019$, $\eta^2_p = 0.05$). Significant differences between women and men occurred at 45 minutes when women exhibited greater IL-6 increases compared to men (Δ 45 minutes for women: $\bar{x} = 0.17 \pm 0.04$ versus men: $\bar{x} = 0.01 \pm 0.03$, $p = 0.002$, 95% CI [0.65, 0.27]). This difference was sustained at 75 minutes (Δ 75 minutes for women: $\bar{x} = 0.26 \pm 0.06$ versus men: $\bar{x} = 0.06$

± 0.04, p = 0.004, 95% CI [0.06, 0.34]). The adjusted IL-6 changes in the two sexes are depicted in Figure 5.2

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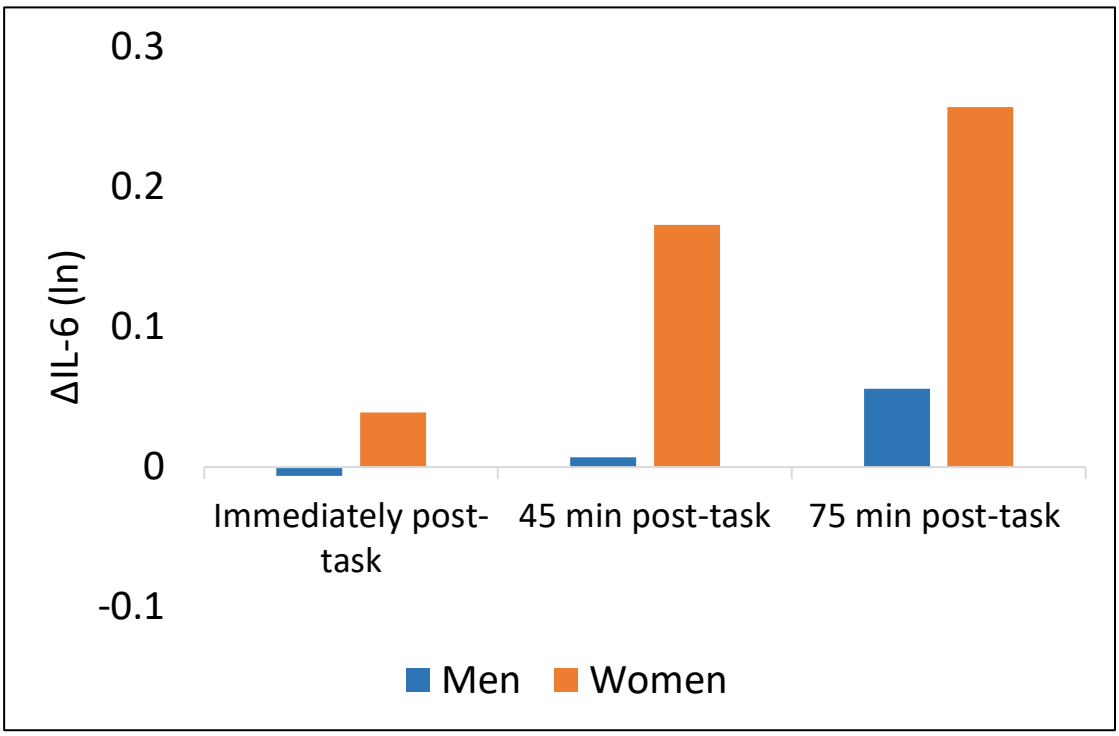

Figure 5.2. Mean changes in interleukin-6 (log-n) from pre-stress to the three time points after stress in men and women with type 2 diabetes (*n* = 92). Values are adjusted for age, household income, smoking status, body mass index, glycated hemoglobin, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, depression symptoms, and time of testing. IL-6 = interleukin-6; ln = log-n; min = minutes; *n* = number; Δ = delta (change) score.

Using a secondary model, analyses were carried out to control for the potential effects of ethnicity, body fat %, WHR, aspirin intake, and stress perception. These variables were selected as they were associated with sex in univariate analyses. These were included in a separate ANCOVA model and the sex by time interaction remained significant ($F(1.78, 147.39) = 4.05$, $p = 0.023$, $\eta^2p = 0.05$). Despite being not statistically significant, pre-stress IL-6 levels were higher for men than women, as illustrated in Figure 5.1. Adjusting for pre-stress IL-6 levels attenuated the sex by time interaction ($F(1.84, 163.89) = 2.69$, $p = 0.075$, $\eta^2p = 0.03$). Finally, additional analysis was carried out to test for the effect of age in the magnitude of IL-6 stress responses in women. IL-6 levels over time did not interact with age in women ($F(2.17, 77.95) = 0.06$, $p = 0.955$, $\eta^2p = 0.002$). Examining IL-6 levels over time separately for younger and older

women (≤ 55, > 55 years) revealed a greater main effect of time for older women (F(3, 21) = 4.74, p = 0.011, η^2p = 0.40) versus younger women (F(2.08, 60.41) = 8.88, p < 0001, η^2p = 0.23).

5.4 Discussion

This study explored differences between sexes in IL-6 stress responses in older-aged men and women with T2D. Women with T2D exhibited significantly larger IL-6 increases from pre-task to 45 minutes and 75 minutes post-task compared to men. Significant changes in women were detectable 45 minutes after the end of the two tasks, with IL-6 levels continuing to rise reaching their highest levels at 75 minutes post-task. In contrast, IL-6 changes in men were more delayed, showing observable, albeit not significant increases at 75 minutes post-task. Significant differences between women and men in IL-6 responses were independent of age, household income, smoking status, BMI, HbA1c, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, depressive symptoms, and time of testing. Findings from this study shed light on different inflammatory stress response processes between older women and men with T2D.

The lack of previous studies in participants with T2D or pre-diabetes does not allow comparisons with people with similar clinical characteristics. Nevertheless, these findings are consistent with three previous studies on healthy, middle- and older-aged participants (Endrighi et al., 2016; Lockwood et al., 2016; Steptoe, Owen, et al., 2002), and one study in patients with CAD (Sullivan et al., 2018). Results from Study 2 contradict findings from one previous study with smaller sample size (N = 40) and younger participants (mean age 21 years old) that reported delayed IL-6 response for

women compared to men (Edwards et al., 2006). As mentioned previously in Chapter 1, one hypothesis is that the post-menopausal reduction in reproductive hormones in women might contribute to sex differences in IL-6 stress responses that are more consistently observed in older participants. It was not possible to directly test this hypothesis because menopausal status data were not collected during the Diabetes Study. Nevertheless, menopause usually occurs between 45 and 55 years of age, and the mean age for a woman to reach the menopause in the UK is 51 (National Health System [NHS] Choices UK, 2018). The mean age of the women in this study was 63 years old (SD = 6.70). Only three out of the 45 women in this study were ≤ 51 years old and eight of them were ≤ 55 years old. Hence, it is conceivable that the great majority of women participants were in the post-menopause phase. In secondary analyses, a greater main effect of time was observed for older (> 55) versus younger women (≤ 55). The possibility that post-menopausal women with T2D may exhibit greater inflammatory stress responses than pre-menopausal women with T2D warrants direct testing in future studies.

Stress-related mediators, including IL-6, cortisol, and catecholamines act synergistically to maintain homeostasis (Karatsoreos & McEwen, 2010) and therefore, human studies on sex differences in cortisol and catecholamines responsivity would be informative. With regards to sex differences in cortisol reactivity, animal studies have demonstrated higher GC stress responsivity for females compared to males (Seale, Wood, Atkinson, Bate, et al., 2004), possibly because of oestrogens displaying an exacerbation effect on the HPA axis (Novais et al., 2017) as well as due to a sex-dimorphic expression of GC receptors in the brain (Kitraki, Kremmyda, Youlatos,

Alexis, & Kittas, 2004). With regard to catecholamines stress responsivity, a 2019 review of sex differences in arousal systems that integrated both human and animal studies suggested that the female LC area of the brain has a greater capacity to synthesise and release noradrenaline in target regions due to its bigger size and morphological characteristics. Along with the oestrogen-related increase in noradrenaline under stress, these brain differences could increase biological stimulation, including inflammatory arousal, in women in response to acute stress (Bangasser, Eck, & Ordones Sanchez, 2019). Future studies need to examine how the interaction of these factors may influence sex differences in inflammatory stress responsivity in people with T2D.

Interestingly, a previous study showed that the sensitivity of immune cells to the anti-inflammatory effects of GCs rises one hour after acute stress in men but not in women, even though men and women had similar free cortisol stress responses (Rohleder, Schommer, Hellhammer, Engel, & Kirschbaum, 2001). Such sensitivity in men may facilitate a sufficient inhibition of IL-6 and other pro-inflammatory cytokines production, and therefore terminate the inflammatory response timely. However, it unknown whether these cellular sensitivity differences can be generalised to people with T2D. Additionally, in this previous study of Rohleder and colleagues (Rohleder et al., 2001) men and women were exposed to a psychosocial stressor (the TSST) instead of a mental stressor. As mentioned previously on page 62, the TSST has a strong social evaluation component and is characterised by increased uncontrollability, therefore is more likely to induce elevations in cortisol secretion than mental stressors. Therefore, it is unlikely that mental stressors activate cortisol in the same way as a

psychosocial stressor; thus, the study by Rohleder and colleagues (Rohleder et al., 2001) is not directly comparable to this study of sex differences in IL-6 stress responsivity.

Men and women of the study shared a similar sociodemographic, behavioural, and clinical profile. Specifically, men and women did not differ in key characteristics including smoking status, frequency of physical exercise, glucose levels, anti-diabetic or cardiovascular medication use, or depressive symptoms. Previous studies showed that women with diabetes remain less likely to achieve high-density lipoprotein cholesterol targets, have a higher prevalence of obesity (Franzini et al., 2013; Go et al., 2013; Ogden, Carroll, Kit, & Flegal, 2014), and have a lower frequency of aspirin use than men (Winston, Barr, Carrasquillo, Bertoni, & Shea, 2009). Indeed, significant differences between sexes were found in body fat % and aspirin use, but when including these variables in a secondary model the significant sex by time interaction effect was maintained. Whether existing sex differences in IL-6 responses are magnified by sex differences in sociodemographic, lifestyle, or clinical factors requires consideration.

Interestingly, women rated the tasks as being more stressful than men did. It is plausible that the effect of the two tasks on participants' mood was reflected in biological activation. A laboratory stress testing study with middle-aged, healthy participants found that greater increases in anxiety after stress predicted larger increases in IL-6 (Carroll et al., 2011). Nevertheless, including stress perception in a secondary model did not alter the significant sex by time interaction. Also, the stress perception by time interaction effect was non-significant. It is also possible that

different stress paradigms could have resulted in different stress appraisals and/or different inflammatory reactivity patterns between women and men. In the recent meta-analysis by Marsland et al. (Marsland et al., 2017) the magnitude of IL-6 responsivity did not vary as a function of task type (IL-6 responses were compared for social stressors versus other stressors). However, analyses were carried out separately for men and women, therefore they did not allow direct comparisons between sexes related to the effects of different task types.

Another possible explanation of the sex differences observed in this study would be that men and women have different levels of background chronic stress, since background chronic stress has long been recognised as a primary influencer of biological (re)activity (McEwen, 1998). The Diabetes Study lacks information on stressful life events but sex differences in depressive symptoms scores were tested in univariate analyses as an indicator of chronic stress exposure. Depressive symptoms score did not differ in women and men, and the IL-6 response difference between sexes was sustained despite inclusion of depressive symptoms as a covariate in main analysis.

The clinical impact of IL-6 stress responsivity in people with T2D remains unclear. In this study, IL-6 concentrations were somewhat higher in men versus women at all four time points, although these differences in absolute levels were not significant. Consequently, despite greater IL-6 changes in women, female participants did not end up with greater IL-6 concentrations than male participants overall. It is not yet certain whether the heightened stress responsivity of women coupled with lower absolute levels or the higher absolute values among men coupled with smaller changes post-

stress is more hazardous to health in the long-term since no follow-up studies have yet tested the longitudinal association between IL-6 stress responses and health outcomes in people with T2D (this link is examined in Study 4, Chapter 8). It is also plausible that these different response patterns bias women and men with T2D towards different pathologies since older women with T2D are rendered more susceptible to macrovascular complications while men with T2D are more prone to microvascular complications, as described previously in Chapter 1. Notably, evidence from laboratory stress testing studies with a follow-up design supports that heightened inflammatory (including IL-6) stress responsivity is associated with the development of CVD risk factors (Brydon & Steptoe, 2005; Ellins et al., 2008; Ellins et al., 2017; Steptoe et al., 2016) and depressive symptoms (Aschbacher et al., 2012) in initially healthy participants. However, it is not yet known whether the same conclusions can be drawn for people with T2D. Study 4 presented in Chapter 8 addresses this gap by investigating the links between IL-6 stress responsivity and health outcomes in people with T2D.

Finally, although the inflammatory response has been recognised as an integral part of the stress response (Chrousos, 1998; Marsland et al., 2017; Steptoe et al., 2007), IL-6 is secreted from multiple sources, such as the liver (Heinrich, Castell, & Andus, 1990) or from muscle tissue during exercise (Pedersen, 2012; Shephard, 2002), and these responses are not part of the inflammatory response. However, simultaneous increases in IL-6 and other pro- or anti-inflammatory factors, such as MCP-1 and IL-1ra, have been reported in individual laboratory stress studies (Hackett, Hamer, Endrighi, Brydon, & Steptoe, 2012; Steptoe, Willemsen, Owen, Flower, & Mohamed-

Ali, 2001), including the Diabetes Study (Panagi et al., 2019a). These results suggest a simultaneous upregulation of inflammatory factors after laboratory stress and therefore support the notion that IL-6 activation found in this study represents changes within the inflammatory signaling cascade.

This study has strengths and limitations. Firstly, this is a relatively large laboratory study. Nevertheless, full biological data were collected for only about three quarters of the sample due to practical difficulties in blood sampling during the insertion of the cannula in some participants. These difficulties were mostly related to obesity. Additionally, this study involved a relatively long post-task blood sampling period. However, a more extended sampling could have provided additional detail particularly in view of evidence of IL-6 responses may continue to increase beyond 75 minutes post-task (Marsland et al., 2017). A wide range of covariates and potential confounders were taken into account including sociodemographic, behavioural, clinical, and psychological factors. The sole focus on IL-6 provides a limited perspective regarding stress-related biological functioning in men and women with T2D. Nevertheless, we recently showed that it is valuable to test IL-6 stress responsivity in people with T2D versus other inflammatory markers. Specifically, we found that IL-1ra and MCP-1 do not increase in response to mental stress in the laboratory in this patient group, in contrast to IL-6 which shows the expected increases (Panagi et al., 2019a). This study, Study 3, is described in Chapter 6. Plasma catecholamines were not assessed as venous measures of these factors can be unreliable indicators of the SNS activation. Additionally, the half-life of catecholamines in plasma is 1-2 minutes and venous concentrations in the forearm have been shown to be primarily due to

local muscle activity (Hjemdahl, 1993). Therefore, in order to assess the dynamic sympathetic function reliably would have required complex, invasive, and costly techniques that were not included in this protocol. Despite not being statistically significant, pre-task IL-6 levels were slightly higher in men than women in this study. It might be more difficult to observe increases in levels when initial values are already elevated (the so-called 'ceiling effect'), but this scenario is unlikely to have happened in this study since pre-task values were not very high in an absolute sense. Adjustment for pre-stress IL-6 levels in secondary analysis attenuated the sex by time interaction. These findings need to be replicated in future studies. Furthermore, given the differences in IL-6 (re)activity between people with T2D and healthy individuals (Steptoe, Hackett, et al., 2014), direct comparisons of sex differences in T2D and a healthy control group would be of interest in future work. Participants of this study were middle-aged and older men and women with T2D and without a history or a doctor diagnosis of CHD, inflammatory diseases, allergies, or mood disorders. They were recruited from London and most of them were of white European ethnicity; thus, it is unknown whether similar patterns would emerge among other cohorts.

Taken together, different inflammatory stress response pathways are present in men and women with T2D, with women producing larger IL-6 stress increases compared to men. Sex differences in the magnitude of IL-6 stress responses might explain the different levels of inflammatory-related disease vulnerability in this population. This hypothesis is directly tested in Study 4 (Chapter 8).

Chapter 6. Study 3: Daily happiness and inflammatory stress responses in people with T2D

Study 3 examined the role of subjective well-being in inflammatory stress responses in people with T2D, using data from the Diabetes Study. This chapter provides an overview of the study background followed by the methods and results of the study and a discussion of the findings. The results presented in this chapter have been the subject of a peer-reviewed publication in *Annals of Behavioural Medicine* (Panagi et al., 2019a).

6.1 Introduction

Evidence from longitudinal studies suggests that subjective well-being, particularly hedonic-well-being, is a protective factor for physical disability, cognitive decline, morbidity, and mortality among older adults (Chida & Steptoe, 2008; Martin-Maria et al., 2017; Steptoe, 2019a). The impact of hedonic well-being on health and longevity may be even more important in populations at increased risk of ill-health and premature mortality, such as those with T2D. In people with T2D, hedonic well-being and other positive constructs have been related to superior physical health outcomes in the long-term. More specifically, findings from longitudinal studies have shown that stress resilience and quality of life are associated with lower HbA1c in people with T2D (Yi et al., 2008), and factors such enjoyment of life, life satisfaction, and self-efficacy are linked with fewer diabetes complications and reduced risk of heart disease and all-cause mortality, independent of covariates (Celano et al., 2013; Huffman et al., 2015; Moskowitz et al., 2008).

As mentioned in the introductory chapter of this thesis, health behaviours do not fully explain the associations between hedonic well-being and longevity (Chida & Steptoe, 2008; Giltay et al., 2006; Kubzansky & Thurston, 2007; Moskowitz et al., 2008; Ostir et al., 2000). Therefore, direct biological mechanisms have been suggested to play a role. Indeed, hedonic well-being has been linked with a more adaptive biological profile, including reduced inflammatory (re)activity (Steptoe, Dockray, & Wardle, 2009). In particular, in a previous study of 2,873 healthy adults, daily happiness, as assessed using EMA (repeated measurements over one day in naturalistic environments), was inversely associated with IL-6 concentrations (Steptoe et al., 2008). Moreover, a nationally representative study in the US demonstrated a significant association between positive events over eight consecutive days and reduced IL-6 levels (Sin, Graham-Engeland, & Almeida, 2015). Similarly, greater decreases in happiness on stressful days were predictive of elevated IL-6 levels (Sin, Graham-Engeland, Ong, & Almeida, 2015). Additionally, in a study of 210 participants with chronic heart failure, lower positive affect was associated with increased IL-6 levels (Brouwers et al., 2013). Cross-sectional associations between lower IL-6 concentrations and other positive factors, such as purpose in life, positive social relationships, and optimism, have also been reported in previous studies (Friedman, Hayney, Love, Singer, & Ryff, 2007; Ikeda et al., 2011). Interestingly, associations were sustained after adjustment for depressive symptoms in some studies (Brouwers et al., 2013; Steptoe et al., 2008). As reported previously in Chapter 1, an important advantage of well-being studies is statistical adjustment for ill-being as it adds value to the distinct, not opposite, effect of well-being on biology and health. Other inflammatory markers, such as IL-1ra and MCP-1 are also relevant to psychological factors. For example, both IL-1ra and MCP-1

have been linked with loneliness in older women (Hackett et al., 2012), and elevated MCP-1 levels have been associated with increased depressive symptoms (Rajagopalan et al., 2001; Suarez, Krishnan, & Lewis, 2003) and chronic psychosocial stress (Asberg et al., 2009). Nevertheless, these previous studies are limited by the fact that they only captured basal inflammatory activity as measurements were only taken under resting conditions, providing no details on the role of well-being on the dynamic inflammatory reactivity to stress.

Albeit scarce, laboratory stress testing studies have provided consistent findings on the role of subjective well-being and related attributes on circulating inflammatory stress responsivity. Specifically, greater daily happiness, self-compassion, and maintaining a positive outlook (characterised by positive affect and cognition) during the stress tasks have been linked with smaller inflammatory increases following laboratory stress (Aschbacher et al., 2012; Breines et al., 2014; Steptoe et al., 2005), after adjusting for covariates. Details from these studies are presented in Chapter 1. Two of the three previous studies (Aschbacher et al., 2012; Breines et al., 2014) tested IL-6 stress responses but included a small sample size (< 41 participants). The study by Steptoe and colleagues (Steptoe et al., 2005) involved a larger sample size (N = 226) and a naturalistic measurement of happiness over the day but effects on fibrinogen reactivity were not controlled for negative affect (e.g. daily sadness). Previous laboratory stress testing studies have also shown negative factors, such as loneliness, to be associated with greater IL-1ra stress responses in healthy, middle-aged women, further supporting the direction of association between psychological factors and inflammatory reactivity (Hackett et al., 2012). To the best of my knowledge, none of

previous studies have tested associations between subjective well-being and IL-1ra or MCP-1 stress responses.

Elevated inflammatory (re)activity has been prospectively associated with increased illness risk. As mentioned previously in Chapter 1, both absolute inflammatory levels and inflammatory stress responses are of clinical interest. For example, increased IL-6 levels have been prospectively linked to macrovascular events and early deaths in those with established T2D (Lowe et al., 2014), and increased inflammatory stress responses in the laboratory have been associated with cardiovascular risk factors and depressive symptoms at follow-up in initially healthy participants (Brydon & Steptoe, 2005; Ellins et al., 2008; Ellins et al., 2017; Steptoe et al., 2016). Apart from IL-6, heightened basal levels of IL-1ra and MCP-1 are also relevant to T2D pathology. For example, increased IL-1ra and MCP-1 concentrations have been prospectively linked to newly-diagnosed CHD in initially healthy adults (Danesh et al., 2008; Herder et al., 2006; Tang et al., 2007; van Minkelen et al., 2009). These findings are particularly relevant to people with T2D because of the greater vulnerability to CHD in this population. Therefore, efforts should be made in order to better understand the factors that are linked to decreased inflammatory (re)activity in this patient group.

Reduced inflammatory (re)activity might be a mechanism through which hedonic well-being protects against future disease risk and mortality in people with established T2D. Considering the lack of research in this patient group, the aim of this study was to explore the role of daily happiness in inflammatory (re)activity in people with T2D. The objective of this study was to examine the relationship between daily happiness and three inflammatory factors, IL-6, IL-1ra, and MCP-1, before and after laboratory

stress testing in people with T2D. It was hypothesised that happier participants will have lower IL-6, IL-1ra, and MCP-1 levels before and after stress exposure and will show smaller IL-6, IL-1ra, and MCP-1 responses post-stress.

Daily happiness ratings were selected as the measure of hedonic well-being in the current study. More precisely, happiness ratings were recorded once a day (every evening, before going to bed) for seven consecutive days prior to the laboratory session. This method was used to provide an aggregated measure of daily happiness over the week. This strategy has been introduced by Cohen and colleagues (Cohen, Doyle, Turner, Alper, & Skoner, 2003) and has been used more recently (Bostock, Hamer, Wawrzyniak, Mitchell, & Steptoe, 2011). Previous studies have indicated that positive emotional factors assessed using naturalistic methods form wider associations with biological stress responses compared with a retrospective measurement of affect over the previous week using a standardised mood scale or questionnaire (Bostock et al., 2011). Furthermore, repeated measures that are taken under naturalistic environments might provide a more reliable estimate of happiness that is not biased by transient moods on a single day or recall bias, as well as involve a smaller burden for participants compared with EMA-derived measures (Steptoe et al., 2005).

6.2 Method

6.2.1 Participants

Participants from the Diabetes Study took part in this project of daily happiness and inflammatory processes. Full information regarding the Diabetes Study is provided in Chapter 4. For this study, data were analysed for 122 participants who provided full

data on exposure variable and all covariates. Twenty-seven out of 122 participants had missing data for at least one IL-6 measurement, 27 participants for at least one IL-1ra measurement, and 26 participants for at least one MCP-1 measurement, owing to blood sampling difficulties related to obesity. Sensitivity analysis was conducted to test for significant differences in participants' characteristics between those with full data on inflammatory markers and those with missing data on at least one marker and for at least one time point. Sensitivity analysis was carried out using independent samples *t*-tests for continuous variables and Chi-square tests for categorical variables. As expected, participants with higher BMI were more likely to have missing data on inflammatory markers ($t(38.75) = -2.50$, $p = 0.017$, $d = 0.57$, 95% CI [-6.14, -0.06]) but no other significant differences were detected between those with and without missing data on inflammatory markers ($ps \geq 0.091$).

6.2.2 Study measures

6.2.2.1 Exposure variable: Daily happiness

Daily happiness was measured using ratings made at the end of each day (using a daily diary) for seven consecutive days prior to the laboratory session. Participants reported the extent to which they had been feeling happy on that day using a 5-point Likert-type scale from 0 (not at all) to 4 (a lot). The mean daily happiness score over the week was calculated to be used as the exposure variable in this study. Items were assessed using the continuous range of scores, with higher scores indicating greater daily happiness. The mean coefficient of variation over the week was 0.28 suggesting a relatively small data variability on average.

6.2.2.2 Outcome variables: IL-6, IL-1ra, MCP-1

Plasma inflammatory markers were measured at four time points: pre-task, immediately post-task, at 45 minutes post-task, and at 75 minutes post-task. For each marker, four absolute scores and three change scores were used as outcome variables. The four absolute scores were the mean values at the four time points. The three change scores were calculated as the mean difference in markers from pre-task to the three post-task measurements: immediately post-task minus pre-task levels (Δ immediately post-task), 45 minutes post-task minus pre-task levels (Δ 45 minutes), and 75 minutes post-task minus pre-task levels (Δ 75 minutes). Higher positive change scores in each marker indicated greater increases from pre- to post-task measurements. Detailed information on the blood sample collection and assay methods, the minimum limit of detection for each marker, and intra- and inter-assay coefficients of variation are presented in Chapter 4.

6.2.2.3 Covariates

Covariates were selected *a priori*, based on previous research indicating their association with inflammatory (re)activity: age (Steptoe, Owen, et al., 2002), sex (Endrighi, Hamer, & Steptoe, 2016; Lockwood, Marsland, Cohen, & Gianaros, 2016; Steptoe, Owen, et al., 2002), household income (Stringhini et al., 2013), smoking status (Marsland et al., 2017), BMI (McInnis et al., 2014), oral anti-diabetic medication, insulin or other injectable anti-diabetic medication (Steptoe et al., 2007), and time of testing (Vgontzas et al., 2005). Additionally, in univariate analyses I found that daily happiness was marginally associated with marital status ($p = 0.054$), therefore marital status was also included as a covariate. To test whether the association between daily

happiness and inflammatory (re)activity is independent of negative affect, significant findings were further adjusted for daily sadness in sensitivity analysis. These measures were described in more detail in Chapter 4.

Other measures included in univariate analyses were ethnicity, educational level, hours of moderate or vigorous physical activity per week, HbA1c, and cardiovascular medication (anti-hypertensive medication, cholesterol-lowering drugs, aspirin, and beta blockers) at the time of stress testing. These further variables were chosen in order to give a broader description of the characteristics of the study sample. Anti-hypertensive medication or beta blocker use were not significantly associated with inflammatory levels or stress responses in preliminary analyses; therefore, these factors were not included as covariates. Subjective stress levels throughout the session (pre-task, immediately post-task, 45 minutes post-task, and 75 minutes post-task) were also assessed in order to verify the stressful nature of the stress tasks. Full information on these variables is reported in Chapter 4.

6.2.3 Statistical analysis

MCP-1 values were normally distributed but the distribution of IL-6 and IL-1ra values were skewed; thus, log-n transformation was applied (all IL-6 and IL-1ra values presented in the Results section are transformed, except those presented in Table 6.2 for ease of interpretation). As reported previously in Chapter 5, the distribution of IL-6 concentrations was positively skewed (skewness > 1 for all four IL-6 measurements [values between 1.27 and 1.63] and kurtosis values between 1.53 and 2.98). Log-n transformation corrected the skewness of the data (skewness between -0.30 and 0.27 for all four IL-6 values after log-n transformation). The distribution of IL-1ra

concentrations was positively skewed (skewness > 1 for all four IL-1ra measurements [values between 1.27 and 2.01] and kurtosis values between 1.60 and 5.31). Log-n transformation corrected the skewness of the data (skewness was between 0.12 and 0.40 for all four IL-1ra values after log-n transformation).

Univariate analyses were first conducted to check for associations between daily happiness and sample characteristics using Pearson's *r* correlations for continuous variables and independent samples *t*-tests and one-way between-subjects ANOVA for categorical variables. Univariate analyses were also carried out to test associations between pre-task inflammatory markers (absolute levels) and their patterns of responsivity (change scores) using Pearson's *r* correlations.

To test for the effects of stress tasks on subjective stress and inflammatory levels, subjective stress ratings and inflammatory levels were examined across the four time points (pre-task, immediately post-task, 45 minutes post-task, and 75 minutes post-task) using one-way repeated measures ANOVA.

In main analyses, the association between daily happiness and inflammatory *levels* was tested using multivariable linear regressions on the pre-task and post-task absolute values for all three inflammatory markers (four absolute levels for each inflammatory marker). The association between daily happiness and inflammatory *stress responses* was tested using multivariable linear regressions on the mean changes between pre-task and the three post-task values for all three inflammatory markers (three change scores for each inflammatory marker). Separate regression analyses were conducted for each inflammatory marker and for each time point (resulting in 21 separate regression models). Models were adjusted for age, sex,

household income, marital status, smoking status, BMI, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, and time of testing. Significant results were further adjusted for daily sadness in sensitivity analysis.

Results are reported as means and SDs or standard errors (SEs) for continuous variables or numbers and percentages for categorical variables. Data from the regression analyses are reported as unstandardised coefficients (*B*), *p* values, and 95% CIs. The level of significance was set at $p < 0.05$, though exact *p* values are reported throughout. Statistical analyses were carried out using SPSS version 24 (SPSS, Chicago, IL).

6.3 Results

6.3.1 Sample characteristics and associations with daily happiness

Data were analysed for 122 participants (36% women) who provided full data on predictor variable (daily happiness) and all covariates (age, sex, household income, marital status, smoking status, BMI, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, and time of testing). Table 6.1 presents characteristics of the sample and associations with mean daily happiness score. Participants were 63.67 (SD = 7.02) years old on average, mostly of White ethnicity (80%), and with relatively low incomes (61% < £40.000 household annual income). The majority of participants (64%) held a degree or above. The mean BMI was within the obese range (30.54 kg/m^2, SD = 5.59) and the mean HbA1c was 7.32% (SD = 1.49; equals to 56.50 mmol/mol, SD = 13.93). The majority of participants were taking oral anti-diabetic medication (80%), anti-hypertensive medication (70%), and cholesterol-lowering drugs (77%).

The mean daily happiness score was 2.61 (SD = 0.87), with scores ranging from 0 to 4. One participant had the minimum score of 0 and eight participants scored 4. Daily happiness was not related to age (p = 0.643), sex (p = 0.868), ethnicity (p = 0.376), household income (p = 0.678), or educational level (p = 0.345). The association between happiness and marital status approached significance ($F(2, 119) = 2.99$, p = 0.054, η^2_p = 0.05). No significant associations were found between happiness and smoking status (p = 0.315), physical activity (p = 0.162), BMI (p = 0.463), HbA1c (p = 0.173), time of testing (p = 0.576), or medication use (for oral anti-diabetic medication: p = 0.430; for insulin or other injectable anti-diabetic medication: p = 0.120; for anti-hypertensive medication: p = 0.330; for cholesterol-lowering drugs: p = 0.117; for aspirin: p = 0.115; for beta blockers: p = 0.193). A significant negative correlation between daily happiness and daily sadness was observed ($r(118) = 0.34$, $p < 0.001$).

Table 6.1

Sample characteristics and associations with daily happiness (N = 122)

Characteristic	n (%) or M ± SD	Associations with happiness: Effect size, *p* value
Daily happiness [score]	2.61 ± 0.87	-
Age [years]	63.67 ± 7.02	$r = 0.04$, $p = 0.634$
Sex [women]	44 (36.1)	$d = 0.03$, $p = 0.868$
Ethnicity		$\eta^2_p = 0.03$, $p = 0.376$
White	97 (79.5)	
Asian	12 (9.8)	
Afro-Caribbean	7 (5.7)	
Other	6 (4.9)	
Household income [£]		$\eta^2_p = 0.01$, $p = 0.678$
<£20,000	51 (41.8)	
£20,000-40,000	34 (27.9)	
£40,000-60,000	11 (9.0)	
>£60,000	26 (21.3)	
Marital status		$\eta^2_p = 0.05$, $p = 0.054$
Married	62 (50.8)	
Single	26 (21.3)	
Divorced, separated or widowed	34 (27.9)	
[a]Educational level		$\eta^2_p = 0.04$, $p = 0.345$
No qualifications	10 (8.3)	
Up to O-levels	21 (17.5)	
A-levels or ONC	12 (10)	
Degree or above	77 (64.2)	
Smoking status [smokers]	18 (14.8)	$d = 0.26$, $p = 0.315$
[b]Moderate or vigorous physical activity per week [hours]	4.61 ± 8.56	$r = 0.13$, $p = 0.162$
BMI [kg/m^2]	30.54 ± 5.59	$r = 0.07$, $p = 0.463$
[c]HbA1c [%]	7.32 ± 1.49	$r = -0.13$, $p = 0.173$
Oral anti-diabetic medication [yes]	98 (80.3)	$d = 0.43$, $p = 0.077$
Insulin or other injectable anti-diabetic medication [yes]	15 (12.3)	$d = 0.12$, $p = 0.661$
Anti-hypertensive medication [yes]	85 (69.7)	$d = 0.33$, $p = 0.077$
Cholesterol-lowering drugs [yes]	94 (77.0)	$d = 0.12$, $p = 0.554$

Characteristic	n (%) or M ± SD	Associations with happiness: Effect size, *p* value
Aspirin [yes]	44 (36.1)	*d* = 0.12, *p* = 0.557
Beta blockers [yes]	11 (9.0)	*d* = 0.19, *p* = 0.620
Time of testing [morning]	55 (45.1)	*d* = 0.10, *p* = 0.576
[d]Daily sadness [score]	0.74 ± 0.73	*r* = -0.34, *p* = < 0.001

Note. Associations between mean happiness score and sample characteristics were tested using Pearson's *r* correlations for continuous variables and independent sample *t*-tests and one-way between-subjects analysis of variance for categorical variables. AM = after midnight; BMI = body mass index; HbA1c = glycated hemoglobin; kg/m^2 = kilograms per square metre; M = mean; min = minutes; N = number; n = number; ONC = ordinary national certificate; SD = standard deviation. [a]*n* =120, [b]*n* = 112, [c]*n* = 118, [d]*n* = 120.

Pre-task IL-6 levels were inversely correlated with IL-6 stress responses immediately post-task (*r*(108) = -0.21, *p* = 0.028) and at 75 minutes post-task (*r*(94) = -0.37, *p* < 0.001). Pre-task IL-6 was not significantly correlated with the 45-minute response (*p* = 0.068). Similarly, pre-task MCP-1 was negatively correlated with the 75-minute MCP-1 response (*r*(95) = -0.29, *p* = 0.004). No significant correlations were found between pre-task MCP-1 and MCP-1 stress responses immediately post-task (*p* = 0.257) or at 45 minutes post-task (*p* = 0.211), or between pre-task IL-1ra and IL-1ra stress responses post-task (*p*s ≥ 0.111).

6.3.2 Subjective stress and inflammatory levels across the laboratory session[52]

A significant main effect of stress tasks was found for the subjective stress ratings (*F*(1.79, 206.36) = 285.52, *p* < 0.001, η^2_p = 0.71). Specifically, there were marked increases in subjective stress immediately post-task compared with pre-task levels (*p* < 0.001, 95% CI [-3.28, -2.47]) and recovery levels (45 minutes versus immediately post-task levels: *p* < 0.001, 95% CI [2.47, 3.30]; 75 minutes versus immediately post-

[52] 95% CI presented in this sub-section is 95% CI for difference.

task levels: $p < 0.001$, 95% CI [2.58, 3.39]). Values returned to pre-task levels during the recovery period so that 45-minute and 75-minute levels were not significantly different from pre-task levels (*ps* = 1.000).

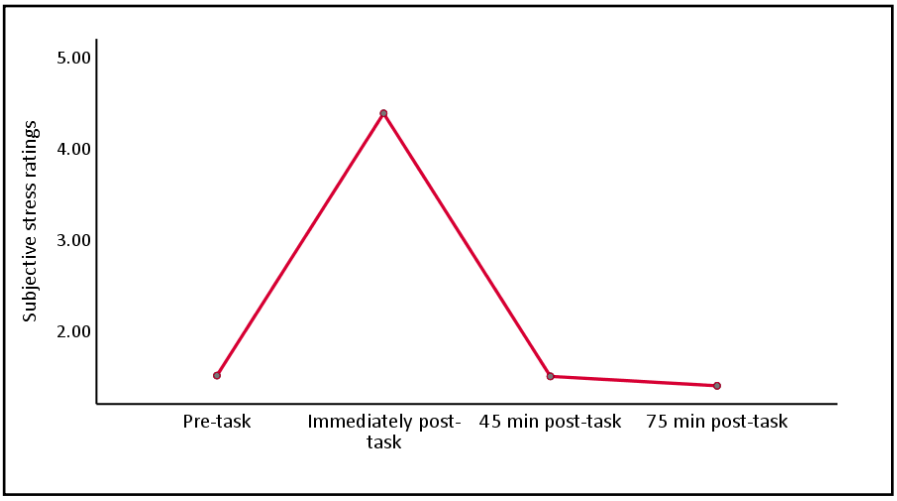

Figure 6.1. Mean subjective stress levels throughout the laboratory session in 116 participants with type 2 diabetes. min = minutes.

Significant main effects of the two tasks were also found for IL-6 levels ($F(2.47, 231.68) = 5.15$, $p = 0.004$, $\eta^2_p = 0.05$). Specifically, IL-6 increased over time and was significantly higher at 75 minutes compared with pre-task levels ($p = 0.038$, 95% CI [0.01, 0.47]) and immediate post-task values ($p = 0.009$, 95% CI [0.04, 0.39]). The average increase from pre-stress to 75 minutes levels (where the greatest change was observed) was 0.24 pg/ml (SE = 0.09). No significant effects of the stress tasks were observed for IL-1ra (F (2.665, 250.530) = 0.16, $p = 0.903$, $\eta^2_p = 0.002$). In contrast, and contrary to expectations, MCP-1 concentrations showed a progressive decline across the session (F (2.67, 253.26) = 7.14, $p < 0.001$, $\eta^2_p = 0.07$). More precisely, MCP-1 values at 75 minutes were significantly lower than pre-task ($p < 0.001$, 95% CI [-12.09, -2.96]) and immediately post-task values ($p = 0.004$, 95% CI [-12.37, -1.67]). The average decrease from pre-task to 75 minutes (where the greatest change was observed) was 7.52

pg/ml (SE = 1.69). Table 6.2 presents participants' subjective stress and inflammatory levels across the duration of the laboratory session.

Table 6.2

Subjective stress and inflammatory levels throughout the laboratory session

	N	Baseline M ± SD	Immediately post-task M ± SD	45 min post-task M ± SD	75 min post-task M ± SD
Subjective stress [score]	116	[a]1.51 ± 0.91	[b]4.38 ± 1.52	[a]1.50 ± 0.95	[a]1.40 ± 0.80
IL-6 [pg/ml]	95	[a]2.06 ± 1.14	[a]2.08 ± 1.11	2.20 ± 1.26	[b]2.30 ± 1.23
IL-1ra [pg/ml]	95	812.59 ± 409.10	813.90 ± 393.59	818.44 ± 404.48	820.05 ± 414.98
MCP-1 [pg/ml]	96	[a]115.58 ± 33.80	[a]115.08 ± 38.83	111.67 ± 37.54	[b]108.06 ± 33.02

Note. Differences in levels across time were tested using one-way repeated measures analysis of variance. Values in rows with different superscripts ([a], [b]) are significantly different from one another ($p < 0.05$). IL-1ra = interleukin-1 receptor antagonist; IL-6 = interleukin-6; MCP-1 = monocyte chemoattractant protein-1; M = mean; min = minutes; N = number; pg/ml = picograms per mililitre; SD = standard deviation.

6.3.3 Daily happiness and inflammatory levels and stress responses[53]

6.3.3.1 IL-6

Higher daily happiness predicted significantly lower IL-6 absolute levels at all four time points: pre-task (B = -0.11, p = 0.043, 95% CI [-0.22, -0.004]), immediately post-task (B = -0.10, p = 0.045, 95% CI [-0.20, -0.002]), 45 minutes post-task (B = -0.12, p = 0.028, 95% CI [-0.23, -0.01]), and 75 minutes post-task (B = -0.11, p = 0.033, 95% CI [-0.22, -0.01]). These effects were adjusted for age, sex, household income, marital status, smoking status, BMI, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, and time of testing. Further adjustment for daily sadness did not

[53] All results presented in this sub-section are adjusted for age, sex, household income, marital status, smoking status, BMI, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, and time of testing (unless otherwise indicated).

alter the significant effect of daily happiness on pre-task IL-6 levels (*B* = -0.15, *p* = 0.015, 95% CI [-0.26, -0.03]), immediately post-task levels (*B* = -0.15, *p* = 0.006, 95% CI [-0.25, -0.04]), 45-minute levels (*B* = -0.14, *p* = 0.018, 95% CI [-0.26, -0.03]), or 75-minute levels (*B* = -0.15, *p* = 0.007, 95% CI [-0.26, -0.04]). Significant results from the regression analysis are presented in Table 6.3. No significant associations were found between daily happiness and IL-6 change scores (*B* values between -0.01 and -0.02, and *p* values ≥ 0.410), indicating no significant effect of daily happiness on IL-6 reactivity to stress.

6.3.3.2 IL-1ra

Daily happiness was not related to IL-1ra levels either pre- or post-stress (*B* values between -0.03 and -0.04, and *p* values ≥ 0.352). Similarly, happiness was not associated with IL-1ra changes in response to stress (*B* values between -0.01 and -0.03, and *p* values ≥ 0.072).

6.3.3.3 MCP-1

Happier individuals with T2D had significantly lower MCP-1 concentrations before stress after adjusting for age, sex, household income, marital status, smoking status, BMI, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, and time of testing (*B* = -7.82, *p* = 0.041, 95% CI [-15.31, -0.32]). Associations between daily happiness and MCP-1 levels post-task were not significant (*B* values between 5.82 and -8.17, and *p* values ≥ *p* = 0.062). After the inclusion of daily sadness in the model, results for pre-task MCP-1 were rendered non-significant (*B* = -6.31, *p* = 0.131, 95% CI [-14.54, 1.92]). No significant associations were observed

between happiness and MCP-1 changes in response to stress (*B* values between 1.05 and 0.17, and *p* values ≥ 0.606).

Table 4

Multivariable linear regression on daily happiness predicting pre- and post-task IL-6 absolute levels, and pre-task MCP-1

Model	Unstandardised coefficients B	SE	Standardised coefficients ß	95% CI	*p* value
IL-6 [ln]					
Pre-task (*n* = 111)					
Daily happiness [score]	-0.15	0.06	-0.23	-0.26 to -0.03	0.015
Age [years]	0.004	0.01	0.05	-0.01 to 0.02	0.571
Sex [ref. cat.: men]	-0.19	0.10	-0.17	-0.39 to 0.004	0.055
BMI [kg/m^2]	0.04	0.01	0.44	0.03 to 0.06	< 0.001
Smoking status [ref. cat.: non-smoker]	0.27	0.14	0.18	-0.003 to 0.55	0.053
Household income [ref. cat.: < £20.000]	-0.06	0.05	-0.14	-0.15 to 0.03	0.166
Marital status [ref. cat.: married]	0.03	0.06	0.04	-0.10 to 0.15	0.698
Oral anti-diabetic medication [ref. cat.: yes]	0.05	0.12	0.04	-0.18 to 0.28	0.683
Insulin or other injectable anti-diabetic medication [ref. cat.: no]	-0.04	0.15	-0.02	-0.33 to 0.26	0.813
Time of testing [ref. cat.: morning]	0.14	0.10	0.13	−0.05 to 0.34	0.138
Daily sadness [score]	-0.10	0.07	−0.14	−0.24 to 0.03	0.140
Immediately post-task (*n* = 108)					
Daily happiness [score]	-0.15	0.05	-0.24	-0.25 to -0.04	0.006
Age [years]	0.01	0.01	0.11	-0.01 to 0.02	0.213
Sex [ref. cat.: men]	-0.09	0.09	-0.08	-0.27 to 0.08	0.296

Model	Unstandardised coefficients B	SE	Standardised coefficients ß	95% CI	*p* value
BMI [kg/m^2]	0.05	0.01	0.53	0.04 to 0.07	< 0.001
Smoking status [ref. cat.: non-smoker]	0.28	0.13	0.18	0.02 to 0.53	0.032
Household income [ref. cat.: < £20.000]	-0.07	0.04	-0.16	-0.16 to 0.01	0.084
Marital status [ref. cat.: married]	-0.04	0.06	-0.07	-0.15 to 0.07	0.465
Oral anti-diabetic medication [ref. cat.: yes]	0.09	0.11	0.07	-0.12 to 0.29	0.412
Insulin or other injectable anti-diabetic medication [ref. cat.: no]	-0.01	0.13	-0.01	-0.28 to 0.25	0.927
Time of testing [ref. cat.: morning]	0.26	0.09	0.24	0.08 to 0.43	0.004
Daily sadness [score]	-0.15	0.07	–0.20	-0.28 to -0.02	0.025
45 min post-task (*n* = 99)					
Daily happiness [score]	-0.14	0.06	-0.230	-0.26 to -0.03	0.018
Age [years]	0.01	0.01	0.10	-0.01 to 0.02	0.318
Sex [ref. cat.: men]	-0.03	0.10	-0.02	0.22 to 0.17	0.795
BMI [kg/m^2]	0.05	0.01	0.46	0.03 to 0.07	< 0.001
Smoking status [ref. cat.: non-smoker]	0.21	0.14	0.14	-0.07 to 0.48	0.134
Household income [ref. cat.: < £20.000]	-0.10	0.05	-0.21	-0.19 to -0.01	0.038
Marital status [ref. cat.: married]	0.05	0.06	-0.08	-0.17 to 0.08	0.446

Model	Unstandardised coefficients B	SE	Standardised coefficients ß	95% CI	*p* value
Oral anti-diabetic medication [ref. cat.: yes]	0.06	0.11	0.05	-0.17 to 0.28	0.601
Insulin or other injectable anti-diabetic medication [ref. cat.: no]	-0.01	-0.01	-0.01	-0.31 to 0.30	0.957
Time of testing [ref. cat.: morning]	0.25	0.10	0.23	0.05 to 0.44	0.014
Daily sadness [score]	-0.07	0.08	-0.10	-0.22 to 0.08	0.333
75 min post-task (*n* = 95)					
Daily happiness [score]	-0.15	0.06	-0.26	-0.26 to -0.04	0.007
Age [years]	0.01	0.01	0.07	-0.01 to 0.02	0.494
Sex [ref. cat.: men]	-0.02	0.09	-0.02	-0.20 to 0.16	0.819
BMI [kg/m^2]	0.05	0.01	0.48	0.03 to 0.07	< 0.001
Smoking status [ref. cat.: non-smoker]	0.24	0.12	0.18	-0.01 to 0.48	0.061
Household income [ref. cat.: < £20.000]	-0.07	0.04	-0.16	-0.15 to 0.02	0.120
Marital status [ref. cat.: married]	-0.06	0.06	-0.10	-0.17 to 0.05	0.305
Oral anti-diabetic medication [ref. cat.: yes]	0.11	0.10	0.10	-0.09 to 0.31	0.291
Insulin or other injectable anti-diabetic medication [ref. cat.: no]	0.02	0.14	0.01	-0.27 to 0.30	0.899
Time of testing [ref. cat.: morning]	0.25	0.09	0.25	0.07 to 0.42	0.007

Model	Unstandardised coefficients B	SE	Standardised coefficients ß	95% CI	*p* value
Daily sadness [score]	-0.17	0.07	-0.25	-0.31 to 0.03	0.016
MCP-1 [pg/ml]					
Baseline (*n* = 117)					
Daily happiness	-7.82	3.78	-0.19	-15.31 to -0.32	0.041
Age	0.89	0.49	0.18	-0.07 to 1.85	0.070
Sex [ref. cat.: men]	-2.96	6.85	-0.04	-16.53 to 10.62	0.667
BMI	0.89	0.65	0.14	-0.40 to 2.19	0.175
Smoking status [ref. cat.: non-smoker]	8.19	9.49	0.08	-10.62 to 27.00	0.390
Household income [ref. cat.: < 20.000]	-2.65	3.13	-0.09	-8.84 to 3.55	0.399
Marital status [ref. cat.: married]	1.96	4.23	0.05	-6.41 to 10.34	0.643
Oral anti-diabetic medication [ref. cat.: yes]	-4.72	8.12	-0.06	-20.82 to -11.38	0.562
Insulin or other injectable anti-diabetic medication [ref. cat.: no]	4.24	9.87	0.04	-15.32 to 23.80	0.688
Time of testing [ref. cat.: morning]	3.58	6.58	0.51	-9.46 to -6.62	0.587

Note. IL-6 model is adjusted for covariates including daily sadness. MCP-1 model is adjusted for covariates except daily sadness. B = unstandardised beta coefficient; BMI = body mass index; CI = confidence interval; IL-6 = interleukin-6; ln = log-n; MCP-1 = monocyte chemoattractant protein-1; *n* = number; pg/ml = picograms per mililitre; SE = standard error; ß = standardized beta coefficient.

6.4 Discussion

This study investigated the relationship between daily happiness and three inflammatory markers before and after stressful tasks in older people with T2D. One

of the first outcomes of the study was that the stress tasks elicited significant increases in subjective stress and IL-6 levels. In particular, subjective stress increased immediately after the tasks and returned to initial levels during recovery period. IL-6 values were not significantly different from resting levels until 75 minutes post-task. This finding is in agreement with the meta-analytic evidence of relatively delayed IL-6 increases in response to laboratory stress (Marsland et al., 2017). Interestingly, the stress of the two tasks did not translate into significant increases in IL-1ra or MCP-1 in this patient group, in contrast to findings in healthy participants (Hackett et al., 2012; Marsland et al., 2017; Steptoe et al., 2007). It is plausible that the condition of diabetes in this sample has accounted for these findings. It is also possible that the type of the tasks or the timing of post-stress sampling played a role in these findings. For example, in a previous laboratory study, IL-1ra reached a peak at 90 minutes following social stress test (Rohleder et al., 2006). Additionally, in another study, IL-1ra showed significant increases only two hours following the tasks (Steptoe et al., 2001), whilst our measurements were taken up to 75 minutes post-task. Further studies looking at the time course of IL-1ra and MCP-1 stress responses over a longer post-task period would allow for a more detailed investigation of stress reactivity in these markers. Nevertheless, IL-6 is one of the most frequently examined factors in stress testing studies (Marsland et al., 2017), and these findings add to the value of assessing IL-6 stress responsivity in adults with T2D.

It was hypothesised that participants with greater daily happiness will have lower IL-6, IL-1ra, and MCP-1 concentrations before and after stress, and will show reduced increases in these markers post-stress. Despite the fact that not any differential

response to stress was found in people varying in happiness over the day, greater daily happiness predicted significantly lower IL-6 levels pre-stress and at all three time points post-stress, and lower pre-stress MCP-1. These effects were independent of age, sex, household income, marital status, smoking status, BMI, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, and time of testing. Notably, the association between happiness and IL-6 levels throughout remained significant after further adjustment for daily sadness.

The majority of previous studies looking at positive psychological attributes and IL-6 levels involved healthy participants (Friedman et al., 2007; Ikeda et al., 2011), and only one study included participants with chronic heart failure (Brouwers et al., 2013). Findings from these studies consistently demonstrated lower IL-6 concentrations in people reporting greater well-being. These findings are extended in individuals with T2D, showing that similar patterns exist in men and women with T2D. The association between happiness and lower IL-6 levels was independent of covariates, including daily sadness. This finding lends further support to the notion that the effects of happiness may be beyond the effects of sadness and consequently, even in the presence of sadness, happiness may have stronger links with biological processes. This independent association between happiness and biological processes is in line with the independent relationships observed between subjective well-being and physical health, as reported previously in Chapter 1.

Greater happiness was not associated with smaller inflammatory stress responses in this study. This finding contradicts previous research showing reduced inflammatory stress responses in people with greater daily happiness, self-compassion, and positive

outlook (Aschbacher et al., 2012; Breines et al., 2014; Steptoe et al., 2005). In the current study, IL-6 levels increased in parallel in people reporting higher and lower happiness. Consequently, absolute levels of IL-6 were greater in less happy people overall, both before and after stress testing, but changes in levels from pre-task to post-task time points were similar in magnitude across different happiness scores. As described in detail in Chapter 1, this sample of people with T2D has been recently characterised by biological dysregulation across multiple systems, manifested as significant differences in biological mechanisms, including inflammatory processes, compared with healthy participants. These differences between the samples were seen both under resting conditions and in response to stress tasks in the laboratory (Steptoe, Hackett, et al., 2014). These biological disturbances in people with T2D might have accounted for the null association between happiness and IL-6 stress responses found in the current study.

Happier participants had significantly lower MCP-1 levels before stress. This result add to the evidence of MCP-1 being sensitive to psychological factors (Asberg et al., 2009; Hackett et al., 2012; Rajagopalan et al., 2001; Suarez et al., 2003). Contrary to hypothesis, no significant association was observed between happiness and resting IL-1ra. Nevertheless, happiness represents only one dimension of hedonic well-being. It is possible that different dimensions of well-being reflect distinct biological processes and are, therefore, linked with different biological markers. For example, in the study with patients with chronic heart failure, different aspects of positive affect were associated with different markers of inflammation (Brouwers et al., 2013). The examination of multiple well-being constructs and their links with different

biomarkers would be informative in future studies. No significant relationships were observed between happiness and IL-1ra or MCP-1 levels post-stress, or stress responses. Neither of the two markers showed increases after stress though, contrary to IL-6. Future studies need to replicate findings relating to IL-1ra and MCP-1 reactivity in people with T2D.

The precise pathways linking greater happiness with lower circulating inflammatory factors are unclear. Nonetheless, ample evidence supports a reciprocal relationship between mood and markers of inflammation. The inflammation – mood direction is supported by pre-clinical evidence that shows that inflammatory cytokines, including IL-6, act centrally to induce mood symptoms (Rosenblat, Cha, Mansur, & McIntyre, 2014). Additionally, in both animal and human studies, the administration of inflammatory cytokines increases the incidence of depressive symptoms (Rosenblat et al., 2014). On the other direction, the mood – inflammation relationship is supported by a variety of laboratory studies that showed that stress exposure induces reliable inflammatory responses (Marsland et al., 2017; Steptoe et al., 2007). In line with this concept, greater happiness over the day might be associated with more adaptive coping mechanisms when faced with daily stressors, such as more adaptive appraisals about the stressor (e.g. perceive the stressor as a challenge rather than as a problem), resulting in lower subjective stress and ultimately a favourable basal activity of the stress systems overall, including the immune system and its parametres.

Given the health-damaging effects of increased inflammatory levels, the lower IL-6 and MCP-1 concentrations recorded in people with T2D might contribute to reduced progression of diabetes in processes such as insulin resistance and macrovascular

complications. The examination of the mediating role of inflammatory levels in the link between happiness and health outcomes in T2D was one of the objectives of Study 4 presented in Chapter 8.

This study has numerous strengths. Mood recordings were assessed repeatedly over several consecutive days. Additionally, three different inflammatory markers were measured before and after stress tasks using a standard protocol. This method allowed the examination of both absolute inflammatory levels and stress responses. Furthermore, a variety of potential confounders were taken into account, including daily sadness. Despite the fact that this is a relatively large laboratory study, full biological data were collected for only about three quarters of participants due to difficulties in blood sampling, particularly in obese individuals, as mentioned previously. Albeit including a long blood sampling period that lasted up to 75 minutes post-task, an extended sampling could have provided important information as well. The present study is also limited by its cross-sectional nature; it does not involve a repeated examination of stress responsivity, hence causal relationships between greater happiness in daily life and lower inflammatory markers in the long-term cannot be drawn. The generalisability of our findings is limited to middle- and older-aged participants with T2D, of white European ethnicity, and with no history or a diagnosis of CHD, inflammatory diseases, allergies, or mood disorders.

In conclusion, this study revealed happier participants with T2D have lower basal IL-6 levels throughout the stress session and lower pre-session MCP-1 concentrations. These findings provide evidence for a protective mechanism that might link daily happiness with better health and longevity in people with T2D.

Chapter 7. The Diabetes Follow-up Study: Data and methods

This chapter describes the data and methods of the Diabetes Follow-up Study. The Diabetes Follow-up Study data were collected by myself and were used for the analyses presented in Chapter 8.

7.1 The Diabetes Follow-up Study

The Diabetes Follow-up Study is a follow-up investigation of the original Diabetes Study. It is an observational study with an average follow-up period of 7.5 years. Analyses presented in Study 4 (see Chapter 8) were carried out using data from the Diabetes Follow-up Study. Study 4 examined sex differences and the effect of daily happiness, as measured during the initial Diabetes Study, on physical and mental health outcomes at follow-up in people with T2D, and the mediating role of inflammatory stress responses. The methods of the Diabetes Follow-up Study are described below, though more specific information about Study 4 (e.g. exposure and outcome measures, covariates, statistical analyses) is provided in Chapter 8.

7.2 Participants

All living participants of the baseline study were invited to take part in this follow-up study carried out between January 2019 and July 2019. Therefore, all participants also took part in the baseline Diabetes Study. No exclusion criteria were applied, assuming that the participant was confirmed alive by their primary care practice manager or physician. Specifically, before any contact was made with the potential participants,

Dr Ruth A. Hackett, who had permission to access participants' personal data[54], was requested by the ethics committee to contact primary care practices to ascertain whether the participant was still alive. Nine out of 16 practices responded to this initial request. One participant was confirmed deceased; hence 139 invitation letters were initially posted. The flow chart of the study sample is depicted in Figure 7.1. All participants of the follow-up study gave fully informed written consent to take part in the study and ethical approval was obtained by the National Research Ethics Service.

[54] All participants of the Diabetes Study had given permission to Dr Ruth A. Hackett to have access to their personal information including home address details and information on their primary care practices.

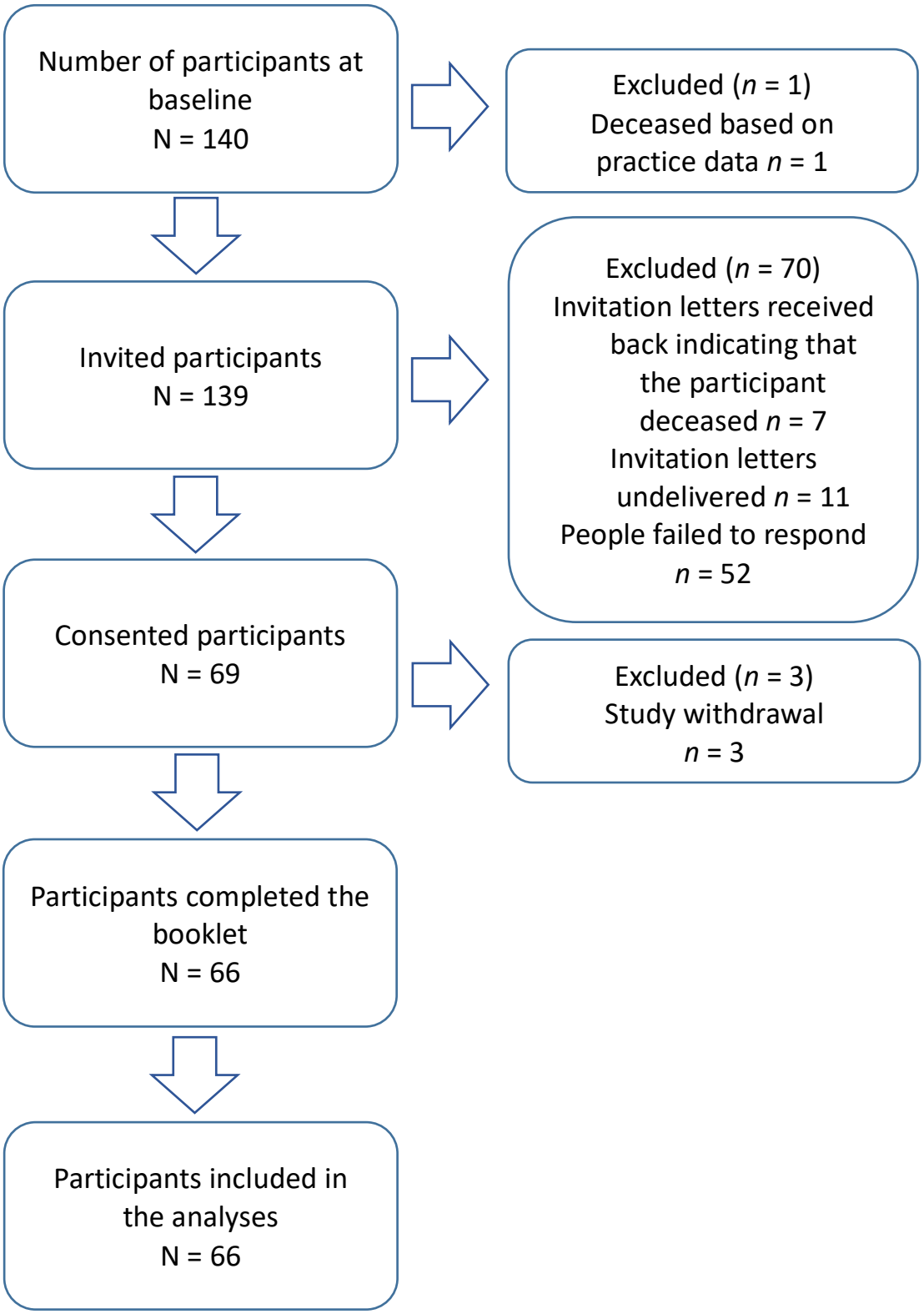

Figure 7.1. The flow diagram of the Diabetes Follow-up Study showing the number of participants at each stage of the study. N = number; *n* = number.

7.3 Procedure

The Diabetes Follow-up Study was a questionnaire-based study consisted of a single stage, the completion of a questionnaire booklet. Participants initially received by post an invitation letter, a participant information sheet, a consent form, and a contact details form. Subsequently, consented participants (confirmation of study participation was ascertained on receipt of a singed consent form) were posted the questionnaire booklet to complete at home and to return to UCL using a freepost envelope. Participants were encouraged to contact me for any questions they might have regarding any aspect of the study (my full contribution to the Diabetes Follow-up Study is presented on page 192). Parts of the questionnaire booklet along with the study consent form and the participant information sheet can be found in Appendix C.

7.4 The Diabetes Follow-up Study measures

The questionnaire booklet consisted of questions and standardised scales aimed primarily to capture the current physical and mental health condition of the individual as well as any changes in their health since the baseline assessment. For simplicity, only the variables that have been used for the purposes of this thesis are described in the next paragraphs. These variables were used in analyses presented in Chapter 8, though the full list of the follow-up study measures is also presented in Table 7.1.

7.4.1 Physical and mental health-related variables

Physical health variables were measured by self-report and included doctor-diagnosed CVD, number of hospital admissions over the 7.5-follow-up period, and physical health-related quality of life. More precisely, information was collected on doctor-

diagnosed CVD: angina or long-term heart problems (yes, no), MI (yes, no), and stroke (yes, no). Participants were free from CVD at baseline, and during the follow-up assessment they were asked to indicate the date (month, year) of diagnosis. Moreover, information was collected on the number of hospital admissions over the last seven years including both day cases and overnight stays. Again, participants were asked to indicate the date (month, year) of hospitalisation. The physical health component of the SF-36 Health Survey (Ware & Sherbourne, 1992) was applied to measure current physical health-related quality of life. Finally, T2D duration was also measured by self-report. Participants were asked to indicate the date (year) of T2D diagnosis. The duration of diabetes was calculated by subtracting the year of diagnosis from 2019. Mental health-related quality of life was assessed using the mental health component of the SF-36 Health Survey (Ware & Sherbourne, 1992). More details on how these variables were handled are presented in Chapter 8.

Table 7.1

List of variables assessed during the Diabetes Follow-up Study[55]

Sociodemographic measures	Employment status Marital status Personal income Household income Financial strain (Pearlin, Lieberman, Menaghan, & Mullan, 1981)
Lifestyle measures	BMI Smoking status Physical activity Alcohol consumption Medication adherence Sleep hours Sleep quality (Jenkins, Stanton, Niemcryk, & Rose, 1988)
Physical health-related measures	**Doctor-diagnosed macrovascular complications** Doctor-diagnosed microvascular complications Doctor-diagnosed illnesses commonly observed in T2D such as depression, anxiety, and Alzheimer's disease **Number of hospital admissions** Medication use Diabetes medication change Diabetes duration[56] **Physical health-related quality of life** (Ware & Sherbourne, 1992) Deceased status
Mental health-related and psychosocial measures	Depressive symptoms (Radloff, 1977) **Mental health-related quality of life** (Ware & Sherbourne, 1992) Daily happiness (Cohen et al., 2003) Psychological well-being (Ryff, 1989) Loneliness (Russell, Peplau, & Cutrona, 1980) Optimism (Scheier, Carver, & Bridges, 1994) Cynical hostility (Cook & Medley, 1954) Self-esteem (Rosenberg, 1979)

Note. Bold text indicates that the specific variable has been used for the purposes of Study 4 presented in Chapter 8. BMI = body mass index; T2D = type 2 diabetes.

[55] The content and wording of the questionnaire booklet was mutually established between myself and my supervisors. Further details on my contribution to the Diabetes Follow-up Study are presented on page 192.

[56] Diabetes duration was not assessed during the baseline study, but it was measured at follow-up by self-report. Moreover, information on age and time-invariant variables such as sex, ethnicity, and educational level were not re-assessed in order to decrease the burden for participants.

7.5 Plans to access medical data from primary care practices

The initial strategy regarding the study measures was to collect both self-reported and clinical records-based data relevant to health. Clinical variables would include indicators of current physical health such as the latest blood pressure results and the latest biochemistry report for information on HbA1c and lipids profile, as well as doctor confirmation of any physical or mental illness diagnosis over the last years. Participants of the follow-up study gave me written consent to access specific parts of their medical files and provided me with information about their current primary care practice. As a result, 31 practices were contacted via electronic mails and phone calls with information about the study. As a first step, practices needed to review and sign the Health Research Authority (HRA) Statement of Activities for participating NHS organisations in England. This document acts as a confirmation of the practice agreement to take part in the study. At the time of writing this thesis, nine out of 31 practices agreed to take part in the study, covering 13 out of the 69 consented participants. Given the small number of responding practices and due to time constraints clinical data were not collected for the purposes of this thesis, but further attempts will continue to be made in order to successfully obtain medical data.

7.6 Data storage and handling

The Diabetes Follow-up Study was registered with the UCL Data Protection Office. This is a secure system at UCL designed for to handle identifiable data. It has been certified to the ISO27001 information security standard and conforms to the NHS Information Governance Toolkit. Considering the sensitive nature of information collected, the data were treated as strictly confidential. Personal identification information

(included in the consent form and the contact details form) were stored separately from the questionnaire data, ensuring that all data remain strictly anonymous. Personal identification information was stored in the Data Safe Haven, a 'walled garden' online system where personal data are stored, processed, and managed securely, avoiding the complexity of assured end-point encryption. Access to the Data Safe Haven was limited to myself[57] and Dr Ruth A. Hackett. The NHS Code of Confidentiality was followed to ensure that personal information is handled fairly, lawfully, and as transparently as possible.

All participant questionnaires were labelled using a unique anonymised participant identification number (e.g. OO1). All hard copies of the data were stored in a locked filing cabinet (located under my office desk at UCL) with access to keys being limited to the authorised study researchers[58]. The questionnaire data that were entered into the computer from hard copies were also anonymised, using the assigned participant number. Electronic questionnaire data were kept on password-protected computers with access only available to the authorised study team. The new dataset was stored on a secure network drive accessible only to the study team, so that any transfer of data between the study team was avoided. The study master file will be archived at UCL for the stipulated time period of 20 years and in line with all relevant legal and statutory requirements. Concluding, data storage and management was conducted in accordance to ethical procedures.

[57] All participants of the follow-up study gave me permission to have access to their personal data.
[58] Authorised researchers consisting the study team were myself, Dr Ruth A. Hackett, Dr Lydia Poole, and Professor Andrew Steptoe.

7.7 My contribution to the Diabetes Follow-up Study

The research questions and, therefore, the design of the Diabetes Follow-up Study were both established after discussions with my supervisors, Professor Andrew Steptoe, Dr Lydia Poole, and Dr Ruth A. Hackett. The following paragraphs provide specific details on my role and contribution during the different stages of the project.

7.7.1 Research training

In order to successfully complete this project, I attended several statistical training courses offered to PhD students by the UCL Doctoral School. Also, to ensure that all personal and research data collected for this project were handled appropriately, I completed training relating to the data protection legislation, including the NHS Digital Data Security Awareness course (carried out on annual basis) and the General Data Protection Regulation online training for students and researchers.

7.7.2 Regulatory approvals

The Diabetes Follow-up Study was deemed to require regulatory approval from several authorities. As a result, my first responsibility was to apply to three different bodies in order to receive 1) UCL sponsorship, 2) ethical approval, and 3) HRA approval. These formal procedures required the preparation and submission of different sets of documents. These documents included a study protocol that highlights the rationale of the proposed study, the scientific hypotheses to be resolved, the anticipated research outputs with their research and clinical impact, and the study methodology. With regards to study methods, a comprehensive list of the study measures was also submitted and scientific justification for the variables to be

collected was provided. Information on study participants, statistical analyses, assessment and management of risk was also described explicitly.

7.7.3 Public involvement

Public involvement is encouraged by the UCL Joint Research Office, and I was responsible for involving members of the public in this study. In particular, I asked two members of the public to review the participant information sheet, the consent form, and the questionnaire booklet. Both individuals were of old age (mean age 65 years), are native speakers of English language, and have T2D. They both verbally confirmed that the information, instructions, and questions included in the study documents were clear. Moreover, the two members of the public calculated how long it took them to fully complete the questionnaire booklet at home in order for me to give study participants an estimation about the time needed to fill out the booklet.

7.7.4 Data collection

I prepared all study documents with input from my supervisors. These documents included the invitation letter, the participant information sheet, the consent form, the contact details form, the thank you letter, and two reminder letters. The content and wording of the questionnaire booklet was mutually established between me and my supervisors. After preparation, I was responsible for posting and receiving all sets of mail-outs to consented participants. I was also responsible for responding to participants' electronic mails and phone calls on questions they might have regarding any aspect of the study.

7.7.5 Data cleaning and statistical analyses

I set up the dataset for the study, carried out all the data entry, and cleaned the dataset to remove any data entry errors. I also merged the new dataset with the baseline dataset based on the participants' identification number. I performed all statistical analyses for Study 4, presented in Chapter 8, with input from my supervisors.

7.7.6 Data storage and handling

I was fully responsible for keeping all personal and questionnaire data, both hard copies and electronic data, strictly confidential and to handle them in accordance with ethical procedures, as described previously in this chapter.

7.7.7 Interpretation and planned dissemination of findings

I interpreted the findings from the follow-up study, as presented in Chapter 8, in line with the relevant scientific knowledge. I am also expected to disseminate the results of the follow-up study through conference presentations and publications in peer-reviewed scientific journals, as well as internally at UCL via this thesis.

Chapter 8. Study 4: Sex and daily happiness as predictors of future health outcomes in T2D: The mediating role of IL-6 stress responses

Study 4 is concerned with the role of sex and daily happiness on physical and mental health outcomes over 7.5 years in people with T2D. Also, Study 4 directly tests the mediating role of IL-6 stress responsivity on these links. This chapter provides a brief overview of the study background, followed by the methods used, the results of the study, and a discussion of the findings.

8.1 Introduction

Stress-related biological markers form a plausible mediating pathway linking demographic and psychological factors with prospective health outcomes in people with T2D. Studies 2 and 3, presented in Chapters 5 and 6, respectively, have demonstrated that sex and daily happiness are related to inflammatory (re)activity in this population. Briefly, in Study 2 it was found that women with T2D show increased IL-6 stress responses compared with men with T2D (Panagi et al., 2019a). In Study 3, daily happiness was associated with lower IL-6 absolute levels before and after stress in this sample (Panagi et al., 2019b). One of the main limitations of these previous studies is that they had a cross-sectional design. Therefore, the temporal relationship between baseline IL-6 stress responsivity and future health outcomes in people with T2D is yet to be established.

Existing longitudinal studies have elucidated that heightened inflammatory reactivity due to mental stress in the laboratory may herald more rapid progression of CVD-related risk factors over time (Aschbacher et al., 2012; Brydon & Steptoe, 2005; Ellins

et al., 2008; Ellins et al., 2017; Steptoe et al., 2016). These laboratory stress testing studies have been described in more detail in Chapter 1. Albeit informative, these studies are limited by the fact that they only included healthy samples and they mostly assessed physical disease risk factors (e.g. carotid artery stiffness) rather than disease-specific endpoints (e.g. stroke). To the best of my knowledge, only a single previous study considered mental health outcomes (depressive symptoms). Additionally, this was the only study to directly test the mediating role of inflammatory (IL-1b) stress responses in the association between positive psychological factors (positive outlook during stress tasks) and depressive symptoms at follow-up (Aschbacher et al., 2012). Nevertheless, this study is limited by its small sample size (N = 35) and short follow-up period (one year). Also, this previous study was carried out in a healthy sample of women participants. The long-term effects of inflammatory reactivity on health might be more apparent in people with existing disease, but the longitudinal association between inflammatory stress responses and health outcomes in people with existing T2D remains to be established. Moreover, no previous studies have directly tested the mediating role of inflammatory stress responses in linking sex and daily happiness with physical and mental health outcomes in people with T2D.

The aim of this follow-up study was to better understand the biological mechanisms that potentially link sex and daily happiness with physical and mental health outcomes in people with T2D. The objectives of this study were 1) to investigate the total effect of sex and happiness, measured at baseline, on physical and mental health outcomes at 7.5-year follow-up, 2) to test the association between IL-6 stress responses, as assessed at baseline, with physical and mental health outcomes at follow-up, and 3)

to test the plausible mediating role of IL-6 stress responsivity on the association between of sex, happiness and future health outcomes. I hypothesised that male sex and greater happiness will be associated with better physical and mental health outcomes at follow-up in people with T2D. I also hypothesised that smaller IL-6 stress responses will be associated with better physical and mental health outcomes at follow-up in this sample. Finally, I hypothesised that the associations between sex and happiness with health outcomes would be mediated (at least partly) by IL-6 stress responses. IL-6 responsivity was chosen based on previous research indicating the importance of measuring IL-6 responses over other inflammatory markers (e.g. IL-1ra, MCP-1) in this sample. Since happiness was related to IL-6 absolute levels rather than stress responses in Study 3, the four IL-6 levels (pre- and three time points post-stress) were also considered in analyses.

8.2 Method

8.2.1 Participants

Sixty-six participants of the Diabetes Follow-up Study took part in this project. Full information regarding the Diabetes Follow-up Study participants, the procedure, exclusion criteria, and the study's flow chart is presented in Chapter 7. Out of 66 participants at the follow-up, five participants had missing data on baseline daily happiness (*n* = 61), four participants had missing data on pre-task IL-6 (*n* = 62), eight participants had missing data on immediately post-task IL-6 (*n* = 58), 13 participants had missing data on IL-6 at 45 minutes post-task (*n* = 53), and 18 participants had missing data on IL-6 at 75 minutes post-task (*n* = 48). With regards to follow-up

(outcome) measures, missing data occurred in self-reported CVD diagnosis for six participants (n = 60). Separate numbers for each analysis are given in footnotes.

Out of 140 participants of the initial study, 53% were lost to follow-up. Univariate analyses, using independent samples t-tests for continuous variables and Chi-square tests for categorical variables, were carried out to test for significant differences in sample characteristics between people who participated in the Follow-up Study (N = 66) and those who were lost to follow-up (N = 74). Results showed that participants of lower education ($\chi^2(3) = 14.26$, $p = 0.003$, $V = 0.32$), higher depressive symptoms ($t(122.77) = -2.54$, $p = 0.012$, $d = 0.43$, 95% CI [-6.63, -0.82]), and higher IL-6 absolute levels at three time points (pre-task: $t(119.95) = -2.07$, $p = 0.041$, $d = 0.36$, 95% CI [-0.90, -0.02]; 45 minutes post-task: $t(97.80) = -2.84$, $p = 0.006$, $d = 0.52$, 95% CI [-1.20, -0.21]; 75 minutes post-task: $t(93.87) = -2.34$, $p = 0.021$, $d = 0.45$, 95% CI [-1.02, -0.08]) were more likely to be lost to follow-up. In addition, participants who were taking cholesterol-lowering medication during the initial study were more likely to take part at follow-up ($\chi^2(1) = 11.92$, $p = 0.001$, $\phi = -0.30$). No other significant differences were observed between those who participated at follow-up and lost to follow-up (ps ≥ 0.077). Full findings from these analyses are presented in Appendix D.

8.2.2 Study measures

8.2.2.1 Model 1: Sex and daily happiness as predictors of physical and mental health outcomes

8.2.2.1.1 Predictor variables: Sex and daily happiness at baseline

Self-reported information on sex and the daily happiness measured over one week were used as predictor variables in this study. Full information on the happiness

measure is presented in Chapter 4. Separate analyses were carried out for each predictor variable.

8.2.2.1.2 Outcome variables: Physical and mental health at follow-up

Physical and mental health at follow-up were assessed using four different measures. 1) Self-reported CVD diagnosis: Self-reported information on CVD diagnosis was collected by asking participants whether they had been diagnosed by a doctor with angina or long-term heart problems (yes, no), MI (yes, no), or stroke (yes, no) since their participation in the initial Diabetes Study. A combined variable of doctor-diagnosed angina or long-term heart problems, and/or MI, and/or stroke was created and used as an outcome variable. All participants were free from CVD during the initial Diabetes Study, and the date of diagnosis (year/month) was self-reported by participants. 2) Number of hospital admissions: Participants were asked to report the number of hospital admissions including both day cases and overnight stays since the initial study. Date of hospitalisation (year/month) was self-reported by participants. A 3-level categorical variable was created to reflect 0 admissions, 1 admission, and ≥ 2 admissions over the follow-up period. 3) Physical health-related quality of life: The physical health component of the SF-36 Health Survey (Ware & Sherbourne, 1992) was applied to measure physical health-related quality of life. This physical health dimension was also administered during the initial study (Cronbach's α for the initial study = 0.75). As described previously in Chapter 4, the SF-36 is a self-reported 36-item survey of patient's health. The physical health dimension includes subscales on physical functioning, bodily pain, role limitations due to physical health problems, and general health perceptions, all of which were used to calculate an average physical

health-related quality of life score for each participant. Greater scores indicated better physical health-related quality of life. The Cronbach's α for the physical health component was good in this study (α = 0.80). 4) Mental health-related quality of life: Mental health-related quality of life was assessed using the mental health component of the SF-36 Health Survey (Ware & Sherbourne, 1992). The mental health component of the SF-36 was also completed during the initial study (Cronbach's α for the initial study = 0.78). The mental health dimension includes subscales relating to role limitations due to personal or emotional problems, general mental health, social functioning, and energy, fatigue, or vitality, all of which were used to calculate an average mental health-related quality of life score. Greater scores were indicative of better mental health-related quality of life. The Cronbach's α of the mental health component was acceptable in this study (α = 0.68).

8.2.2.1.3 Covariates

Covariates were chosen based on previous research indicating their effect on physical and/or mental health. All covariates were assessed during the baseline study. Covariates included age, sex[59], smoking status, and BMI. Analyses predicting physical health-related quality of life and mental health-related quality of life were further adjusted for baseline scores on these measures. In sensitivity analysis, significant findings were further adjusted for T2D duration (T2D duration was self-reported at follow-up). Full information on these measures is given in Chapter 4.

8.2.2.2 Model 2: IL-6 stress responses as predictors of physical and mental health outcomes

[59] Sex was used as a covariate in analyses testing happiness and health outcomes (but as a predictor variable in analyses testing sex and health outcomes).

8.2.2.2.1: Predictor variables: IL-6 stress responses at baseline

During the initial Diabetes Study, IL-6 was measured in plasma at four time points: before stress tasks, immediately post-task, 45 minutes post-task, and 75 minutes post-task. The three change scores were used as predictor variables in this study. As reported previously in Chapter 4, these three change scores were calculated to reflect the mean difference/change in IL-6 from pre-task to the three post-task measurements. Higher positive change scores indicated greater increases in IL-6 from pre-task to post-task measurements. Separate analyses were carried out for each time point. In secondary analyses, the four IL-6 absolute scores were also used to test for associations with physical and mental health outcomes at follow-up. Detailed information on the blood sample collection and IL-6 assay methods, the minimum limit of detection, and intra-assay coefficients of variation are presented in Chapter 4.

8.2.2.2.2: Outcome variables: Physical and mental health at follow-up

Physical and mental health at follow-up were assessed using the four different measures, as described on page 199: self-reported CVD diagnosis, number of hospital admissions, physical health-related quality of life, and mental health-related quality of life.

8.2.2.2.3: Covariates

In this second model of IL-6 stress responses predicting health outcomes at follow-up, covariates were chosen based on previous research indicating their effect on physical and/or mental health. All covariates were measured during the initial Diabetes Study. Covariates included age, sex, smoking status, and BMI. Analyses predicting physical health-related quality of life and mental health-related quality of life were further

adjusted for baseline scores on these measures. In sensitivity analysis, significant findings were further adjusted for pre-task IL-6 levels and T2D duration. Full information on these measures is given in Chapter 4 of the thesis.

8.2.2.3 Model 3: Mediation model of sex, IL-6 stress responses, and mental health outcomes

Based on the findings of Models 1 and 2 (shown below in the Results section), the mediation model was limited to the association between sex (predictor variable) and mental health-related quality of life at follow-up (outcome variable), mediated by the 45-minute IL-6 stress response. Mediation analysis was adjusted for age, smoking status, BMI, and mental health-related quality of life (all covariates were measured at baseline).

8.2.2.4 Other measures

Other measures included in univariate analyses were ethnicity, household income, marital status, education level, physical activity, body fat %, WHR, oral anti-diabetic medication, insulin or other injectable anti-diabetic medication, anti-hypertensive medication, cholesterol-lowering medication, aspirin, beta blockers, and pre-test subjective stress. These variables were measured during the initial Diabetes Study and along with the exposure measures and all covariates (also measured during the initial study) were used to compare baseline characteristics between individuals who took part at follow-up and those lost to follow-up (see Appendix D). Full information on these measures is reported in Chapter 4.

8.2.3 Statistical analysis

The distribution of IL-6 concentrations was positively skewed (skewness > 1 for all four IL-6 measurements [values between 1.27 and 1.63] and kurtosis values between 1.53 and 2.98). Therefore, log-n transformation was applied in all analyses, except the descriptive statistics presented in Table 7.2 for ease of interpretation. After log-n transformation, skewness levels were between -0.30 and 0.27 for all four values, hence transformation corrected the skewness of the data.

Three statistical models were tested in this follow-up study. For the first model, sex and daily happiness (as measured during the initial study) were used as predictor variables and physical and mental health outcomes (as measured at follow-up) were used as outcome variables. For the second model, IL-6 stress responses (as assessed during the initial study) were used as predictor variables and physical and mental health outcomes (as assessed at follow-up) were used as outcome variables. For the third (mediation) model, the 45-minute IL-6 stress response was used as the mediator variable to test whether the association between sex and mental health-related quality of life was mediated by IL-6 responsivity.

Descriptive statistics for baseline exposure measures and covariates and follow-up outcome data were first carried out (see Table 7.2). One-way repeated measures ANOVA was also used to test for significant differences in physical and mental health-related quality of life between baseline and follow-up assessments. Associations between sex and happiness with long-term health outcomes (Model 1) were examined using multivariable binary logistic regression for binary outcomes, multivariable multinomial logistic regression for categorical outcomes, and

multivariable linear regression for continuous outcomes. Separate regression analyses were carried out for each exposure and outcome measure adjusting for covariates. Associations between IL-6 stress responses and long-term health outcomes (Model 2) were explored using multivariable binary logistic regression for binary outcomes, multivariable multinomial regression for categorical outcomes, and multivariable linear regression for continuous outcomes. In secondary analyses, I examined the relationship between absolute IL-6 concentrations at the four time points and health outcomes adjusting for covariates. Separate regression analyses were carried out for each exposure and outcome measure adjusting for covariates. Findings from the regression analyses are presented as ORs or unstandardised coefficients (*B*), *p* values, and 95% CI, as appropriate.

In the last set of main analyses (Model 3), I explored whether IL-6 responsivity at 45 minutes post-task mediated the association between sex and mental health-related quality of life adjusting for covariates. Mediation was initially tested by conducting a series of linear regression analyses (Baron & Kenny, 1986). The significance of the indirect pathway was assessed with the Sobel first-order test for mediation using the method set out by Preacher and Hayes (Preacher & Hayes, 2004, 2008). The bootstrapping technique (5000 replications) was also employed using PROCESS version 3.5 by Andrew F. Hayes as a validated method to estimate the bias-corrected CIs of the indirect effect (MacKinnon, 2008).

Results of this study are presented as means and SDs for continuous variables or numbers and percentages for categorical variables. The level of significance was set at

$p < 0.05$, though exact p values are reported throughout. Statistical analyses were carried out using SPSS version 25 (SPSS, Chicago, IL).

8.3 Results

8.3.1 Sample characteristics

Sixty-six participants (41% women) from the initial Diabetes Study took part in this Follow-up Study. Table 7.2 presents baseline and follow-up characteristics of the follow-up sample (N = 66). In 2011 - 2012, when the initial study was carried out, follow-up participants were 63.70 (SD = 6.65) years old on average. The mean BMI was within the obese range (30.79 kg/m^2, SD = 5.31) and the majority of participants were non-smokers (86%). The average IL-6 levels pre-task were 1.91 pg/ml (SD = 1.03). Follow-up participants had an average daily happiness score of 2.66 (SD = 0.82). Physical and mental health-related quality of life at baseline was 72.75 (SD = 19.54) and 75.29 (SD = 15.07), respectively. Mean duration of T2D, as reported at follow-up assessment, was 15.66 years (SD = 7.52). Four participants (7%) reported having been diagnosed with either angina or long-term heart problems, and/or MI, and/or stroke over the follow-up period. Thirty-three participants (50%) reported being admitted to hospital at least once since the initial study either as a day case or overnight stay, with 19 participants (29%) reporting more than two admissions. Physical and mental health-related quality of life at follow-up was 70.55 (SD = 22.78) and 74.99 (SD = 19.52), respectively.

Table 7.2

Sample characteristics at baseline (2011/2) and follow-up (2019) in 66 participants from the Diabetes Follow-up Study

Sample characteristics at baseline	
Characteristic	n (%) or M ± SD
Age [years]	63.70 ± 6.65
Sex [women]	27 (40.9)
Smoking status [smokers]	9 (13.6)
BMI [kg/m^2]	30.79 ± 5.31
[a]Pre-task IL-6 [pg/ml]	1.91 ± 1.03
[b]Immediately post-task IL-6 [pg/ml]	1.93 ± 0.95
[c]45 min IL-6 [pg/ml]	1.99 ± 1.03
[d]75 min IL-6 [pg/ml]	2.02 ± 0.93
[c]Δ immediately post-task [pg/ml]	0.02 ± 0.38
[b]Δ 45 min [pg/ml]	0.10 ± 0.42
[d]Δ 75 min [pg/ml]	0.26 ± 0.66
[e]Daily happiness [score]	2.66 ± 0.82
Physical health quality of life [score]	72.75 ± 19.54
Mental health quality of life [score]	75.29 ± 15.07
Sample characteristics at follow-up	
Characteristic	n (%) or M ± SD
CVD case [yes]	4 (6.7)
Hospital admissions	
0 admissions	33 (50.0)
1 admission	14 (21.2)
≥ 2 admissions	19 (28.8)
Physical health quality of life [score]	70.55 ± 22.78
Mental health quality of life [score]	74.99 ± 19.52

Note. IL-6 values presented in Table 7.2 are unlogged values. BMI = body mass index; IL-6 = interleukin-6; kg/m^2 = kilograms per square metre; M = mean; min = minutes; N = number; n = number, pg/ml = picogram per millilitre; SD = standard deviation; Δ = delta (change) score. [a]*n* = 62; [b]*n* = 54; [c]*n* = 59; [d]*n* = 51; [e]*n* = 61.

8.3.2 Model 1: Sex and daily happiness as predictors of physical and mental health outcomes

The first model tested the effect of sex and daily happiness on physical and mental health outcomes at the 7.5-year follow-up.

8.3.2.1 Doctor-diagnosed CVD

Four participants (7%) reported having been diagnosed with either angina or long-term heart problems, and/or MI, and/or stroke over the follow-up period. No significant sex differences were found in the prevalence of doctor-diagnosed CVD at follow-up after adjusting for age, smoking status, and BMI (OR = 4.48, p = 0.290)[60]. There was no significant effect of baseline daily happiness on doctor-diagnosed CVD prevalence at follow-up after adjusting for age, sex, smoking status, and BMI (OR = 5.40, p = 0.066)[61].

8.3.2.2 Number of hospital admissions

Thirty-three participants (50%) reported being admitted to hospital at least once since the initial study either as a day case or overnight stay, and 19 participants (29%) reported more than two admissions. No significant sex differences were observed in the number of hospital admissions over the follow-up period after adjusting for age, smoking status, and BMI (for 1 versus 0 admissions: OR = 0.91, p = 0.887, 95% CI [0.25, 3.36]; for ≥ 2 versus 0 admissions: OR = 0.70, p = 0.567, 95% CI [0.97, 1.17])[62]. The effect of daily happiness on the number of hospital admissions was not significant adjusting for age, sex, smoking status, and BMI (for 1 versus 0 admissions: OR = 1.16,

[60] n = 60.
[61] n = 55.
[62] n = 66.

p = 0.713, 95% CI [0.53, 2.56]; for ≥ 2 versus 0 admissions: OR = 1.30 p = 0.511, 95% CI [0.59, 2.85])[63].

8.3.2.3 Physical health-related quality of life

There was no significant effect of time (from baseline to follow-up) for the physical health-related quality of life score (F(1.00, 65.00) = 1.14, p = 0.290, η^2_p = 0.02). There were no significant associations between sex and physical health-related quality of life adjusting for age, smoking status, BMI, and baseline physical health-related quality of life (B = -6.34, p = 0.131, 95% CI [-14.64, 1.95)[64]. The relationship between daily happiness and physical health-related quality of life was non-significant after adjusting for age, sex, smoking status, BMI, and baseline physical health-related quality of life (B = 0.76, p = 0.761, 95% CI [-4.23, 5.75])[65].

8.3.2.4 Mental health-related quality of life

Mental health-related quality of life did not significantly change from baseline to follow-up (F(1.00, 65.00) = 0.02, p = 0.886, η^2_p = 0.00). Significant sex differences in mental health-related quality of life were found between men and women in this study (B = -8.76, p = 0.033, 95% CI [-16.79, -0.73])[66]. Specifically, women with T2D scored significantly lower in the mental health component of the SF-36 scale, reflecting worse mental health-related quality of life at follow-up compared with men with T2D. These findings were adjusted for age, smoking status, BMI, and mental health-related quality of life as assessed at baseline. These results are presented in

[63] n = 61.
[64] n = 66.
[65] n = 61.
[66] n = 66.

Table 7.3 below. After inclusion of diabetes duration as an additional covariate, the significant sex differences in quality of life were sustained (*B* = -9.29, *p* = 0.040, 95% CI [-18.12, -0.46])[67]. In contrast, daily happiness was not significantly linked with mental health-related quality of in analyses adjusted for age, sex, smoking status, BMI, and baseline mental health-related quality of life (*B* = 0.99, *p* = 0.685, 95% CI [-3.88, 5,87])[68].

Table 7.3

Multivariable linear regression on sex predicting mental health-related quality of life at follow-up (N = 66)

Model	Unstandardised Coefficients		Standardised Coefficient	95% CI	*p* value
	B	SE	ß		
Sex [ref. cat.: men]	-8.76	4.01	-0.22	-16.79 to -0.73	0.033
Age [years]	-0.24	0.31	-0.08	-0.87 to 0.38	0.440
Smoking status [ref. cat.: non-smokers]	3.90	5.73	0.07	-7.57 to 15.36	0.499
BMI [kg/m^2]	-0.62	0.38	-0.17	-1.38 to 0.15	0.112
Baseline mental health quality of life [score]	0.71	0.13	0.55	0.44 to 0.98	< 0.001

Note. B = unstandardised beta coefficient. BMI = body mass index; CI = confidence interval; kg/m^2 = kilograms per square metre; ref. cat. = reference category; SE = standard error; ß = standardised beta coefficient.

8.3.3 Model 2: IL-6 stress responses as predictors of physical and mental health outcomes

8.3.3.1 Doctor-diagnosed CVD

[67] *n* = 62.
[68] *n* = 61.

IL-6 stress responses immediately post-task, at 45 minutes, or at 75 minutes post-task did not significantly predict the prevalence of doctor-diagnosed CVD adjusting for age, sex, smoking status, and BMI (ORs between 0.06 and 0.40, and *p* values ≥ 0.230)[69].

8.3.3.2 Number of hospital admissions

IL-6 stress responses at the three post-task time points were not significantly associated with the number of hospital admissions over the follow-up period after adjusting for age, sex, smoking status, and BMI (for 1 versus 0 admissions: ORs between 1.55 and 7.63, and *p* values ≥ 0.152; for ≥ 2 versus 0 admissions: ORs between 0.13 and 1.00, and *p* values ≥ 0.178)[70].

8.3.3.3 Physical health-related quality of life

No significant relationships were found between IL-6 stress responses at the three time points post-task and physical health-related quality of life adjusting for age, sex, smoking status, BMI, and baseline physical health-related quality of life (*B* values between 2.521 and -7.880, and *p* values ≥ 0.431)[71].

8.3.3.4 Mental health-related quality of life

A significant association was observed between IL-6 stress responses and mental health-related quality of life at follow-up adjusting for covariates. More precisely, higher IL-6 stress responses at 45 minutes post-task significantly predicted lower mental health-related quality of life after adjusting for age, sex, smoking status, BMI,

[69] For immediately post-task changes: *n* = 53; for 45 minutes changes: *n* = 49; for 75 minutes changes: *n* = 44.
[70] For immediately post-task changes: *n* = 58; for 45 minutes changes: *n* = 53; for 75 minutes changes: *n* = 48.
[71] For immediately post-task changes: *n* = 58; for 45 minutes changes: *n* = 53; for 75 minutes changes: *n* = 48.

and baseline mental health-related quality of life (*B* = -21.45, *p* = 0.026, 95% CI [-40.23, -2.67]; see Table 7.4)[72]. The model remained significant after further adjustment for pre-task IL-6 levels (*B* = -21.26, *p* = 0.043, 95% CI [-41.82, -0.70])[73] and T2D duration (*B* = -25.90, *p* = 0.019, 95% CI [-47.28, -4.52])[74]. Neither immediately post-task changes in IL-6 (*B* = -6.718, *p* = 0.491, 95% CI [-26.14, 12.70])[75] nor 75-minute changes (*B* = -3.79, *p* = 0.593, 95% CI [-17.99, 10.41])[76] showed a significant effect on mental health-related quality of life at follow up controlling for covariates.

Table 7.4 5

Multivariable linear regression on IL-6 stress responses in 2011/2 predicting mental health-related quality of life in 2019 (n = 53)

Model	Unstandardised Coefficients		Standardised Coefficient	95% CI	*p* value
	B	SE	ß		
45 min IL-6 response [ln]	-21.44	9.33	-0.26	-40.23 to -2.67	0.026
Age [years]	-0.27	0.36	-0.09	-1.00 to 0.46	0.462
Sex [ref. cat.: men]	-9.51	4.72	-0.23	-19.01 to -0.01	0.050
Smoking status [ref. cat.: non-smokers]	8.54	6.45	0.15	-4.45 to 21.52	0.192
BMI [kg/m^2]	-0.60	0.42	-0.17	-1.44 to 0.24	0.158
Baseline mental health quality of life [score]	0.72	0.15	0.55	0.41 to 1.02	< 0.001

Note. B = unstandardised beta coefficient; BMI = body mass index; CI = confidence interval; IL-6 = interleukin-6; kg/m^2 = kilograms per square metre; ln = log-n; min = minutes; *n* = number; ref. cat. = reference category; SE = standard error; ß = standardised beta coefficient.

[72] *n* = 53.
[73] *n* = 53.
[74] *n* = 50.
[75] *n* = 58.
[76] *n* = 48.

8.3.3.5 Secondary analyses: IL-6 absolute levels as predictors of physical and mental health outcomes

The associations between IL-6 absolute scores at the four time points and follow-up physical and mental health outcomes adjusting for covariates were not significant (for CVD[77]: ORs between 0.04 and 0.56, and *p* values ≥ 0.147; for number of hospital admissions[78]: 1 versus 0 admissions: ORs between 1.13 and 5.67, and *p* values ≥ 0.069; ≥ 2 versus 0 admissions: ORs between 0.91 and 1.74, and *p* values ≥ 0.337; for physical health-related quality of life[79]: *B* values between 0.26 and 2.45, and *p* values ≥ 0.637; for mental health-related quality of life[80]: *B* values between -0.788 and 2.815, and *p* values ≥ 0.442).

8.3.4 Model 3: Mediation model of sex, 45-minute IL-6 response, and mental health-related quality of life

A mediation model was performed using the 45-minute IL-6 stress response as a plausible mediator of the sex – mental health quality of life relationship. Firstly, and as shown in Model 1, sex significantly predicted mental health-related quality of life in the absence of the mediator (adjusted *B* = -8.76, *p* = 0.033, 95% CI [-16.79, -0.73])[81]. Secondly, sex did not significantly affect the 45-minute IL-6 stress response (adjusted *B* = -0.09, *p* = 0.218, 95% CI [-0.24, 0.06])[82]. Nevertheless, significant sex differences in the 45-minute IL-6 response were evident in this sample (see Results section of Study

[77] For baseline IL-6: *n* = 57; for immediately post-task IL-6: *n* = 53; for 45-minute IL-6: *n* = 49; for 75-minute IL-6: *n* = 44.
[78] For baseline IL-6: *n* = 62; for immediately post-task IL-6: *n* = 58, for 45-minute IL-6: *n* = 53, for 75-minute IL-6: *n* = 48.
[79] For baseline IL-6: *n* = 62; for immediately post-task IL-6: *n* = 58; for 45-minute IL-6: *n* = 53; for 75-minute IL-6: *n* = 48.
[80] For baseline IL-6: *n* = 62; for immediately post-task IL-6: *n* = 58; for 45-minute IL-6: *n* = 53; for 75-minute IL-6: *n* = 48.
[81] Findings are adjusted for age, smoking status, BMI, and baseline mental health-related quality of life (*n* = 66).
[82] Findings are adjusted for age, smoking status, and BMI (*n* = 53).

2 presented in Chapter 5). Thirdly, and as shown in Model 2, the 45-minute IL-6 response had a significant effect on mental health-related quality of life when sex was included in the regression model (adjusted *B* = -21.45, *p* = 0.026, 95% CI [-40.23, -2.67])[83]. At the same time, sex was also a significant predictor in the model (adjusted *B* = -9.511, *p* = 0.049, 95% CI [-19.014, -0.007])[84]. Therefore, the significant effect of sex on mental health-related quality of life was not attenuated on the inclusion of the 45-minute IL-6 response. The Sobel test for mediation revealed no significant indirect pathway in the model (Sobel test statistic = 1.10, SE = 1.76, *p* = 0.272). The bootstrapping technique using PROCESS by Andrew F. Hayes confirmed no significant indirect effect (adjusted *B* = 1.91, SE = 1.55, 95% CI [-1.67, 4.73])[85].

8.4 Discussion

This study extends previous research by investigating the longitudinal relationship between sex and daily happiness with physical and mental health outcomes in T2D, and the mediating role of inflammatory stress responsivity. Evidence from this study showed that 1) women with T2D are more likely to experience lower mental health-related quality of life at follow-up compared with their male counterparts, 2) greater IL-6 stress responses at 45 minutes following stress predict lower mental health-related quality of life at follow-up, and 3) increased IL-6 stress responses at 45 minutes post-stress do not mediate the association between sex and quality of life.

[83] Findings are adjusted for age, sex, smoking status, BMI, and baseline mental health-related quality of life (*n* = 53).
[84] Findings are adjusted for age, smoking status, BMI, baseline mental health-related quality of life, and the 45-minute IL-6 stress response (*n* = 53).
[85] Findings are adjusted for age, smoking status, BMI, and baseline mental health-related quality of life (*n* = 53).

Unfortunately, the associations between daily happiness at baseline and follow-up health outcomes were not statistically significant.

With regards to the first set of main analyses, my first hypothesis was partially supported. Sex differences in mental health-related quality of life were observed, measured using the mental health component of the SF-36 questionnaire. The mental health component included questions on role limitations due to personal or emotional problems, general mental health, social functioning, and energy, fatigue, or vitality. Results showed that women with T2D experience significantly worse mental health quality of life at follow-up compared with men with T2D after adjusting for age, smoking status, BMI, baseline mental health quality of life, and diabetes duration. These findings are in agreement with previous research. As reported in the introductory chapter of the thesis, results from cross-sectional studies demonstrate that men with T2D experience greater quality of life and subjective well-being compared to women with diabetes, live more effectively with the condition, experience lesser depression and anxiety, have more energy, and use more active problem-oriented and solving approach strategies when faced with every-day challenges. Although the impact of coping styles is not yet fully explored, strategies classified as problem-focused coping (e.g. active planning) seem to enhance adjustment to T2D compared to emotion-focused coping (Julien, Senécal, & Guay, 2009). What is more, women with diabetes are less satisfied with their management of the disease and experience more social worry (Siddiqui et al., 2013). In keeping with the findings from the current study, two large cross-sectional studies involving 755 and 1,353 participants with T2D, respectively, showed that women were more likely

to score lower in mental health-related quality of life using questionnaire measures, including the SF-12 (Landman et al., 2010; Schunk et al., 2015). Not only self-reported mental health-related quality of life but also clinical disorders like anxiety and eating disorders are more common in women than men with T2D (Kautzky-Willer et al., 2016). Mental disorders and low quality of life may adversely affect treatment adherence, ultimately leading to poorer lipids and glucose control in women with T2D and the development of complications (Billimek et al., 2015; Kautzky-Willer et al., 2016). The increased mental health burden in women with T2D may be related to a variety of underlying causes such as lower perceived family support (Kautzky-Willer et al., 2016), lower self-efficacy (Kautzky-Willer et al., 2016), increased concerns about potential anti-diabetic treatment side effects (Inzucchi et al., 2012), or a sense of failure because of poorer disease control (Mohamed et al., 2013; Shibayama et al., 2011). We recently found that female participants with T2D show increased perceived stress coupled with enhanced IL-6 stress responses in the laboratory compared with male participants with T2D (Panagi et al., 2019b). Such psychobiological, particularly psycho-immunological, differences in stress responsivity may be another plausible mechanism explaining the sex differences in mental health-related quality of life observed at follow-up. This hypothesis was directly tested in the current study (see page 219 below).

Contrary to hypothesis, no significant sex differences were found in the prevalence of doctor-diagnosed CVD at follow-up, number of hospital admissions over the follow-up period, or physical health-related quality of life at follow-up. Similarly, baseline daily happiness did not significantly predict physical or mental health outcomes in this

cohort, in contrast to previous research. One possible explanation of the null findings is the small sample size of this study. Also, only four participants (7%) reported having been diagnosed with CVD over the follow-up period in this study. Therefore, the effect of sex or happiness on CVD diagnosis may have been missed because of insufficient prevalence.

With regards to the second set of main analyses, and in line with the second hypothesis, participants with greater IL-6 stress responses at 45 minutes were observed to have significantly lower mental health quality of life 7.5 years later. These findings were adjusted for age, sex, smoking status, BMI, and baseline mental health quality of life score. Significant findings were sustained in sensitivity analysis after further adjusting for pre-stress IL-6 levels and T2D duration. To the best of my knowledge, this is the first laboratory stress testing study to explore the longitudinal association between inflammatory responsivity and later mental health outcomes in people with T2D. This study corroborates findings from a previous cross-sectional study in this same sample of participants with T2D that found that more hostile participants with T2D show enlarged IL-6 stress responses adjusting for covariates (Hackett et al., 2015). However, this previous study did not include a follow-up examination. The current study, therefore, adds to this evidence demonstrating a temporal relationship between heightened IL-6 responses to stress and later decline in mental functioning in people with T2D, in line with one previous study in 35 healthy women (Aschbacher et al., 2012). It is notable that an independent association between the IL-6 response and mental health was only observed with change in IL-6 at 45 minutes post-task and not with immediate post-task changes or 75-minute

changes. In previous laboratory stress testing studies, IL-6 responses were solely assessed at either 30 minutes (Lockwood et al., 2016) or 45 minutes post-stress (Brydon & Steptoe, 2005; Ellins et al., 2008); thus it is difficult to draw conclusions on the 45-minute response as a more important time point than others. Nevertheless, two previous laboratory stress studies demonstrated a stronger effect of the fibrinogen 45-minute response on future health outcomes versus the immediate response (Ellins et al., 2008; Ellins et al., 2017), in agreement with the current finding. As shown previously in this sample, stress-induced IL-6 levels increase significantly only at 45 minutes post-stress and continue to rise up to 75 minutes following stress (Panagi et al., 2019a). It is unclear why the 75-minute response did not significantly predict mental health outcomes in this study. As reported previously in Chapter 4, increased missing IL-6 data occurred at this time point due to technical difficulties in repeated blood sampling. Specifically, 18 out of 66 participants had missing data on 75-minute IL-6 levels. Missing 75-minute data led to a smaller sample size in analyses of this time point and this might explain the non-significant effect of 75-minute responses on later health outcomes.

The mechanisms through which IL-6 stress responses and mental health are related are not clear. Hypothetically, inflammatory hyperactivity may be associated with sickness behaviour (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008) that may be reflected in impaired mental functioning in the long-term. Ample evidence also supports the direct effect of inflammatory biomarkers on mood and well-being. For example, pre-clinical studies have shown that pro-inflammatory cytokines, including IL-6, act centrally to induce negative mood symptoms (Rosenblat et al., 2014). In both

animal and human studies, the administration of pro-inflammatory cytokines, such as INF-γ, increases the incidence of depressive symptoms (Rosenblat et al., 2014) and fatigue (Majer et al., 2008; Malik, Makower, & Wadler, 2001). Additionally, two reviews of the literature evident that the administration of pro-inflammatory cytokines inhibitors, such as TNF-α antagonists and IL-1 inhibitors, reduces depressive symptoms, anxiety, and fatigue, and increases vitality and mental health-related quality of life across different patient populations (Soczynska et al., 2009; Yadlapati & Efthimiou, 2016). The immune system may also be related to subsequent well-being indirectly via the HPA axis activity. To support this notion, there is evidence that mood symptoms are subserved by disturbances in interacting inflammatory and neuroendocrine networks (Soczynska et al., 2009). Indeed, pro-inflammatory cytokines may initiate, promote, and maintain immune-stimulus-associated HPA reactivity and variations in HPA axis responses have been associated with chronic pain, psychological distress, and major depressive disorder (Hurwitz & Morgenstern, 2001). Future studies need to elucidate the precise mechanisms that link IL-6 stress processes with poorer mental health in people with T2D.

There were no significant associations between IL-6 stress responses and doctor-diagnosed CVD, number of hospital admissions, or physical health quality of life. Also, secondary analyses indicated no significant effect of IL-6 absolute levels at the four time points on physical or mental health outcomes. The dynamic inflammatory changes rather than absolute levels may provide a more accurate measure of future disease risk in this patient group, particularly with regards to mental health outcomes.

However, given the small sample size of the current study, replication of these findings is warranted.

No evidence was found to support the third hypothesis of the study. In particular, the 45-minute IL-6 stress response was not a significant mediator of the relationship between sex and mental health-related quality of life, suggesting that the lower mental health quality of life observed in women with T2D is not mediated by the increased IL-6 responsivity to stress. Although small sample sizes have been used in mediation analyses in some previous investigations, larger sample sizes are suggested for small indirect paths' effects and in order to maintain 0.8 power (Fritz & Mackinnon, 2007). Future studies involving greater sample sizes need to further explore the mediating role of inflammatory stress responses in associations between sex and health outcomes in this population.

This study has several strengths. The follow-up design of the study allowed the examination of longitudinal associations between baseline and follow-up measurements, and the mediating role of inflammatory stress responsivity. Daily happiness at baseline was recorded repeatedly over seven consecutive days, and a standard stress testing protocol was applied to assess biological responsivity to stress tasks. Outcome variables were based on self-report. Self-reported clinical data may increase the likelihood of missing data and recall errors, especially in cases of long recall periods (Bhandari & Wagner, 2006; Short et al., 2009). Nevertheless, self-reported clinical data are increasingly used in health research as they have been found to be accurate for hospital admissions-related data (Raina, Torrance-Rynard, Wong, & Woodward, 2002; Seidl et al., 2016) and well-defined chronic conditions such as MI

and stroke (Bush, Miller, Golden, & Hale, 1989; Jamrozik et al., 2014; Kehoe, Wu, Leske, & Chylack, 1994; Okura, Urban, Mahoney, Jacobsen, & Rodeheffer, 2004; Paganini-Hill & Chao, 1993; Tretli, Lund-Larsen, & Foss, 1982), independently of age, sex, ethnicity, and education (Kehoe et al., 1994). A key limitation of this follow-up study is the fact that 54% of the initial sample was lost to follow-up (with 6% of them known to have deceased over the follow-up period while 8% of them did not receive the participation invitation letter [study's flow chart is presented in Chapter 7). Out of 66 participants of the follow-up, up to 18 participants had missing data on post-task IL-6 due to difficulties in blood sampling, as mentioned previously (see Chapter 5). Comparisons between participants who took part at follow-up and those who were lost to follow-up showed that those lost to follow-up were more likely to have lower education, higher depressive symptoms, lower mental health-related quality of life, and higher IL-6 values at three time points, and were more likely to not take cholesterol-lowering medication at the time of testing. This pattern of non-response suggests that the observed associations may have been reduced. This limitation is discussed in more detail in Chapter 9. Results of this study were adjusted for potential confounders. Nevertheless, the small sample size restricted the number of covariates included in the regression models. Therefore, residual confounding as a consequence of unmeasured variables cannot be wholly discounted. Participants of the current study were middle- and older-aged women and men with T2D and without a history or a diagnosis of CHD, inflammatory diseases, allergies, or mood disorders during the baseline assessment. They were recruited from London area and the majority of them were of white European ethnicity, hence it is unknown whether these findings can be generalised in other cohorts.

In conclusion, this study showed that women with T2D experience poorer mental health-related quality of life over time compared with men with T2D and strengthened the predictive value of IL-6 stress responsivity for mental health-related outcomes. Particular focus is needed on how to improve the mental health of women with T2D. Also, further longitudinal research is required in order to explore the role of biological processes linking demographic and positive psychological factors with future health outcomes in people with T2D. These implications are discussed more broadly in the following chapter.

Chapter 9. Discussion

9.1. PhD findings

This PhD consisted of four studies with an overarching aim to assess the associations between two individual factors (sex and subjective well-being), stress-related inflammatory processes, and T2D. To fulfil its aim, this thesis used data from an epidemiological cohort study, a laboratory stress testing study, and an observational follow-up to the laboratory trial. This chapter summarises the findings of the four studies, highlights their contribution to the existing knowledge, and discusses their implications for research and practice, acknowledging at the same time the limitations of this thesis.

9.1.1 Study 1: Enjoyment of life predicts T2D incidence over 12 years: Findings from ELSA

In Study 1 (presented in Chapter 3) data were analysed from 4134 diabetes-free participants who were followed-up biennially from 2004/05 up to 2016/17. Participants were drawn from ELSA, a nationally representative cohort study of adults 50 years and older living in England. I sought to examine the longitudinal association between two types of subjective well-being (hedonic and eudaimonic well-being) with incident T2D and to estimate the extent to which these associations could be explained by sociodemographic, behavioural, and clinical risk factors. The foundation of this study was research that has shown hedonic and eudaimonic well-being to be associated with better health and longevity in older adults (Cohen et al., 2016; Kim et al., 2019; Westerhof et al., 2012; Zaninotto & Steptoe, 2019). Studies examining associations between subjective well-being and T2D risk are scarce and the majority

of them have tested a single dimension of well-being or used a blended measure of overall well-being. None of the previous studies differentiated between hedonic and eudaimonic well-being in relation to T2D. Indeed, it is not common for the different aspects of well-being to be considered simultaneously within a single sample, preventing the ability to determine whether differential effects are evident for individual attributes of well-being. Nevertheless, consideration of the individual contribution of the different types of well-being is suggested in order to specify the role of well-being on certain health conditions (Hernandez et al., 2017; Steptoe, 2019). Therefore, this study has provided novelty to the field by assessing how the relationship between subjective well-being and T2D incidence could vary according to type of well-being. Additionally, even though previous studies demonstrated an independent association between subjective well-being and T2D (Boehm et al., 2015; Okely & Gale, 2016; Poole et al., 2019; Tsenkova et al., 2016), what was still unclear is the extent to which the link between well-being and T2D can be explained by sociodemographic, behavioural, and clinical characteristics. As described in Chapter 1, these factors are relevant to both subjective well-being and T2D, forming plausible mechanisms through which subjective well-being links to superior health. Study 1 addressed this gap in the field by estimating, for the first time, the degree to which the protective effects of well-being can be explained by sociodemographic, behavioural, and clinical risk factors.

Results from Study 1 revealed a protective relationship between hedonic well-being (as indexed by enjoyment of life score) and T2D rate over 12 years of follow-up after adjusting for several covariates. Sociodemographic, behavioural, and clinical

characteristics accounted for 27%, 27%, and 18% of the relationship between enjoyment of life and incident diabetes, respectively, compared to the unadjusted model. In contrast, the association between eudaimonic well-being (as indexed by purpose in life score) and T2D was attenuated in adjusted analyses, suggesting that there is no direct relationship between purpose in life and diabetes. One possible explanation for the null results is that the assessment of purpose in life was weak in this study. This limitation was discussed in Chapter 3 and is reiterated in the next sections of this chapter (see page 235 below). Nevertheless, these results add credence to value of testing these different dimensions separately. With regards to my secondary analyses, findings for enjoyment of life were maintained after the inclusion of depression in the secondary model. Also, associations between subjective well-being and T2D did not differ by age or sex.

Overall, Study 1 made a significant contribution to the field by showing, for the first time, that T2D risk is differentially related to hedonic and eudaimonic well-being. Additionally, Study 1 added to our understanding of the mechanisms through which subjective well-being is linked to reduced risk of T2D by demonstrating the role of sociodemographic, behavioural, and clinical pathways. Furthermore, this study built upon previous research by indicating that the protective effect of enjoyment of life is above and beyond the adverse effects of depression. Finally, the study supported a general protective effect of enjoyment of life which is not moderated by age or sex differences.

9.1.2 Study 2: Sex differences in IL-6 stress responses in people with T2D

Moving from T2D onset to prevalent T2D, Study 2 (presented in Chapter 5) used data from 121 patients with existing T2D drawn from the Diabetes Study (Steptoe, Hackett, et al., 2014). I sought to examine whether women and men with T2D respond differently to stress, in terms of inflammatory levels, particularly IL-6. The rationale for this study was that post-menopausal women with T2D show higher rates of some inflammatory-related conditions compared to their male counterparts, including CVD (Peters et al., 2014a, 2014b). These sex differences are not fully explained by conventional risk factors (Espeland et al., 2018; Raparelli et al., 2017). A small number of previous laboratory stress testing studies revealed that women show heightened IL-6 stress responses (Edwards et al., 2006; Endrighi et al., 2016; Lockwood et al., 2016; Steptoe, Owen, et al., 2002; Sullivan et al., 2018), suggesting that sex differences in stress-related inflammatory reactions may expose women to a higher risk of inflammatory-related conditions in the long-term. However, no previous laboratory stress testing studies were conducted in people with established T2D. Therefore, this study added to the literature by investigating sex differences in IL-6 stress responses in women and men with T2D.

Results from Study 2 showed that, following acute stress in the laboratory, IL-6 stress responses are greater in women with T2D compared to men with T2D after adjusting for covariates. Briefly, significant changes in women were detectable 45 minutes after the end of the tasks with IL-6 levels continuing to rise, reaching their peak at 75 minutes post-task. In contrast, IL-6 changes in men were more delayed, showing observable, albeit not significant increases at 75 minutes post-task. Secondary

analyses revealed that female participants perceived the tasks as more stressful than male participants did. Evidence from secondary analyses also showed that the effect of the two tasks on IL-6 stress responses was greater in older than younger women.

Overall, Study 2 made a novel contribution to the field by examining, for the first time, sex differences in inflammatory stress responses in people with established T2D. Sex differences in both subjective stress and objective inflammatory responses were evident, with women being more profoundly impacted by the stress experience than men. Importantly, this study formed the basis of the Diabetes Follow-up Study (presented in Chapter 8 and summarised on page 229 below).

9.1.3 Study 3: Daily happiness and inflammatory stress responses in people with T2D

In Study 3 (presented in Chapter 6) data were analysed from 122 participants with T2D drawn from the Diabetes Study. I sought to examine whether daily happiness can predict inflammatory levels and stress responses in people with T2D. The evidence that drove this study was that, in adults with existing T2D, subjective well-being and related constructs are longitudinally associated with more favourable health outcomes as well as longer survival (Celano et al., 2013; Huffman et al., 2015; Moskowitz et al., 2008; Yi et al., 2008). Interestingly, lifestyle factors do not fully explain the health-protective effect of subjective well-being (Chida & Steptoe, 2008; Giltay et al., 2006; Kubzansky & Thurston, 2007; Moskowitz et al., 2008; Ostir et al., 2000). Also, a limited number of previous studies showed that positive psychological factors, including hedonic well-being, are cross-sectionally associated with lower inflammatory (absolute) levels (Brouwers et al., 2013; Sin et al., 2015; Steptoe et al.

2008) and can predict diminished inflammatory stress responses in the laboratory (Aschbacher et al., 2012; Breines et al., 2014; Steptoe et al., 2005), suggesting that reduced inflammatory (re)activity might be a plausible underlying mechanism through which hedonic well-being may enhance better health and longevity. However, previous laboratory studies were carried out in healthy volunteers. Considering that inflammatory processes play a key role in the risk and progression of T2D (as described in Chapter 1), translation of this previous research in people with T2D would be important. Study 3 has contributed to the field in several key ways. Firstly, this is the first study to investigate associations between daily happiness and inflammatory (re)activity in people with T2D. Secondly, none of the previous studies recorded happiness ratings once-a-day for seven consecutive days in order to provide an aggregated measure of daily happiness over the week. In the study by Steptoe et al. (Steptoe et al., 2005), daily happiness was assessed by aggregating momentary experience ratings over a single working day. This strategy used by Steptoe et al. has improved ecological validity, since ratings are accomplished under naturalistic conditions. The measurement method used for Study 3 extended this approach, by collecting repeated measurements over several days; this is thought to provide a more reliable estimate of affect that is not biased by transient moods on a single day. What is more, Study 3 is the first to examine associations between subjective well-being and three different markers of inflammation (IL-6, IL-1ra, MCP-1) before and after laboratory stress. Considering that IL-1ra and MCP-1 are a) sensitive to psychological factors (Asberg et al., 2009; Hackett et al., 2012; Rajagopalan et al., 2001; Suarez et al., 2003), b) stress-responsive (Hackett et al., 2012; Marsland et al., 2017; Steptoe et al., 2007), and c) relevant to diabetes complications, particularly CVD (Danesh et al.,

2008; Herder et al., 2006; Tang et al., 2007; van Minkelen et al., 2009), these inflammatory factors were also selected as interesting markers to explore.

Results from Study 3 showed that the stress tasks elicited significant increases in subjective stress levels and in IL-6, lending support to the validity of the mental stress tasks used in the Diabetes Study. However, the two tasks did not translate into significant increases in IL-1ra or MCP-1. Regarding the main findings of the study, greater daily happiness significantly predicted lower IL-6 levels pre-stress and post-stress, and reduced MCP-1 levels pre-stress, independent of covariates. No significant relationships were observed for happiness and IL-1ra levels. Nevertheless, this study assessed only one dimension of subjective well-being, and as reported earlier, consideration of the different types of well-being is meaningful in health research. It can be, for example, that IL-1ra shows more consistent associations with other aspects of well-being, but this hypothesis requires direct testing in future studies[86]. Contrary to hypothesis, changes in markers were similar in magnitude across different happiness levels. It is not clear why well-being does not influence inflammatory responses in this patient group. Inflammatory dysregulation in this population, reflected in both pre-task absolute levels and IL-6 stress responses compared to healthy people (Steptoe, Hackett, et al., 2014), may have accounted for the null findings, but replication of these results is warranted.

Overall, Study 3 shed light on some previously unexplored associations by examining, for the first time, the effect of daily happiness on three different inflammatory markers throughout laboratory stress testing. The study showed that, in people with

[86] Further suggestions for future research are provided on page 244.

T2D, subjective stress and IL-6 increase after stress tasks. Notably, the current study extended previous literature by demonstrating, for the first time, that happier individuals with T2D have lower IL-6 and MCP-1 levels pre-stress (for both markers) and post-stress (for IL-6). Importantly, Study 3 formed the basis of the Diabetes Follow-up Study that is summarised below.

9.1.4 Study 4: Sex and daily happiness as predictors of future health outcomes in T2D: The mediating role of IL-6 stress responses

Study 4 (presented in Chapter 8) is a follow-up investigation of the Diabetes Study that built upon findings from the two preceding laboratory studies, studies 2 and 3. Data were collected and analysed from people with T2D (N = 66) who had previously taken part in the Diabetes Study, 7.5 years earlier. Taking the results of studies 2 and 3 into consideration, I sought to examine their long-term health implications, by testing associations between my baseline predictors of interest (sex, daily happiness) and future health outcomes (doctor-diagnosed CVD, number of hospital admissions over the previous years, physical and mental health-related quality of life), and by exploring the potential mediating role of IL-6 (re)activity (my primary focus was on IL-6 *stress responses* but IL-6 absolute levels were also studied in secondary analysis).

Study 4 makes a novel and significant contribution to the field for numerous reasons. Firstly, the majority of the research on mechanisms published to date has been cross-sectional, and relatively few longitudinal studies linking inflammatory stress responses to future health have been published. Additionally, even though existing laboratory studies with a follow-up design demonstrated significant associations between increased inflammatory stress responses and poorer CVD risk profile in the long-term

(Brydon and Steptoe, 2005; Ellins et al., 2008; Ellins et al., 2017; Steptoe et al., 2016), none of these studies were carried out in people with T2D, who face a substantially elevated risk of CVD. Also, while these previous studies suggested that increased inflammatory responsivity may be a mechanism through which psychological factors contribute to ill-health, only one study formally tested the mediating role of inflammatory responsivity using mediation analysis (Aschbacher et al., 2012). Additionally, Study 4 considered mental health outcomes (as indexed by mental health-related quality of life). Mental health-related outcomes have received less attention in follow-up studies of biological stress reactivity. Given that increased inflammatory cytokines, including IL-6, can induce negative mood symptoms (Rosenblat et al., 2014), mental health outcomes are important to be considered, especially in people with T2D, who face an increased risk of mental health disturbances and subsequently lower quality of life (Arditi et al., 2019; Fisher et al., 2008; Jannoo et al., 2017; Rotella & Mannucci, 2013; Smith et al., 2013). The study by Aschbacher and collegues (Aschbacher et al., 2012), which tested inflammatory (IL-1β) stress responses in relation to follow-up depressive symptoms, is limited by its sample size (N = 35 healthy, post-menopausal women) and short follow-up period of one year. In contrast, Study 4 included a larger sample size of both women and men with T2D and a follow-up of 7.5 years on average.

Study 4 confirmed previous research by demonstrating that women with T2D experience worse mental health-related quality of life versus men with T2D after adjusting for covariates. No sex differences were observed for the other outcomes of interest, and no significant associations were found between daily happiness and

physical or mental health outcomes. Interestingly, reduced IL-6 stress responses at 45 minutes (but not the other time points post-stress) predicted superior mental health-related quality of life at follow-up after adjusting for covariates. Opposed to my expectations, IL-6 stress responses were not associated with physical health outcomes. Similarly, IL-6 absolute levels did not predict physical or mental health outcomes in this study. Finally, mediation analysis (carried out to test whether sex differences in mental health-related quality of life are mediated by IL-6 stress responses at 45 minutes) was not significant. Therefore, the sex – mental health relationship was not mediated by IL-6 stress responses in this study.

Findings from Study 4 should be interpreted with caution. Despite its longitudinal design, this study may have low statistical power (low power increases the probability that the test will accept the null hypothesis when it is false [Kang, 2013]). Indeed, one of the major limitations of this study is the fact that 74 out of 140 initial participants did not take part at follow-up, resulting in a final sample of just 66 individuals. This limitation and ways to minimise loss to follow-up are discussed more broadly in the sections below (pages 241 and 249, respectively). Nevertheless, findings from Study 4 significantly added to evidence in the field by introducing, for the first time, the prognostic value of IL-6 stress responsivity in the risk of mental health disturbances in people with T2D.

Taken together, this PhD work has shed light on some previously opaque avenues, and in combination these studies contribute to the knowledge linking individual factors such as sex and subjective well-being, stress-related inflammatory processes, and T2D risk and progression.

9.2. Methodological issues and limitations

The results presented in this thesis need to be interpreted in light of their limitations. The shortcomings of the individual studies were mentioned at the end of each chapter. In this section, the most important methodological issues and limitations of the studies comprising this PhD will be highlighted and discussed.

9.2.1 The study samples

For this PhD, two different study samples were used. Study 1 participants were drawn from ELSA, a nationally representative cohort study of adults aged 50 years and older who live in England. Study 1 involved a large sub-sample of ELSA, including 4134 participants free from diabetes at baseline (2004/05). The samples for Study 2 (N = 121) and Study 3 (N = 122) were drawn from the Diabetes Study. The Diabetes Study was conducted by the Psychobiology Group at UCL in 2011/2 and included participants with established T2D. The Diabetes Follow-up Study (Study 4) was a follow-up examination of the Diabetes Study involving 66 participants who responded to a follow-up invitation.

Participant samples are not without limitations. In ELSA, while the entire sample is nationally representative, missing baseline and follow-up data substantially reduced the size of the analytic sample in Study 1. The analytic sample (N = 4134), consisting of participants with full data on exposure and outcome variables and all covariates, was compared to those excluded due to missing data (N = 4646). Significant differences were observed in sociodemographic, behavioural, and clinical characteristics between the two samples, and such differences may have introduced bias to the study results. For example, compared to those excluded due to missing

data, participants of this study were younger, wealthier, and healthier on average, and they were more likely to engage in health behaviours such as more frequent physical activity and not smoking.

The Diabetes Study involved strict inclusion criteria. More precisely, enrollment was limited to people with T2D and no history or previous diagnosis of CHD, inflammatory diseases, allergies, or mood disorders. Even though strict inclusion criteria may minimise the risk of extraneous factors being present, these may also restrict the external validity of the results (Patino & Ferreira, 2018). For example, by restricting the sample to people with T2D and without CHD raises issues about the representativeness of the study, since a large number of older people with T2D (up to 29.1%) have comorbid CHD (Einarson, Acs, Ludwig, & Panton, 2018).

The number of participants who met the inclusion criteria at the outpatient diabetes clinics was low (10%), and as a result the majority of participants were recruited from primary care practices. However, the study response rate was difficult to assess as primary care practices differed substantially in their ability to record the number of screened medical records. For example, patients' paper records were hand searched by the study researchers in some practices. In others, the practice shared the addresses of eligible participants, while in the remainder, the practice administrators themselves searched for eligible individuals and sent out the recruitment letters independent of the study group[87]. Subsequently, for many practices, the study researchers could not estimate the number of eligible participants or the number of

[87] The Diabetes Study was carried out before the General Data Protection Regulation was in place, therefore it allowed for the specific recruitment strategies to be performed.

invitation letters sent out before an interested person contacted the study team at UCL.

All primary care practices that were approached to provide participants for the Diabetes Study were located in the London borough of Camden. The fact that recruitment was limited to a single area of London raises issues about the representativeness of the study sample. More specifically, evidence from the Camden Joint Strategic Needs Assessment (Camden Joint Strategic Needs Assessment, 2014) demonstrates a large difference between the number of people with T2D and the number of expected T2D cases in Camden, and that this chasm is significantly larger compared to London or England as a whole. This suggests that a significant number of individuals with T2D in Camden are undiagnosed hence these individuals were not identified using the recruitment strategy applied in the Diabetes Study.

Selection bias can alter study findings in cases where potential participants with certain characteristics are more or less likely to be selected for the study, rendering them over- or under-represented in the study than they are in the general population. Participants of the Diabetes Study were blind to study hypotheses, reducing the risk of selection bias. Additionally, it is unlikely that individuals with a greater vulnerability to stress were more prone to be selected since people with a diagnosed mood disorder were excluded from the study. Nevertheless, mood disorder diagnosis (along with the other inclusion criteria of the study) was self-reported by the participants instead of being screened using questionnaire measures of depression. Specifically, potentially eligible participants were asked during a telephone interview and on the

day of stress testing whether they had any co-morbidities that would exclude them from participating in the study.

All participants of the Diabetes Study were fluent in the English language. Fluency in English was necessary in order for participants to be able to successfully complete the study's self-reported questionnaires. Therefore, the sample is representative of the wider London community. Most participants (80%) of the Diabetes Study were of white ethnicity. This limitation is important, especially considering the fact that people of South Asian and African-Caribbean origin who live in London have a significantly greater risk of diabetes than white ethnic groups (Tillin et al., 2013). Similarly, and with regards to Study 1, there are very few ethnic minority participants in ELSA (1%), as establishing representative oversamples of ethnic minority groups was prohibitively expensive (ELSA, 2019). The small number of ethnic minorities in ELSA is still nationally representative. However, because of the lack of diversity within study samples, it was not possible to test for ethnic differences in study findings. Nevertheless, my PhD research was limited to analysing data from existing datasets, therefore it was not likely for me to amend the recruitment processes. Additionally, adjusting for ethnicity in analyses of studies 1, 2, and 3 did not change the pattern of results observed.

9.2.2 The study measures

The ELSA study could be criticised for its single purpose in life measure, as a source of measurement error and consequently, residual confounding. Indeed, as suggested by previous research (Czekierda et al., 2017), a combined measure of meaning in life and meaning-related sense of harmony, peace, and well-being shows more robust associations with physical health outcomes compared to a single measure that focuses

solely on meaning in life. The purpose in life measure of the Ryff Psychological Well-being Scales, which has been widely used in previous studies, was only administered to a sub-sample of ELSA participants hence it would result in a much smaller sample size had it been selected for inclusion in Study 1. Additionally, one-off assessments of enjoyment of life and purpose in life were used in Study 1. Repeated measurements could provide a more reliable estimate of well-being over time that reflects chronic rather than situational well-being. Nevertheless, the temporal stability of subjective well-being has been previously documented (Charles et al., 2001; Lucas, & Gohm, 2000). In ELSA, doctor diagnosis of diabetes over the follow-up was self-reported instead of being assessed objectively using HbA1c measurements. Self-reported outcome measures rely on the completeness and accuracy of the recorded information (Song & Chung, 2010). The reason why self-reported diagnoses rather than clinical assessments of blood glucose were chosen is because the latter are only carried out every four years (in ELSA's clinical waves). Furthermore, HbA1c data are missing for many participants in ELSA, hence assessing T2D with HbA1c data would result in a smaller sample size. Excellent agreement between self-reported doctor diagnosis and validated diagnosis of diabetes has been reported in a study of more than 1,000 older participants independent of age, education, exiting comorbidities, and cognition (Simpson et al., 2004). Additionally, HbA1c data were used in sensitivity analysis in order to exclude individuals with undiagnosed T2D at baseline and thence minimise the possibility of reverse causality.

With regards to the biological measures of studies 2, 3, and 4, these included three inflammatory markers (IL-6, IL-1ra, MCP-1). All three markers have been used in the

analyses presented in Study 3. Findings from Study 3 demonstrated that, in people with T2D, IL-6 is the only marker to show significant increases in response to two mental stress tasks in the laboratory, adding value to measuring IL-6 stress responsivity in this patient group. Considering this finding, Study 2 and Study 4 were solely focused on IL-6 (re)activity. One of the key limitations of these studies is that missing inflammatory data occurred for about one quarter of participants due to technical difficulties with blood sampling, especially in obese individuals. As expected, sensitivity analyses showed that participants with missing inflammatory data were more likely to be obese compared to participants with full biological data. Complete case analysis was used for the main analyses, but such a strategy subsequently resulted in a smaller sample size in all three studies. Evidence from previous laboratory stress testing studies has shown that blunted cardiovascular and neuroendocrine stress responses are cross-sectionally related to obesity (Jones et al., 2012; Phillips, 2011), as well as increase the likelihood of becoming obese longitudinally (Carroll et al., 2008; de Rooij, 2013). If participants with T2D and higher obesity show blunted inflammatory stress responses, missing data from obese individuals may have produced biased estimates in this study. 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', which refers to a transient elevation in biological levels that is exhibited in response to observation during measurement. So, although pre-task blood sampling was taken 30 minutes into the rest period, it is possible that the white coat effect could have impacted the measurements of the study. Also, even though the consideration of three different markers of inflammation is an important

advantage of the Diabetes Study, one limitation of studies 2, 3, and 4 is that they only focused on inflammatory processes. Given the interplay between the immune system and other stress-responsive biological systems (as described in Chapter 1), the assessment of other biomarkers would have been valuable. For example, potentially relevant biological markers that were not assessed are glucose and insulin. Stressful tasks in the laboratory induce increases in both glucose and insulin in healthy participants (Nowotny et al., 2010; Picard, Juster, & McEwen, 2014), and may alter glucose control in people with T2D (Faulenbach et al., 2012). Even though glucose metabolism is a key issue in T2D risk and progression, glucose and insulin stress responses were not assessed in the Diabetes Study because these measures were not available in the HSS which focused primarily on healthy individuals rather than people with metabolic disturbances (as reported earlier in Chapter 4, the Diabetes Study was set up as a comparison study to the HSS).

Outcome measures of the Diabetes Follow-up Study were self-reported. These included doctor diagnosis of CVD over the follow-up period, number of hospital admissions over the follow-up period, and physical and mental health-related quality of life at follow-up. Self-reported clinical measures might be susceptible to recall errors (Bhandari & Wagner, 2006; Short et al., 2009). Nevertheless, self-reported clinical data are increasingly used in health research as they have been found to be accurate for hospital admissions-related data (Raina et al., 2002; Seidl et al., 2016) and well-defined chronic conditions such as MI and stroke (Bush et al., 1989; Jamrozik et al., 2014; Kehoe et al., 1994; Okura et al., 2004; Paganini-Hill & Chao, 1993; Tretli et al., 1982), independently of age, sex, ethnicity, and education (Kehoe et al., 1994). For

example, in a nationally representative study of over 10,000 participants in the US, it was found that self-reports of hospitalisations for ischemic heart disease and stroke were confirmed as accurate for 84% and 67% of cases, respectively (Bergmann, Byers, Freedman, & Mokdad, 1998). As reported in Chapter 7, the initial strategy regarding the outcome measures of this follow-up was to collect both self-reported and clinical-records data that are relevant to health. However, at the time of writing this thesis, just nine out of 31 primary care practices had agreed to provide data for the study, covering only 13 out of the 69 consented participants. Given the small number of responding practices and due to time constraints, clinical-records data were not collected for the purposes of this thesis, but efforts to obtain medical data will continue. Finally, although studies 1, 2, and 3 have adjusted their analyses for multiple covariates, the small sample size of the follow-up study limited the number of covariates to be included in analyses, hence increasing the possibility of residual confounding as a ramification of unmeasured variables. Nevertheless, key covariates, such as baseline BMI, were assessed objectively using standardised methods.

9.2.3 Causality

Two out of the four studies presented in this thesis were of cross-sectional nature, something that precludes any inference about causality. For example, Study 2 did not allow the investigation of the background factors that may have contributed to significant sex differences in IL-6 stress responsivity in this patient group. Similarly, in Study 3, the direction of the relationship between happiness and lower IL-6 and MCP-1 concentrations could not be examined. It might be that feelings of happiness have a regulatory effect on stress systems, including the immune system, or, in the opposite

direction, that higher inflammatory levels act centrally to induce mood symptoms (as discussed in Chapter 6). Nevertheless, the two studies generated hypotheses to be investigated using the follow-up design of Study 4.

Although studies 1 and 4 were longitudinal analyses, this does not assure that there is a causal relationship between the exposures and outcomes of interest. For example, we cannot conclude that enjoyment of life or male sex causes more favourable health outcomes; other factors may be involved in the pathway. Indeed, this limitation of observational studies is particularly relevant to subjective well-being, which is embedded in a matrix of other health-related phenomena (Steptoe, 2019b). Controlling for sociodemographic, behavioural, and clinical factors in the relationship between well-being and health does not mean that these factors are not relevant and does not tease out the complexity of these relationships. Naturally this issue also applies to the other studies of this thesis.

Based on the Bradford Hill's criteria, the case for causation is strengthened when 1) the association between exposure and outcome is strong (strength), 2) the same link is reported consistently using a variety of methods and in different populations (consistency), 3) the association is limited or is greatly increased in specific groups of people with a particular environmental exposure (specificity), 4) the exposure precedes the outcome (temporality), 5) a biological gradient or a dose-response relationship is manifested (biological gradient), 6) there is a biological plausibility of the relationship based on current knowledge (biological plausibility), 7) the cause-and-effect interpretation fits with the known facts of the natural history and biology of the

disease (coherence), 8) there is experimental evidence to support the association (experiment), and 9) analogies are present (analogy; Lucas & McMichael, 2005).

The issue of reverse causality (when an outcome of interest is already present, but mistaken, at the time of initial assessment) is particularly relevant in conditions which develop slowly over many years. This hypothesis was unsubstantiated in Study 1, since the results did not attenuate after excluding participants with undiagnosed diabetes at baseline and participants who developed T2D within two years of baseline, adding weight to the temporal sequence between well-being and T2D. Additionally, experimental evidence from Study 3 complements the longitudinal association between hedonic well-being and T2D found in Study 1.

Another well-recognised drawback of observational cohort studies is confounding. Indeed, adjusting for potential confounding factors does not fully exclude the possibility that unmeasured or imperfectly measured factors account for the relationship. This limitation is particularly relevant to Study 4, where the small sample size of the study restricted the number of statistical adjustments, as reported previously in Chapter 8.

Another potential source of bias in cohort studies is loss to follow-up, and this is a key limitation of Study 4. The sample in Study 4 was reached with invitation letters which were posted to participants' home addresses. Information on home addresses (but not telephone numbers) was obtained during the initial Diabetes Study and was available for all 140 participants. However, some participants (*n* = 11) likely moved to a new house in the interim period, as invitation letters for these participants were returned to UCL as undelivered. Therefore, these participants were not recruited to

take part in the follow-up study. Also, at least eight participants had died over the follow-up period, and 52 participants failed to respond to the study invitation, despite the fact that we provided incentives to participate (a £20 retail voucher) as a means to maximise retention of participants. Information on the reasons of the withdrawal should be obtained to be used in analyses. However, given the limited ways of communication between the study team and participants, this was not feasible. Loss to follow-up resulted in a small sample size (N = 66), potentially reducing the statistical power of Study 4. Also, systematic differences relating to the outcome or the exposure measures between people who drop out and those who continue can introduce attrition bias to the study results, thus may reduce the internal validity of the study (Song & Chung, 2010). For example, in Study 4 it was found that individuals who did not participate at follow-up were of lower education, had higher IL-6 levels (pre- and post-task), and experienced higher depressive symptoms during the baseline assessment. Adverse psychosocial and inflammatory profiles may be related to an increased risk of ill-health in the long term, therefore if losses to follow-up were maintained in the study then the study findings may have been different. A general rule of thumb states that > 20% losses to follow-up poses serious threats to validity of observational cohort studies (Merril & Trimmreck, 2006).

9.2.4 Limitations of the laboratory stress testing protocol

The advantages and disadvantages of laboratory stress testing studies have been discussed in the introductory chapter. In brief, laboratory 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, have been previously 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 (Steptoe, Feldman, et al., 2002). However, these tasks are not without limitations. One of the main limitations relating to the stress tasks is that the Stroop task was computer-based. The age range in the Diabetes Study was 50 - 75 years and older participants may have less experience using a computer versus younger participants. Previous experience with computers could have impacted participants' performance and subjective task ratings (e.g. stressfulness, difficulty). In contrast, in the case of the mirror tracing task, computer literacy was unlikely to have influenced performance or subjective task ratings. The two tasks are commonly used in stress research, usually in combination. However, there was no socially evaluative element to the stress session, which could have potentially elicited greater inflammatory responses (Dickerson & Kemeny, 2004). A previous study, for example, showed that IL-1ra levels increase two hours after exposure to a social stress task. Indeed, in order to obtain a comprehensive snapshot of how an individual would respond to a stressor encountered in real-life settings, laboratory stressors should have ecological validity and be representative of experiences in natural settings. In natural settings, exposure to social evaluation is omnipresent; for example, giving presentations and being monitored during the performance of tasks in the workplace are commonplace and involve perceived threats to ones' abilities, traits or competencies (Gruenewald, Kemeny, Aziz, & Fahey, 2004). Therefore, a laboratory paradigm that is representative of these situations would be advantageous. Another limitation of the laboratory stress testing protocol was the timing of post-stress blood sampling. Although the Diabetes Study was designed to capture post-stress reactivity and recovery levels, longer post-stress

sampling could be required for the optimal measurement of IL-6 recovery, since IL-6 is known to peak at 90 minutes post-stress as shown in a meta-analysis of previous laboratory studies (Marsland et al., 2017). Also, as mentioned previously in Chapter 6, there is evidence to support that IL-1ra shows delayed increases, captured at 90 minutes (Rohleder et al., 2006) and 120 minutes (Steptoe et al., 2001) post-stress. Therefore, it is possible that the stress effects in IL-1ra may have been missed due to the limited sampling duration. Nevertheless, repeated blood sampling over a longer period may have been intrusive and stress-inducing, which in itself may also influence biomarker levels.

9.3. Suggestions for future research

Specific suggestions for future studies have been provided in the Discussion section of each chapter, particularly in view of the limitations of each study. In this sub-section, I will highlight ways to address the most important limitations in future studies as well as provide more general ideas for future work in the field.

With regards to Study 1, ELSA is ongoing (Wave 9 data collection was completed in 2019 [ELSA, 2019]), and therefore continues to present numerous possibilities for research. For example, future analyses of ELSA data could make use of the repeated assessments of subjective well-being over time. Indeed, most studies to date have assessed subjective well-being on a single occasion. However, the duration of the exposure may also be important in this relationship. Repeated reports over several years may have a stronger association with physical health outcomes than reliance on a single measurement point, and evidence of a time-dependent exposure effect would increase confidence for a potential causal association. For example, Iob et al. (Iob,

Kirschbaum, & Steptoe, 2020) has assessed the longitudinal persistence of depressive symptoms over a 14-year period using Trait - State - Occasion structural equation modelling. Future studies can use this method to measure the maintenance of subjective well-being over time. Additionally, while some studies (including Study 1) have used a single purpose in life measure, research has suggested that a combined measure of meaning in life and meaning-related sense of harmony, peace, and well-being may be preferable as it shows more robust associations with physical health outcomes compared to a single measure that focuses solely on meaning in life (Czekierda et al., 2017). To add to this point, although most of the previous studies have treated purpose in life and meaning in life as synonymous constructs, emerging work has resulted in the development of a tripartite model of meaning in life, consisting of three aspects: 1) purpose in life, 2) coherence[88], and 3) significance[89] (George & Park, 2016; Martela & Steger, 2016). Therefore, in order to identify the best potential intervention targets for reducing T2D risk, observational cohort studies could also assess the other facets of meaning in life. This also applies to the different facets of hedonic well-being (e.g. enjoyment of life versus happiness), some of which may show stronger associations with T2D. Another key point that requires attention is whether the protective association between enjoyment of life and incident T2D (as observed in Study 1) is mediated via biological factors, such as reduced inflammatory (re)activity. Fibrinogen data are available in ELSA, and considering that fibrinogen (re)activity is sensitive to subjective well-being (Steptoe et al., 2005) and relevant to

[88] Coherence refers to the degree to which people perceive their lives as making sense (George & Park, 2016; Martela & Steger, 2016).
[89] Significance refers to the degree to which people feel that their existence is of significance, importance, and value in the world (George & Park, 2016; Martela & Steger, 2016).

physical health outcomes (Brydon & Steptoe, 2005; Ellins et al., 2008; Ellins et al., 2017; Steptoe et al., 2016), fibrinogen would be an interesting marker to assess in future work. In addition, over the last decades, several studies have demonstrated that cellular aging markers, including telomere length, mitochondrial function, markers of oxidative stress (e.g. 8-hydroxy-2-deoxyguanosine levels, superoxide dismutase), and inflammatory markers intertwined in complex networks that shape their roles in cellular senescence (Liu et al., 2019; Zhu, Liu, Ding, Wang, & Geng, 2019; Zole & Ranka, 2018). These factors have been reported to be associated with multiple age-related conditions, including T2D (D'Mello et al., 2015; Liu et al., 2020; Salpea et al., 2010; Willeit et al., 2014; Zhao et al., 2014). The complex interactions of these markers are also possible avenues of exploration in relation to T2D.

With regards to the Diabetes Study limitations, and for the sake of advancing the quality of future research, recruitment of participants with T2D should be carried out across different areas of London in order for researchers to ensure that the sample is representative of the wider population of individuals with T2D who live in London. For example, a more divergent sample would be expected taken that people of South Asian and African-Caribbean origin who live in London have a significantly greater risk of T2D than white ethnic groups (Tillin et al., 2013). And even though Camden is an ethnically diverse area, as mentioned previously, many people with T2D remain undiagnosed in Camden (Camden Joint Strategic Needs Assessment, 2014). Additionally, as described previously, one of the key limitations of studies 2 and 3 is that full biological data were collected for only about three quarters of the sample due to practical difficulties with blood sampling. Indeed, blood sampling is invasive and

may be burdensome to collect for both researchers and participants. An alternative, non-invasive means to assess systemic inflammation may be the use of salivary cytokines. Research examining the utility of salivary markers of inflammation has grown rapidly over the past five years. A review of laboratory stress testing studies concluded that IL-1β, TNF-α, and IL-6 can be reliably determined from saliva and they show significant increases in response to laboratory stress, with effect sizes ranging from very small to very large (Slavish et al., 2015). Furthermore, salivary markers of inflammation, including CRP and IL-6, have been cross-sectionally associated with psychological characteristics such as daily happiness, vital exhaustion, depressive symptoms, and post-traumatic stress symptoms (Newton, Fernandez-Botran, Miller, & Burns, 2014; Sjögren, Leanderson, Kristenson, & Ernerudh, 2006; Slavish et al., 2019; Wang, Mandel, Levingston, & Young, 2016). There is also evidence for connections between salivary cytokine responses and brain activity. Specifically, in women who experienced a recent loss, salivary IL-1β and soluble TNF receptor II correlated positively with activation of the prefrontal cortex and anterior cingulate cortex after a grief elicitation task (O'Connor, Irwin, & Wellisch, 2009). Albeit limited, evidence from existing studies encourages further investigation of the utility of salivary measures as well as more basic research on the origin of inflammatory markers in saliva and on potential daily rhythm (Out, Hall, Granger, Page, & Woods, 2012). Most importantly, future studies with larger sample sizes should explore the predictive validity of saliva cytokines (re)activity on targeted disease outcomes, as well as demographic and psychological moderators of salivary concentrations and stress responses. Another advantage of salivary collection is that it may allow the application of naturalistic pre-test measurements as a means to rule out the white coat effect. While comparisons

of absolute concentrations of biomarkers obtained through different means can be problematic, this is less critical when examining change in biomarkers over time. In the laboratory, salivary measurements may also enable a more extensive sampling to be carried out. Indeed, a longer sampling period (e.g. up to 120 minutes following stress) would be an important asset for future studies which aim to measure the course of inflammatory markers such as IL-6 or IL-1ra.

With regards to Study 3, future qualitative studies could assess observable happy feelings (e.g. using records of facial signs of emotional experiences under naturalistic conditions) as this could provide a different perspective in understanding the relationship between daily happiness and inflammatory stress reactivity. Additionally, as reported earlier, consideration of the different types of well-being is meaningful in health research. It could be, for example, that IL-1ra shows more consistent associations with different aspects of well-being, and this hypothesis requires direct testing in future studies. Another suggestion for studies of stress reactivity would be to investigate the effects of individual factors (e.g. happiness) on multiple systems' function in people with T2D, and the long-term health implications. Cardiovascular and cortisol data are available in the Diabetes Study, and by examining the effect of a psychological factor upon the whole system's function would give us a more detailed picture of the underlying mechanisms and would pave the way for the examination of the predictive validity of an aggregated biological risk score. It is also important for future investigations to assess glucose and insulin stress responses (these were not measured in the Diabetes Study) as these may also be key predictors of T2D risk and progression. Further inflammatory factors that are relevant to assess, as shown in

laboratory stress testing studies, include IL-1b, fibrinogen, and TNF-α (fibrinogen, in particular, shows consistent associations with physical health outcomes in follow-up studies, as described in Chapter 1). Not only biological markers' levels but also the cellular sensitivity to these markers would also be an interesting pathway to search. Given the anti-inflammatory effects of GCs, the measurement of GC sensitivity would be particularly important in this context. A previous study has shown that the condition of T2D is associated with increased GC sensitivity pre-stress (measured by quantifying the action of dexamethasone on IL-6 levels) compared to a healthy control group. Additionally, in contrast to the control group, which showed decreased GC sensitivity in response to stress, GC sensitivity remained stable in the diabetes group post-stress (Carvalho et al., 2015). These findings are in line with the blunted IL-6 stress responses found in T2D compared to healthy controls (Steptoe, Hackett, et al., 2014). Following on from findings of studies 2 and 4, further sex-specific research on health outcomes in T2D must be stressed. One line of research would be to have clear insight on what contributed to sex differences in inflammatory responsivity in this population, and why women with T2D experience had worse mental health-related quality of life compared to men with the condition. Future studies would benefit from a careful consideration of sex as a potential moderator of the relationship between psychological factors and health outcomes in T2D.

Referring to the follow-up study, future investigations should try to minimise the number of participants who drop out from the study. One useful strategy to prevent losses to follow-up is to ensure good communication between researchers and participants using effective communication channels (e.g. contact to be carried out via

telephone instead of post). For example, future follow-up studies can maintain telephone communication with the initial study participants about plans to be re-contacted for future research. Indeed, participants from the Diabetes Study were not warned that they might be re-approached again in a few years' time. Telephone communication would also enable researchers to track any changes in participants' personal details (e.g. home address details). These strategies certainly imply that a prior permission was provided by the participants, personal data are securely stored, access is limited to authorised researchers, and anonymity is maintained, as required by the General Data Protection Regulation. Another strategy to help prevent losses to follow-up is to limit the amount of data collected in order to reduce the burden for participants. This can be achieved by collecting only the essential information. With regards to outcome measures, objective medical data should be assessed. Objective clinical data can also be expanded to include mental health outcomes (e.g. major depressive disorder diagnosis), while nurse visits at participants' homes would allow objective measurements to be carried out (e.g. blood sampling for HbA1c measurements or objective anthropometric assessments). Nevertheless, such objective measurements carried out by trained staff demand considerable financial investment.

Finally, and with regards to study design and causality, firm conclusions would be strengthened by randomised intervention studies aimed at measuring whether the changes in the exposure result in changes in the outcome, but such a method would require longitudinal trials that involve robust methods of modifying the exposure over prolonged periods. Randomised controlled studies are described in the final section

of this chapter (page 251). In the meantime, observational cohort studies can be complemented by other approaches that might produce more compelling findings than those emerging from observational work, such as Mendelian randomisation and regression discontinuity analyses. Mendelian randomisation studies sit at the interface between conventional observational studies and randomised controlled trials (Davies, Holmes, & Davey Smith, 2018). These use genetic variants, which are fixed at conception, to support, under certain assumptions, causal associations between an exposure and an outcome using observational data (Davies et al., 2018). Comparable to randomised clinical trials, Mendelian randomisation studies create groups that are determined by genotypes and are independent of confounding factors. In theory, if the groups form a significant association with the outcome, the relationship is independent of confounders and is via the exposure (Davey Smith & Hemani, 2014; Kuo, Pilling, Kuchel, Ferrucci, & Melzer, 2019). Mendelian randomisation studies may produce more compelling findings than traditional observational studies as they are less susceptible to confounding effects, measurement errors, or reverse causality bias (Davies et al., 2018; Kuo et al., 2019). Regression discontinuity design is a form of quasi-randomisation in an observational setting that can be used, in some cases, to estimate the impact of an intervention on a specific outcome of interest. Regression discontinuity studies make use of a clinical cut-off point and assume that individuals who are just above the cut-off point and those who are just below belong to the same population. Based on this assumption, the treatment is made available to people who are just above the threshold and is not provided to those who are just below the threshold (O'Keeffe et al., 2014). By

assigning people to well-being interventions based on BMI or HbA1c clinical cut-offs might be a way to harness this design in well-being and health research.

9.4 Implications

9.4.1 Well-being and lifestyle interventions for the prevention of T2D

Results from Study 1 showed that enjoyment of life was associated with a decreased rate of incident T2D over the follow-up period of 12 years. These findings highlight the role of enjoyment of life on glucoregulation and, ultimately, diabetes prevention and have implications for positive psychology interventions to reduce the risk of T2D.

Positive psychology is a fast-moving research area with great promise. Positive psychology interventions aim to boost positive emotions, behaviours, and thoughts via structured exercises such as expressing gratitude, using strengths, savouring positive experiences, performing acts of kindness, and mindfulness (Bolier et al., 2013; Sin & Lyubomirsky, 2009). They differentiate from traditional psychological interventions that were designed to address an emotional or behavioural problem in that positive psychology interventions were developed to enhance subjective well-being across different populations, including those with and without emotional or behavioural difficulties (Feig et al., 2019). This is an important asset of positive psychology interventions compared to other intervention programs. As described in Chapter 1, positive and negative emotions operate on separate *continua*; The absence of negative affect does not by definition imply that one is experiencing psychological well-being (Keyes, 2005). Positive psychology interventions operate from this conceptualisation; Subjective well-being is not just the absence of negative affect or

mental health problems, but instead is the presence of positive psychological resources (Sin & Lyubomirsky, 2009).

There is currently a substantial literature on positive psychology interventions and their efficacy in enhancing subjective well-being in both healthy and diseased individuals. Findings from previous studies showed that positive psychology interventions have favourable effects on reducing psychological distress (symptoms of depression, anxiety, and stress) and increasing well-being, positive affect, quality of life, and life satisfaction (Allen, Wetherell, & Smith, 2020; Bawa et al., 2015; Bolier et al., 2013; Chakhssi, Kraiss, Sommers-Spijkerman, & Bohlmeijer, 2018; Frawley, 2015; Gotink et al., 2015; Hendriks, Schotanus-Dijkstra, Hassankhan, de Jong, & Bohlmeijer, 2019; Sin & Lyubomirsky, 2009; Smith, Thompson, Hall, Allen, & Wetherell, 2018). Notably, a meta-analysis of single-component intervention studies (including activities that target one component of well-being [Boiler et al., 2013]) and a meta-analysis of multi-component interventions (including activities that target more than one construct of well-being [Hendriks et al., 2019]) demonstrated similar effects on increasing well-being and reducing depression symptoms post-interventions. These results suggest that targeting only one component (e.g. enjoyment of life) can be as effective as targeting multiple components. For example, interventions targeting enjoyment of life may include 1) exercises that help individuals to identify those activities they enjoy the most in their daily life and their wider concept; when, where, how, why, with who, 2) exercises that boost full consciousness so as for individuals to learn to be 'at present' when experiencing joyful moments, and 3) enhancement of these activities on a daily basis. Single component interventions may be easier for

participants to grasp and exercise and cheaper to deliver compared to multi-component trials. Given that the well-being programs require a trained interventionist, the feasibility and cost of disseminating such a program in a healthcare setting is important to consider.

As mentioned above, positive psychology interventions seem efficacious for eliciting favourable changes in well-being in both clinical and non-clinical populations. With regards to people with established diabetes, a recent systematic review identified 34 well-being interventions in participants with T1D and/or T2D (mostly group-based interventions in adults with T2D [Massey et al., 2019]). These included positive psychology interventions, mindfulness-based programs, acceptance and commitment therapy, and resilience-based interventions. The overall findings revealed significant benefits across a variety of psychological and physical health outcomes. Depressive symptoms and HbA1c levels were the most consistently improved outcomes post-intervention. Some, but lesser, effects were observed for behavioural (self-care) outcomes. Nevertheless, only four studies were rated as high-quality in this review, and the majority of them involved small sample sizes (less than 50 participants). Although promising, it is not yet known whether the effects of well-being interventions are sufficiently large or enduring to improve prognosis of people with T2D or to prevent the onset of T2D.

Indeed, the effects of positive psychology interventions could be even more important in people at high risk of developing T2D. A recent randomised controlled trial examined the effectiveness of an 8-week mindfulness-based stress reduction program combined with diabetes risk reduction education versus diabetes risk reduction

education alone on reducing the risk of T2D. The sample consisted of 68 African American participants with pre-diabetes and high levels of self-reported life stress. Both groups (mindfulness-based stress reduction and education versus education alone) attended weekly sessions that lasted for two and a half hours, in addition to a 4-hour half-day retreat, plus six 1.5-hour booster sessions at 1-month intervals after the completion of the 8-week intervention. Within-group comparisons demonstrated that both groups achieved reductions in HbA1c at three and six months from baseline (effect sizes for each group are not reported in this study). Also, the mindfulness group showed decreases in calorie, carbohydrate, and fat intake and increases in spiritual well-being which were observed up to six months from baseline. BMI and perceived stress reductions were seen at the 3-month follow-up but were not maintained at six months (Woods-Giscombe et al., 2019). Concluding, this study by Woods-Giscombe et al. showed that mindfulness-based interventions might have biological benefits and might reduce the risk of overt diabetes by modifying clinical risk factors. Nevertheless, this was a small intervention study and follow-up period was short. Therefore, what is still unknown is whether these effects could be sustained over longer time periods and for how long these interventions need to be applied in order for them to have a meaningful impact on biology and thence health.

And although mindfulness is distinct from enjoyment of life, the two constructs present important similarities. For example, the state of awareness, which is at the core of mindfulness practices, might be strongly related to the state of enjoyment. Additionally, mindfulness practices are focused on savouring positive emotions, including enjoyment of life. Findings from a meta-analysis with a heterogeneous

sample of clinical and non-clinical populations showed that psychological interventions (including mindfulness interventions) have positive effects on biological factors relevant to diabetes including fasting blood glucose, ambulatory systolic blood pressure, resting heart rate, heart rate variability, and low density lipoprotein cholesterol (Pascoe, Thompson, & Ski, 2017). Again these findings suggest that mindfulness interventions may be effective in reducing the risk or delaying the onset of T2D.

The benefits of well-being interventions might be extended to improve lifestyle factors. Indeed, not only clinical, but also behavioural pathways, could provide simultaneous targets for well-being interventions studies. For example, two previous interventions (positive affect and mindfulness interventions, respectively) revealed advantageous effects on lifestyle measures, including increased physical activity, in patients with heart disease (Peterson et al., 2012; Younge et al., 2015). Enduring physical activity, either directly or indirectly, might be particularly important for the prevention of T2D (Wu et al., 2014).

Preventing or delaying T2D by directly targeting healthy lifestyle is on the focus of numerous intervention studies. Nine lifestyle randomised controlled trials in individuals who were at elevated risk of T2D (mostly based on measures of glycemia) were reviewed in 2013 (Schellenberg, Dryden, Vandermeer, Ha, & Korownyk, 2013). It was concluded that lifestyle interventions can decrease the risk of diabetes up to 10 years later, as well as having benefits for weight loss and BMI. However, the lifestyle interventions performed by each of the studies included in the review were heterogenous. For example, all lifestyle interventions included both a diet and a

physical activity component, but further components such as counselling, smoking cessation programmes, and weight loss medication, were divergent between studies. A 2016 systematic review of lifestyle interventions with a more similar structure (including physical activity and diet) in diabetes high-risk groups demonstrated that these interventions are associated with reduced rate of diabetes as well as decreased glucose levels and adiposity at follow-up (Howells, Musaddaq, McKay, & Majeed, 2016). Notably, these benefits were more variable in time-limited interventions, highlighting the need for longer-duration trials. Additionally, most interventions included less than six years follow-up, but longer follow-up periods may be required in order to capture additional effects that failed to be assessed in this review (e.g. effects on cardiovascular complications and mortality). Finally, a more recent Cochrane library review of 12 randomised controlled trials provided further information on the separate effects of physical activity and diet interventions in people who are at elevated risk of T2D. Specifically, it was found that physical activity alone or diet alone are not efficient enough to reduce the risk of T2D compared to standard treatments, but in combination these strategies can significantly reduce the incidence of T2D or delay the onset of T2D in participants with impaired glucose tolerance (Hemmingsen et al., 2017). However, it seems that only a small number of people at high-risk for diabetes are taking part in lifestyle intervention studies, therefore more efforts should be made in order to engage these people in intervention trials. Concluding, there is evidence to support the effectiveness of lifestyle interventions for the prevention of T2D. These interventions should be considered in micro-level and macro-level prevention strategies.

9.4.2 Anti-inflammatory interventions for the prevention of mental health disturbances in people with T2D

Study 3 showed a cross-sectional association between greater subjective well-being, as indexed by daily happiness levels, and lower IL-6 levels before and after stress exposure in people with T2D. Adding to these results, Study 4 demonstrated that higher IL-6 stress responses in individuals with T2D are longitudinally linked with lower mental health-related quality of life. Together these findings suggest that IL-6 levels and stress responses could possibly serve as individual biomarkers of excess risk for the development of mental health disturbances in this population and encourage research to investigate the implementation of immunomodulatory interventions in patients with elevated IL-6 levels and exaggerated stress responses.

Psychological interventions (Cognitive Behaviour Therapy [CBT], mindfulness-based interventions, relaxation techniques, medical qigong) seem to have anti-inflammatory effects, as evident in a systematic review and meta-analysis of 19 randomised controlled trials including healthy and diseased individuals (O'Toole et al., 2018). More specifically, a small but significant effect was observed for CRP attenuation immediately following these interventions. In view of evidence that lower concentrations of resting CRP are cross-sectionally associated with reduced IL-6 stress responses in the laboratory (Lockwood et al., 2016), such interventions may have additional benefit in terms of regulating IL-6 reactivity to stress. Nevertheless, the effect of the interventions on CRP was not seen when including studies with follow-up assessments (the mean time from baseline to follow-up was eight months [ranged from three to 16 months]). Indeed, the majority of well-being interventions have measured health outcomes over relatively short periods, and one of the greatest

challenges of such studies are to induce sustained effects that can be translated into long-term improvements in health. Intervention effects are worthwhile but are not likely to promote enhanced health unless sustained for longer periods (Steptoe, 2019b). Moderation analysis on treatment type (CBT versus meditation/mindfulness), number of sessions (from four sessions to 24 sessions [mostly on a weekly basis], and follow-up duration was not statistically significant in the aforementioned meta-analysis (O'Toole et al., 2018). Non-significant findings from these sub-group analyses may in part be explained by the small number of studies included. For example, only four studies were included in analyses testing for follow-up effects. However, the effect for CRP reduction was greater for individuals with higher psychological stress at baseline, suggesting that people with T2D, who have been found to experience increased levels of psychological stress compared to healthy individuals (Steptoe, Hackett, et al., 2014), may experience a stronger anti-inflammatory advantage when participating in psychological interventions. A more recent meta-analysis of 56 randomised controlled trials examined the effect of nine different psychosocial interventions on seven immune outcomes in participants with inflammatory-related conditions (Shields, Spahr, & Slavich, 2020). This study revealed that psychosocial interventions, especially CBT and interventions incorporating multiple psychotherapies, were significantly associated with enhanced immune system function overall. Immune outcomes more consistently associated with the interventions were decreases in inflammatory markers' levels, including IL-6 and CRP, and, secondarily, increases in immune cell counts. The mean length of the interventions was 10 weeks and the effects of the interventions persisted for at least six months following the end of the programmes. Notably, relative to the control

group, psychosocial interventions were associated with an 18% reduction in harmful pro-inflammatory activity. In comparison, a previous randomised clinical trial (Mohler et al., 2008) demonstrated that, compared to a control group, a treatment with 160-milligram dose of darapladib (for the management of atherosclerosis) decreased IL-6 and CRP levels by 12% and 13%, respectively, suggesting that psychosocial interventions can reduce systemic inflammation in a way similar to using anti-inflammatory agents to reduce CVD risk. A previous randomised controlled trial in 214 participants with T2D and sub-clinical depression explored the anti-inflammatory effect of a 5-week CBT versus diabetes education (Hermanns et al., 2015). At the 12-month follow-up both groups showed significant decreases in IL-1ra levels, but no significant differences were observed in serum CRP, IL-6, and total adiponectin within or between groups. Concluding, well-being interventions seem to have an anti-inflammatory impact which may be of clinical importance in patients with T2D.

Physical activity is another example of a natural anti-inflammatory agent. A growing number of studies have demonstrated the role of physical exercise on improving inflammatory parameters in older individuals. A recent review of the literature involving both observational studies and randomised trials suggested that physical activity can successfully modify the inflammatory status of the elderly by decreasing the levels of pro-inflammatory markers including IL-6, IL-1ra, and TNF-α (Fossati et al., 2020). It was also evident that acute periods of exercise result in a transient, mostly pro-inflammatory effect, whereas physical activity on a regular basis is associated with a chronic anti-inflammatory effect (Fossati et al., 2020).

Evidence of the anti-inflammatory impact of physical activity in people with established T2D seems contradictory (Melo et al., 2017). For example, one previous study including 60 participants with impaired glucose tolerance showed that a 12-month aerobic exercise programme (versus usual care) resulted in significant decreases in plasma IL-6 concentrations but only in those participants carrying the SNP K174C allele (Oberbach et al., 2008). In contrast, another study which compared aerobic exercise versus resistance exercise in 48 participants with diabetes found no significant between-groups differences in IL-6 after the 3-month training session (Jorge et al., 2011). Data from another study with a larger sample size (N = 82) showed that high-intensity and supervised aerobic or combined exercise (aerobic and resistance training) can result in a significant reduction in IL-6 and CRP in individuals with T2D and metabolic syndrome after 12 months of training compared with controls, with the effects for CRP being stronger for the combined exercise group (Balducci et al., 2010). In another study of 75 participants with T2D, no significant decrease in IL-6 was reported for the exercise group (moderate-intensity aerobic) versus controls after 12 weeks of training, but the aerobic group achieved a significant reduction in CRP (Choi et al., 2012). It is not clear why physical activity interventions in people with metabolic disturbances present mixed results relating to their effectiveness in reducing inflammatory factors. Differences in sample size, type and intensity of exercise programmes, and the duration of the interventions may have played a role in the inconsistency of findings.

Intervening with anti-inflammatory dietary supplements in people with T2D provides another interesting possibility to consider in this area for the prevention and/or

management of mental health difficulties. A randomised, double-blind, placebo-controlled trial was carried out to examine the effectiveness of vitamin D and probiotic co-supplementation in 60 patients with T2D and comorbid heart disease. It was found that the 12-week intervention had beneficial effects on CRP reduction among other favourable changes (mental health, glycemic control, high-density lipoprotein cholesterol, nitric oxide, and total antioxidant capacity levels (Raygan, Ostadmohammadi, Bahmani, & Asemi, 2018). However, a previous meta-analysis conducted among people with T2D indicated that probiotic use does not have a significant effect on CRP levels (Kasińska & Drzewoski, 2015), therefore it is possible that the CRP-effect seen in the study by Raygan et al. (Raygan et al., 2018) was mainly driven by the consumption of vitamin D. The effects of omega-3 polyunsaturated fatty acids supplementation on reducing inflammatory factors, including IL-6, in patients with the metabolic syndrome, hypertensive obese and/or T2D, were not confirmed in previous individual randomised controlled trials (Ellulu, Khaza'ai, Patimah, Rahmat, & Abed, 2016; Kabir et al., 2007; Satoh et al., 2007; Woodman et al., 2003) with small sample sizes (Ns between 27 and 64). In contrast, a pooled analysis of three trials (with 159 participants in total) showed a beneficial effect of omega-3 supplements in reducing fibrinogen levels by 10% compared with placebo (Hartweg, Farmer, Holman, & Neil, 2009).

Concluding, current findings from well-being programs, physical activity trials, and nutrient supplementation interventions seem promising in reducing levels of IL-6 and other markers of inflammation and should be considered in people with T2D for the prevention of mental health difficulties. Notably, no previous interventions have

directly targeted inflammatory stress *reactivity*, albeit the latter has been found to be predictive of clinical health outcomes as described in Chapter 1. One previous study showed that increased physical fitness (as indexed by exercise heart rate) is associated with reduced IL-6 and TNF-α stress responses in the laboratory after adjusting for covariates (Hamer & Steptoe, 2007). However, there is a lack of intervention studies that have directly targeted inflammatory stress responses. Inflammatory stress responsivity needs to be considered as a modifiable risk factor in future intervention studies.

9.4.3 Interventions for the management of mental health disturbances in women with T2D

Study 4 showed that women with T2D face worse mental health-related quality of life compared to men with the condition. These findings highlight the role of sex as an individual factor that needs to be considered in clinical settings and suggest that the management of mental health-related quality in people with T2D, especially women, should be set as a priority in diabetes care.

Improving quality of life is an ultimate goal of diabetes care. As evident from a recent review of 700 articles published in 61 countries, intervention trials to improve quality of life in people with diabetes have risen over time, but the majority of studies have been carried out in high-income countries (Tran et al., 2020). Common approaches to improve quality of life in people with diabetes include community, family, and e-based interventions (using digital technology) to improve self-management and self-efficacy, lifestyle (physical activity and dietary) programmes, and interventions focused on comorbidities, while only a minority of programmes (4%) are focused on addressing

mental disorders in people with diabetes (Tran et al., 2020). The impact of interventions aiming to improve quality of life in people with diabetes seems to vary according to type of intervention. For example, surgery and pharmacotherapy for the management of complications bear the highest beneficial impact on quality of life, while educational and behavioural interventions have the lowest effect (Zhang, Norris, Chowdhury, Gregg, & Zhang, 2007). Individual studies focused solely on the mental health component of quality of life have shown that mindfulness-based interventions (van Son et al., 2014), community programmes (Markle-Reid et al., 2018), family-oriented self-management interventions (Wichit, Mnatzaganian, Courtney, Schulz, & Johnson, 2017), and vitamin D supplementation (Penckofer et al., 2017) are all impactful means of improving mental health-related quality of life in people with diabetes. However, the above-mentioned intervention studies did not stratify analyses by sex and therefore outcomes are presented for both women and men with diabetes. This does not allow direct comparisons of the effect of the intervention between sexes to help identify of the most beneficial intervention for each sex. As reported previously in the introductory chapter of this thesis, women with diabetes have a more stressful psychological profile than men with the condition, and much of the increased burden felt by women is disease-oriented (Fisher et al., 2008; Inzucchi et al., 2012; Siddiqui et al., 2013). Therefore, women with diabetes may be more benefitted from diabetes management approaches in order to cope with mental health-related problems than men. Sex-specific interventions targeting mental health-related quality of life in people with T2D are warranted.

Intervening with anti-inflammatory agents in people with T2D shows promise in improving mental health and quality of life. For example, two reviews of the literature evident that the administration of pro-inflammatory cytokines inhibitors, such as TNF-α antagonists and IL-1 inhibitors reduces depressive symptoms, anxiety, and fatigue, and increases vitality and mental health-related quality of life across different patient populations (Soczynska et al., 2009; Yadlapati & Efthimiou, 2016). The effect of anti-inflammatory agents may be even more robust in individuals with T2D and comorbid mental disorders, such as major depressive disorder. Indeed, inflammation has been implicated in the pathophysiology, risk, and progression of mood disorders and anti-inflammatory agents found to have anti-depressant effects compared to placebo in people with mood disorders (Husain, Strawbridge, Stokes, & Young, 2017; Rosenblat, Cha, Mansur, & McIntyre, 2014). Notably, anti-inflammatory agents, such as TNF-α antagonists and IL-1 inhibitors, have shown to exhibit metabolic effects as well (Rosenblat et al., 2014), something which opens new avenues for the prevention and management of diabetes (Donath, 2014) and mood comorbidities.

Concluding, as evidence accumulates, subjective well-being might emerge as a novel target for interventions and policies seeking to enhance metabolic health and reduce the risk of T2D. Lifestyle interventions can also be a powerful way to prevent or delay diabetes onset in high-risk populations. Also, interventions targeting inflammatory levels and stress responses should be considered for the prevention of mental health difficulties in people with diabetes. Women with T2D, especially, who face worse mental health-related quality of life ramifications, should be at the forefront of the focus on management interventions.

9.5 Final conclusion

This PhD consisted of four studies aiming to assess the impact of sex and subjective well-being on T2D risk and progression, and the biological processes involved. Data were used from a nationally representative cohort study and a laboratory stress testing study with a follow-up design. Together this body of work contributes to a better understanding of the role of sex and subjective well-being in T2D risk and progression, and the inflammatory mechanisms involved. Considering the limitations of this PhD research, future studies should seek to replicate and expand current findings in order to provide improved modalities for the prevention and management of T2D.

References

Abraham, S., Shah, N. G., Diez Roux, A., Hill-Briggs, F., Seeman, T., Szklo, M., . . . Golden, S. H. (2015). Trait anger but not anxiety predicts incident type 2 diabetes: The Multi-Ethnic Study of Atherosclerosis (MESA). *Psychoneuroendocrinology, 60*, 105-113. doi:10.1016/j.psyneuen.2015.06.007

Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology, 34*(10), 1423-1436.

Aikens, J. E. (2012). Prospective associations between emotional distress and poor outcomes in type 2 diabetes. *Diabetes Care, 35*(12), 2472-2478. doi:10.2337/dc12-0181

Alexander, C., & Rietschel, E. T. (2001). Bacterial lipopolysaccharides and innate immunity. *Journal of endotoxin research, 7*(3), 167-202. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11581570

Allen, A. P., Kennedy, P. J., Dockray, S., Cryan, J. F., Dinan, T. G., & Clarke, G. (2017). The trier social stress test: principles and practice. *Neurobiology of stress, 6*, 113-126.

Allen, S. F., Wetherell, M. A., & Smith, M. A. (2020). Online writing about positive life experiences reduces depression and perceived stress reactivity in socially inhibited individuals. *Psychiatry Research, 284*, 112697.

American Diabetes Association. (2003). Implications of the diabetes control and complications trial. *Diabetes Care, 26 Suppl 1*, S25-S27. doi:10.2337/diacare.26.2007.s25

American Diabetes Association. (2005). Intensive lifestyle intervention or metformin on inflammation and coagulation in participants with impaired glucose tolerance. *Diabetes, 54*(5), 1566-1572.

American Diabetes Association. (2014). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, *37*, S81-S90.

American Diabetes Association. (2017). Classification and diagnosis of diabetes. *Diabetes Care, 40*(suppl. 1), S11-S24. doi: 10.2337/dc17-S005.

American Diabetes Association. (2018). Standards of Medical Care in Diabetes-2018 Abridged for Primary Care Providers. *Clinical diabetes : a publication of the American Diabetes Association, 36*(1), 14-37. doi:10.2337/cd17-0119

American Diabetes Association. (2019). 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. *Diabetes Care, 42*(Suppl 1), S13-S28. doi:10.2337/dc19-S002

American Psychiatric Association. (2020a). *Depression*. Retreived from https://www.psychiatry.org/patients-families/depression/what-is-depression

American Psychiatric Association. (2020b). *Anxiety Disorders*. Retreived from https://www.psychiatry.org/patients-families/anxiety-disorders/what-are-anxiety-disorders

Anderson, R. J., Freedland, K. E., Clouse, R. E., & Lustman, P. J. (2001). The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care, 24*(6), 1069-1078. doi:10.2337/diacare.24.6.1069

Andren, L., Lindstedt, G., Björkman, M., Borg, K., & Hansson, L. (1982). Effect of noise on blood pressure and 'stress' hormones. *Clinical Science, 62*(2), 137-141.

Arditi, C., Zanchi, A., & Peytremann-Bridevaux, I. (2019). Health status and quality of life in patients with diabetes in Switzerland. *Primary care diabetes, 13*(3), 233-241. doi:10.1016/j.pcd.2018.11.016

Asberg, M., Nygren, A., Leopardi, R., Rylander, G., Peterson, U., Wilczek, L., . . . Ekman, R. (2009). Novel biochemical markers of psychosocial stress in women. *PLoS One, 4*(1), e3590. doi:10.1371/journal.pone.0003590

Aschbacher, K., Epel, E., Wolkowitz, O. M., Prather, A. A., Puterman, E., & Dhabhar, F. S. (2012). Maintenance of a positive outlook during acute stress protects against pro-inflammatory reactivity and future depressive symptoms. *Brain, behavior, and immunity, 26*(2), 346-352. doi:10.1016/j.bbi.2011.10.010

Atlantis, E., Vogelzangs, N., Cashman, K., & Penninx, B. J. (2012). Common mental disorders associated with 2-year diabetes incidence: the Netherlands Study of Depression and Anxiety (NESDA). *J Affect Disord, 142 Suppl*, S30-35. doi:10.1016/s0165-0327(12)70006-x

Attvall, S., Fowelin, J., Lager, I., Von Schenck, H., & Smith, U. (1993). Smoking induces insulin resistance—a potential link with the insulin resistance syndrome. *Journal of internal medicine, 233*(4), 327-332.

Aune, D., ó Hartaigh, B., & Vatten, L. (2015). Resting heart rate and the risk of type 2 diabetes: a systematic review and dose–response meta-analysis of cohort studies. *Nutrition, Metabolism and Cardiovascular Diseases, 25*(6), 526-534.

Balducci, S., Zanuso, S., Nicolucci, A., Fernando, F., Cavallo, S., Cardelli, P., . . . Jimenez, A. (2010). Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. *Nutrition, Metabolism and Cardiovascular Diseases, 20*(8), 608-617.

Banerjee, M., & Saxena, M. (2012). Interleukin-1 (IL-1) family of cytokines: role in type 2 diabetes. *Clinica chimica acta; international journal of clinical chemistry, 413*(15-16), 1163-1170. doi:10.1016/j.cca.2012.03.021

Bangasser, D. A., Eck, S. R., & Ordones Sanchez, E. (2019). Sex differences in stress reactivity in arousal and attention systems. *Neuropsychopharmacology, 44*(1), 129-139. doi:10.1038/s41386-018-0137-2

Banks, J., Karlsen, S., & Oldfield, Z. (2003). Socio-economic position. In M. Marmot, J. Banks, R. Blundell, C. Lessof & J. Nazroo (Eds.), *Health, wealth and lifestyles of*

the older population in England: The 2002 English Longitudinal Study of Ageing (pp. 71-125). London: Institute for Fiscal Studies.

Banks, J., Marmot, M., Oldfield, Z., & Smith, J. P. (2006). Disease and disadvantage in the United States and in England. *JAMA, 295*(17), 2037-2045. doi:10.1001/jama.295.17.2037

Barengo, N. C., Katoh, S., Moltchanov, V., Tajima, N., & Tuomilehto, J. (2008). The diabetes-cardiovascular risk paradox: results from a Finnish population-based prospective study. *European heart journal, 29*(15), 1889-1895. doi:10.1093/eurheartj/ehn250

Bargetzi, M. J., Lantz, M., Smith, C. G., Torti, F. M., Olsson, I., Eisenberg, S. P., & Starnes, H. F., Jr. (1993). Interleukin-1 beta induces interleukin-1 receptor antagonist and tumor necrosis factor binding protein in humans. *Cancer research, 53*(17), 4010-4013. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8395334

Baron, R. M., & Kenny, D. A. (1986). The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol, 51*(6), 1173-1182. doi:10.1037//0022-3514.51.6.1173

Bassuk, S. S., & Manson, J. E. (2005). Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease. *Journal of applied physiology, 99*(3), 1193-1204.

Bastard, J.-P., Jardel, C., Bruckert, E., Blondy, P., Capeau, J., Laville, M., . . . Hainque, B. (2000). Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *The Journal of Clinical Endocrinology & Metabolism, 85*(9), 3338-3342.

Baturone, R., Soto, M. J., Márquez, M., Macías, I., de Oca, M. M., Medina, F., . . . Girón-González, J. A. (2009). Health-related quality of life in patients with primary Sjögren's syndrome: relationship with serum levels of

proinflammatory cytokines. *Scandinavian journal of rheumatology, 38*(5), 386-389. doi:10.1080/03009740902973821

Bawa, F. L., Mercer, S. W., Atherton, R. J., Clague, F., Keen, A., Scott, N. W., & Bond, C. M. (2015). Does mindfulness improve outcomes in patients with chronic pain? Systematic review and meta-analysis. *Br J Gen Pract, 65*(635), e387-400. doi:10.3399/bjgp15X685297

Bawa, H., Poole, L., Cooke, D., Panagi, L., Steptoe, A., & Hackett, R. A. (under review). Diabetes-related distress and daily cortisol output in people with type 2 diabetes. *Diabetes Research and Clinical Practice*.

Belalcazar, L. M., Haffner, S. M., Lang, W., Hoogeveen, R. C., Rushing, J., Schwenke, D. C., . . . the Look AHEAD Research Group, C. M. (2013). Lifestyle intervention and/or statins for the reduction of C-reactive protein in type 2 diabetes: from the look AHEAD study. *Obesity, 21*(5), 944-950.

Belkina, A. C., & Denis, G. V. (2010). Obesity genes and insulin resistance. *Current opinion in endocrinology, diabetes, and obesity, 17*(5), 472-477. doi:10.1097/MED.0b013e32833c5c48

Bell, R. A., Smith, S. L., Arcury, T. A., Snively, B. M., Stafford, J. M., & Quandt, S. A. (2005). Prevalence and correlates of depressive symptoms among rural older African Americans, Native Americans, and whites with diabetes. *Diabetes Care, 28*(4), 823-829.

Bennett, J. M., Glaser, R., Andridge, R. R., Peng, J., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2013). Long lasting effects of smoking: breast cancer survivors' inflammatory responses to acute stress differ by smoking history. *Psychoneuroendocrinology, 38*(2), 179-187. doi:10.1016/j.psyneuen.2012.05.012

Berger, A. (2000). Th1 and Th2 responses: what are they? *BMJ (Clinical research ed.), 321*(7258), 424-424. doi:10.1136/bmj.321.7258.424

Bergmann, M. M., Byers, T., Freedman, D. S., & Mokdad, A. (1998). Validity of self-reported diagnoses leading to hospitalization: a comparison of self-reports with hospital records in a prospective study of American adults. *American journal of epidemiology, 147*(10), 969-977.

Best, L. G., Zhang, Y., Lee, E. T., Yeh, J.-L., Cowan, L., Palmieri, V., . . . Tracy, R. P. (2005). C-reactive protein as a predictor of cardiovascular risk in a population with a high prevalence of diabetes: the Strong Heart Study. *Circulation, 112*(9), 1289-1295.

Bhandari, A., & Wagner, T. (2006). Self-reported utilization of health care services: improving measurement and accuracy. *Med Care Res Rev, 63*(2), 217-235. doi:10.1177/1077558705285298

Biessels, G. J., Staekenborg, S., Brunner, E., Brayne, C., & Scheltens, P. (2006). Risk of dementia in diabetes mellitus: a systematic review. *The Lancet. Neurology, 5*(1), 64-74. doi:10.1016/S1474-4422(05)70284-2

Billimek, J., Malik, S., Sorkin, D. H., Schmalbach, P., Ngo-Metzger, Q., Greenfield, S., & Kaplan, S. H. (2015). Understanding disparities in lipid management among patients with type 2 diabetes: gender differences in medication nonadherence after treatment intensification. *Womens Health Issues, 25*(1), 6-12. doi:10.1016/j.whi.2014.09.004

Black, P. H. (2002). Stress and the inflammatory response: a review of neurogenic inflammation. *Brain, behavior, and immunity, 16*(6), 622-653. doi:10.1016/s0889-1591(02)00021-1

Black, P. H. (2003). The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. *Brain, behavior, and immunity, 17*(5), 350-364.

Black, S. V., Cooper, R., Martin, K. R., Brage, S., Kuh, D., & Stafford, M. (2015). Physical Activity and Mental Well-being in a Cohort Aged 60-64 Years. *Am J Prev Med, 49*(2), 172-180. doi:10.1016/j.amepre.2015.03.009

Boehm, J. K., & Kubzansky, L. D. (2012). The heart's content: the association between positive psychological well-being and cardiovascular health. *Psychological bulletin, 138*(4), 655-691. doi:10.1037/a0027448

Boehm, J. K., Chen, Y., Williams, D. R., Ryff, C. D., & Kubzansky, L. D. (2016). Subjective well-being and cardiometabolic health: An 8-11year study of midlife adults. *J Psychosom Res, 85*, 1-8. doi:10.1016/j.jpsychores.2016.03.018

Boehm, J. K., Trudel-Fitzgerald, C., Kivimaki, M., & Kubzansky, L. D. (2015). The prospective association between positive psychological well-being and diabetes. *Health Psychol, 34*(10), 1013-1021. doi:10.1037/hea0000200

Bolier, L., Haverman, M., Westerhof, G. J., Riper, H., Smit, F., & Bohlmeijer, E. (2013). Positive psychology interventions: a meta-analysis of randomized controlled studies. *BMC Public Health, 13*, 119. doi:10.1186/1471-2458-13-119

Bostock, S., Hamer, M., Wawrzyniak, A. J., Mitchell, E. S., & Steptoe, A. (2011). Positive emotional style and subjective, cardiovascular and cortisol responses to acute laboratory stress. *Psychoneuroendocrinology, 36*(8), 1175-1183. doi:10.1016/j.psyneuen.2011.02.009

Boyle, P. A., Buchman, A. S., & Bennett, D. A. (2010). Purpose in life is associated with a reduced risk of incident disability among community-dwelling older persons. *Am J Geriatr Psychiatry, 18*(12), 1093-1102. doi:10.1097/JGP.0b013e3181d6c259

Boyle, P. A., Buchman, A. S., Barnes, L. L., & Bennett, D. A. (2010). Effect of a purpose in life on risk of incident Alzheimer disease and mild cognitive impairment in community-dwelling older persons. *Arch Gen Psychiatry, 67*(3), 304-310. doi:10.1001/archgenpsychiatry.2009.208

Bracha, H. S. (2004). Freeze, flight, fight, fright, faint: adaptationist perspectives on the acute stress response spectrum. *CNS spectrums, 9*(9), 679-685. doi:10.1017/s1092852900001954

Breedlove, S. M., & Watson, N. V. (2013). *Biological Psychology*. Sunderland, MA: Sinauer associates.

Breines, J. G., Thoma, M. V., Gianferante, D., Hanlin, L., Chen, X., & Rohleder, N. (2014). Self-compassion as a predictor of interleukin-6 response to acute psychosocial stress. *Brain, behavior, and immunity, 37*, 109-114. doi:10.1016/j.bbi.2013.11.006

Brotman, D. J., Golden, S. H., & Wittstein, I. S. (2007). The cardiovascular toll of stress. *Lancet (London, England), 370*(9592), 1089-1100. doi:10.1016/S0140-6736(07)61305-1

Brouwers, C., Mommersteeg, P. M., Nyklicek, I., Pelle, A. J., Westerhuis, B. L., Szabo, B. M., & Denollet, J. (2013). Positive affect dimensions and their association with inflammatory biomarkers in patients with chronic heart failure. *Biol Psychol, 92*(2), 220-226. doi:10.1016/j.biopsycho.2012.10.002

Bruehl, H., Wolf, O. T., & Convit, A. (2009). A blunted cortisol awakening response and hippocampal atrophy in type 2 diabetes mellitus. *Psychoneuroendocrinology, 34*(6), 815-821.

Brunner, E. J., Shipley, M. J., Britton, A. R., Stansfeld, S. A., Heuschmann, P. U., Rudd, A. G., . . . Kivimaki, M. (2014). Depressive disorder, coronary heart disease, and stroke: dose-response and reverse causation effects in the Whitehall II cohort study. *Eur J Prev Cardiol, 21*(3), 340-346. doi:10.1177/2047487314520785

Bruun, J. M., Helge, J. W., Richelsen, B., & Stallknecht, B. (2006). Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. *American Journal of Physiology-Endocrinology and Metabolism, 290*(5), E961-E967.

Brydon, L., & Steptoe, A. (2005). Stress-induced increases in interleukin-6 and fibrinogen predict ambulatory blood pressure at 3-year follow-up. *J Hypertens, 23*(5), 1001-1007. doi:10.1097/01.hjh.0000166841.57474.d0

Brydon, L., Strike, P. C., Bhattacharyya, M. R., Whitehead, D. L., McEwan, J., Zachary, I., & Steptoe, A. (2010). Hostility and physiological responses to laboratory stress in acute coronary syndrome patients. *Journal of psychosomatic research, 68*(2), 109-116. doi:10.1016/j.jpsychores.2009.06.007

Bush, T. L., Miller, S. R., Golden, A. L., & Hale, W. E. (1989). Self-report and medical record report agreement of selected medical conditions in the elderly. *Am J Public Health, 79*(11), 1554-1556. doi:10.2105/ajph.79.11.1554

Buske-Kirschbaum, A., & Hellhammer, D. H. (2003). Endocrine and Immune Responses to Stress in Chronic Inflammatory Skin Disorders. *Annals of the New York Academy of Sciences*, *992*, 231-240. doi:10.1111/j.1749-6632.2003.tb03153.x

Camden Joint Strategic Needs Assessment. (2014). *Camden Joint Strategic Needs Assessment 2014/2015 Executive Summary*. Retreived from https://www.camden.gov.uk/documents/20142/1195356/Joint+Strategic+needs+assesments.pdf/49b7214b-df20-592c-969e-b5aa211daf82

Cannon, W. B. (1939). *The Wisdom of the Body* (2nd ed.). Norton, New York, NY: W.W. Norton & Company, Inc.

Cannon, W.B. (1929). B*odily Changes in Pain, hunger, fear and rage*. Oxford, England: Appleton.

Carpenter, L. L., Gawuga, C. E., Tyrka, A. R., Lee, J. K., Anderson, G. M., & Price, L. H. (2010). Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 35*(13), 2617-2623. doi:10.1038/npp.2010.159

Carroll, D., Ginty, A. T., Whittaker, A. C., Lovallo, W. R., & de Rooij, S. R. (2017). The behavioural, cognitive, and neural corollaries of blunted cardiovascular and cortisol reactions to acute psychological stress. *Neuroscience & Biobehavioral Reviews, 77*, 74-86.

Carroll, D., Phillips, A. C., & Der, G. (2008). Body mass index, abdominal adiposity, obesity, and cardiovascular reactions to psychological stress in a large community sample. *Psychosom Med, 70*(6), 653-660. doi:10.1097/PSY.0b013e31817b9382

Carroll, J. E., Low, C. A., Prather, A. A., Cohen, S., Fury, J. M., Ross, D. C., & Marsland, A. L. (2011). Negative affective responses to a speech task predict changes in interleukin (IL)-6. *Brain Behav Immun, 25*(2), 232-238. doi:10.1016/j.bbi.2010.09.024

Carstensen, B., Jørgensen, M. E., & Friis, S. (2014). The epidemiology of diabetes and cancer. *Current diabetes reports, 14*(10), 535-535. doi:10.1007/s11892-014-0535-8

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.

Castillo, J. J., Mull, N., Reagan, J. L., Nemr, S., & Mitri, J. (2012). Increased incidence of non-Hodgkin lymphoma, leukemia, and myeloma in patients with diabetes mellitus type 2: a meta-analysis of observational studies. *Blood, 119*(21), 4845-4850. doi:10.1182/blood-2011-06-362830

CDC. (2014). *Centers for Disease Control and Prevention. National diabetes statistics report: Estimates of diabetes and its burden in the United States, 2014.* Atlanta, GA: Department of Health and Human Services.

Celano, C. M., Beale, E. E., Moore, S. V., Wexler, D. J., & Huffman, J. C. (2013). Positive psychological characteristics in diabetes: a review. *Curr Diab Rep, 13*(6), 917-929. doi:10.1007/s11892-013-0430-8

Chakhssi, F., Kraiss, J. T., Sommers-Spijkerman, M., & Bohlmeijer, E. T. (2018). The effect of positive psychology interventions on well-being and distress in clinical samples with psychiatric or somatic disorders: A systematic review and meta-analysis. *BMC psychiatry, 18*(1), 211.

Champagne, F. A., Weaver, I. C. G., Diorio, J., Dymov, S., Szyf, M., & Meaney, M. J. (2006). Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. *Endocrinology, 147*(6), 2909-2915. doi:10.1210/en.2005-1119

Champaneri, S., Xu, X., Carnethon, M. R., Bertoni, A. G., Seeman, T., Roux, A. D., & Golden, S. H. (2012). Diurnal salivary cortisol and urinary catecholamines are associated with diabetes mellitus: the Multi-Ethnic Study of Atherosclerosis. *Metabolism, 61*(7), 986-995.

Charles, J., Travers, P., Walport, M., & Shlomchik, M. (2001). *Immunobiology* (5th ed.). New York and London: Garland Science.

Charles, S. T., Reynolds, C. A., & Gatz, M. (2001). Age-related differences and change in positive and negative affect over 23 years. *J Pers Soc Psychol*, *80*(1), 136-151. doi:10.1037/0022-3514.80.1.136

Chatterjee, S., Peters, S. A., Woodward, M., Mejia Arango, S., Batty, G. D., Beckett, N., . . . Huxley, R. R. (2016). Type 2 Diabetes as a Risk Factor for Dementia in Women Compared With Men: A Pooled Analysis of 2.3 Million People Comprising More Than 100,000 Cases of Dementia. *Diabetes Care, 39*(2), 300-307. doi:10.2337/dc15-1588

Chavez, M., Seeley, R. J., Green, P. K., Wilkinson, C. W., Schwartz, M. W., & Woods, S. C. (1997). Adrenalectomy increases sensitivity to central insulin. *Physiology & behavior, 62*(3), 631-634.

Chawla, A., Nguyen, K. D., & Goh, Y. S. (2011). Macrophage-mediated inflammation in metabolic disease. *Nature Reviews Immunology, 11*(11), 738-749.

Chida, Y., & Steptoe, A. (2008). Positive psychological well-being and mortality: a quantitative review of prospective observational studies. *Psychosom Med, 70*(7), 741-756. doi:10.1097/PSY.0b013e31818105ba

Chida, Y., & Steptoe, A. (2010). Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: a meta-analysis of prospective evidence. *Hypertension (Dallas, Tex. : 1979), 55*(4), 1026-1032. doi:10.1161/HYPERTENSIONAHA.109.146621

Chiolero, A., Wietlisbach, V., Ruffieux, C., Paccaud, F., & Cornuz, J. (2006). Clustering of risk behaviors with cigarette consumption: a population-based survey. *Preventive medicine, 42*(5), 348-353.

Choi, K. M., Han, K. A., Ahn, H. J., Hwang, S. Y., Hong, H. C., Choi, H. Y., . . . Choi, D. S. (2012). Effects of exercise on sRAGE levels and cardiometabolic risk factors in patients with type 2 diabetes: a randomized controlled trial. *The Journal of Clinical Endocrinology & Metabolism, 97*(10), 3751-3758.

Chrousos, G. P. (1998). Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. *Ann N Y Acad Sci, 851*, 311-335. doi:10.1111/j.1749-6632.1998.tb09006.x

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nature reviews. Endocrinology, 5*(7), 374-381. doi:10.1038/nrendo.2009.106

Chrousos, G. P., & Gold, P. W. (1992). The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA, 267*(9), 1244-1252. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1538563

Chrousos, G. P., & Kino, T. (2007). Glucocorticoid action networks and complex psychiatric and/or somatic disorders. *Stress (Amsterdam, Netherlands), 10*(2), 213-219. doi:10.1080/10253890701292119

Chrousos, G. P., Loriaux, D. L., & Gold, P. W. (1988). The Concept of Stress and Its Historical Development. In *Mechanisms of Physical and Emotional Stress: Advances in Experimental Medicine and Biology*. New York, NY: Plenum Press.

Chung K. F. (2009). Cytokines. In P. Barnes, J. Drazen, S. Rennard, & N. Thomson (Eds.), *Asthma and COPD* (pp. 327-341): Academic Press.

Clore, J., & Thurby-Hay, L. (2009). Glucocorticoid-induced hyperglycemia. *Endocrine Practice, 15*(5), 469-474.

Clouet, F., Excler-Cavailher, G., Christophe, B., Masson, F., & Fasquel, D. (2001). Type 2 Diabetes and Short Form 36-items Health Survey. [Evaluation de la qualité de vie de diabétiques de type 2 par l'échelle internationale SF 36]. *Diabetes & metabolism, 27*(6), 711-717. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11852382

Cohen, J. (1988). *Statistical power and analysis for the behavioral sciences* (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum Associates, Inc.

Cohen, R., Bavishi, C., & Rozanski, A. (2016). Purpose in Life and Its Relationship to All-Cause Mortality and Cardiovascular Events: A Meta-Analysis. *Psychosom Med, 78*(2), 122-133. doi:10.1097/psy.0000000000000274

Cohen, S. (1994). Perceived Stress Scale.

Cohen, S., Doyle, W. J., Turner, R. B., Alper, C. M., & Skoner, D. P. (2003). Emotional style and susceptibility to the common cold. *Psychosom Med, 65*(4), 652-657. doi:10.1097/01.psy.0000077508.57784.da

Collins, M., Corcoran, P., & Perry, I. (2009). Anxiety and depression symptoms in patients with diabetes. *Diabetic Medicine, 26*(2), 153-161.

Connolly, V., & Kesson, C. (1996). Socioeconomic status and clustering of cardiovascular disease risk factors in diabetic patients. *Diabetes Care, 19*(5), 419-422.

Cook, W. W., & Medley, D. M. (1954). Proposed hostility and Pharisaic-virtue scales for the MMPI. *J of Appl Psychol, 38*(6), 414-418. doi:10.1037/h0060667

Couzin-Frankel, J. (2010). Inflammation bares a dark side. *Science (New York, N.Y.), 330*(6011), 1621-1621. doi:10.1126/science.330.6011.1621

Crump, C., Sundquist, J., Winkleby, M. A., & Sundquist, K. (2016). Stress resilience and subsequent risk of type 2 diabetes in 1.5 million young men. *Diabetologia, 59*(4), 728-733. doi:10.1007/s00125-015-3846-7

Czekierda, K., Banik, A., Park, C. L., & Luszczynska, A. (2017). Meaning in life and physical health: systematic review and meta-analysis. *Health Psychol Rev, 11*(4), 387-418. doi:10.1080/17437199.2017.1327325

Dabelea, D., Mayer-Davis, E. J., Saydah, S., Imperatore, G., Linder, B., Divers, J., . . . Study, S. f. D. i. Y. (2014). Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. *JAMA, 311*(17), 1778-1786. doi:10.1001/jama.2014.3201

Dalsgaard, E.-M., Vestergaard, M., Skriver, M. V., Maindal, H. T., Lauritzen, T., Borch-Johnsen, K., . . . Sandbaek, A. (2014). Psychological distress, cardiovascular complications and mortality among people with screen-detected type 2 diabetes: follow-up of the ADDITION-Denmark trial. *Diabetologia, 57*(4), 710-717. doi:10.1007/s00125-014-3165-4

Danaei, G., Finucane, M. M., Lu, Y., Singh, G. M., Cowan, M. J., Paciorek, C. J., . . . Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating, G. (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. *Lancet (London, England), 378*(9785), 31-40. doi:10.1016/S0140-6736(11)60679-X

Danesh, J., Kaptoge, S., Mann, A. G., Sarwar, N., Wood, A., Angleman, S. B., . . . Gudnason, V. (2008). Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med, 5*(4), e78. doi:10.1371/journal.pmed.0050078

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci, 9*(1), 46-56. doi:10.1038/nrn2297

Davey Smith, G., & Hemani, G. (2014). Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet, 23*(R1), R89-98. doi:10.1093/hmg/ddu328

Davey Smith, G., & Hemani, G. (2014). Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Human molecular genetics, 23*(R1), R89-R98.

Davidson, K. W., Mostofsky, E., & Whang, W. (2010). Don't worry, be happy: positive affect and reduced 10-year incident coronary heart disease: the Canadian Nova Scotia Health Survey. *Eur Heart J, 31*(9), 1065-1070. doi:10.1093/eurheartj/ehp603

Davies, M. J., D'Alessio, D. A., Fradkin, J., Kernan, W. N., Mathieu, C., Mingrone, G., . . . Buse, J. B. (2018). Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care, 41*(12), 2669-2701. doi:10.2337/dci18-0033

Davies, N. M., Holmes, M. V., & Davey Smith, G. (2018). Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *Bmj, 362*, k601. doi:10.1136/bmj.k601

de Brouwer, S. J. M., van Middendorp, H., Kraaimaat, F. W., Radstake, T. R. D. J., Joosten, I., Donders, A. R. T., . . . Evers, A. W. M. (2013). Immune responses to stress after stress management training in patients with rheumatoid arthritis. *Arthritis research & therapy, 15*(6), R200-R200. doi:10.1186/ar4390

de Rooij, S. R. (2013). Blunted cardiovascular and cortisol reactivity to acute psychological stress: a summary of results from the Dutch Famine Birth

Cohort Study. *Int J Psychophysiol, 90*(1), 21-27. doi:10.1016/j.ijpsycho.2012.09.011

Demakakos, P., Biddulph, J. P., Bobak, M., & Marmot, M. G. (2016). Wealth and mortality at older ages: a prospective cohort study. *J Epidemiol Community Health, 70*(4), 346-353. doi:10.1136/jech-2015-206173

Demakakos, P., Pierce, M. B., & Hardy, R. (2010). Depressive symptoms and risk of type 2 diabetes in a national sample of middle-aged and older adults: the English longitudinal study of aging. *Diabetes Care, 33*(4), 792-797. doi:10.2337/dc09-1663

Demakakos, P., Zaninotto, P., & Nouwen, A. (2014). Is the association between depressive symptoms and glucose metabolism bidirectional? Evidence from the English Longitudinal Study of Ageing. *Psychosom Med, 76*(7), 555-561. doi:10.1097/psy.0000000000000082

Demmer, R. T., Gelb, S., Suglia, S. F., Keyes, K. M., Aiello, A. E., Colombo, P. C., . . . Kubzansky, L. D. (2015). Sex differences in the association between depression, anxiety, and type 2 diabetes mellitus. *Psychosom Med, 77*(4), 467-477. doi:10.1097/psy.0000000000000169

Derry, H. M., Fagundes, C. P., Andridge, R., Glaser, R., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2013). Lower subjective social status exaggerates interleukin-6 responses to a laboratory stressor. *Psychoneuroendocrinology, 38*(11), 2676-2685. doi:10.1016/j.psyneuen.2013.06.026

Deshmane, S. L., Kremlev, S., Amini, S., & Sawaya, B. E. (2009). Monocyte chemoattractant protein-1 (MCP-1): an overview. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research, 29*(6), 313-326. doi:10.1089/jir.2008.0027

Di Dalmazi, G., Pagotto, U., Pasquali, R., & Vicennati, V. (2012). Glucocorticoids and type 2 diabetes: from physiology to pathology. *Journal of nutrition and metabolism, 2012*.

Diabetes UK. (2020). *Diabetes Prevalence 2019*. Retreived from https://www.diabetes.org.uk/professionals/position-statements-reports/statistics/diabetes-prevalence-2019

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull, 130*(3), 355-391. doi:10.1037/0033-2909.130.3.355

Dinh, K. M., Kaspersen, K. A., Mikkelsen, S., Pedersen, O. B., Petersen, M. S., Thørner, L. W., . . . Erikstrup, C. (2019). Low-grade inflammation is negatively associated with physical Health-Related Quality of Life in healthy individuals: Results from The Danish Blood Donor Study (DBDS). *PLoS One, 14*(3), e0214468-e0214468. doi:10.1371/journal.pone.0214468

D'Mello, M. J., Ross, S. A., Briel, M., Anand, S. S., Gerstein, H., & Paré, G. (2015). Association between shortened leukocyte telomere length and cardiometabolic outcomes: systematic review and meta-analysis. *Circ Cardiovasc Genet, 8*(1), 82-90. doi:10.1161/circgenetics.113.000485

Donath, M. Y. (2014). Targeting inflammation in the treatment of type 2 diabetes: time to start. *Nature reviews Drug discovery, 13*(6), 465-476.

Duncan, B. B., Schmidt, M. I., Pankow, J. S., Ballantyne, C. M., Couper, D., Vigo, A., . . . Heiss, G. (2003). Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes, 52*(7), 1799-1805.

Eapen, D. J., Manocha, P., Patel, R. S., Hammadah, M., Veledar, E., Wassel, C., . . . Epstein, S. E. (2013). Aggregate risk score based on markers of inflammation, cell stress, and coagulation is an independent predictor of adverse cardiovascular outcomes. *Journal of the American College of Cardiology, 62*(4), 329-337. doi:10.1016/j.jacc.2013.03.072

Eckel, R. H., Alberti, K. G., Grundy, S. M., & Zimmet, P. Z. (2010). The metabolic syndrome. *Lancet, 375*(9710), 181-183. doi:10.1016/s0140-6736(09)61794-3

Edwards, K. M., Burns, V. E., Ring, C., & Carroll, D. (2006). Sex differences in the interleukin-6 response to acute psychological stress. *Biol Psychol, 71*(3), 236-239. doi:10.1016/j.biopsycho.2005.06.006

Egede, L. E., & Zheng, D. (2003). Independent factors associated with major depressive disorder in a national sample of individuals with diabetes. *Diabetes Care, 26*(1), 104-111.

Ehses, J. A., Ellingsgaard, H., Böni-Schnetzler, M., & Donath, M. Y. (2009). Pancreatic islet inflammation in type 2 diabetes: from α and β cell compensation to dysfunction. *Archives of physiology and biochemistry, 115*(4), 240-247.

Einarson, T. R., Acs, A., Ludwig, C., & Panton, U. H. (2018). Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007-2017. *Cardiovasc Diabetol, 17*(1), 83. doi:10.1186/s12933-018-0728-6

Elenkov, I. J., Kvetnansky, R., Hashiramoto, A., Bakalov, V. K., Link, A. A., Zachman, K., . . . Wilder, R. L. (2008). Low- versus high-baseline epinephrine output shapes opposite innate cytokine profiles: presence of Lewis- and Fischer-like neurohormonal immune phenotypes in humans? *Journal of immunology (Baltimore, Md. : 1950), 181*(3), 1737-1745. doi:10.4049/jimmunol.181.3.1737

Elenkov, I. J., Wilder, R. L., Chrousos, G. P., & Vizi, E. S. (2000). The sympathetic nerve--an integrative interface between two supersystems: the brain and the immune system. *Pharmacological reviews, 52*(4), 595-638. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11121511

Eliasson, M., Asplund, K., Evrin, P.-E., & Lundblad, D. (1995). Relationship of cigarette smoking and snuff dipping to plasma fibrinogen, fibrinolytic variables and serum insulin. The Northern Sweden MONICA Study. *Atherosclerosis, 113*(1), 41-53.

Ellins, E. A., Rees, D. A., Deanfield, J. E., Steptoe, A., & Halcox, J. P. (2017). Increased fibrinogen responses to psychophysiological stress predict future endothelial dysfunction implications for cardiovascular disease? *Brain Behav Immun, 60*, 233-239. doi:10.1016/j.bbi.2016.10.017

Ellins, E., Halcox, J., Donald, A., Field, B., Brydon, L., Deanfield, J., & Steptoe, A. (2008). Arterial stiffness and inflammatory response to psychophysiological stress. *Brain Behav Immun, 22*(6), 941-948. doi:10.1016/j.bbi.2008.01.009

Ellulu, M. S., Khaza'ai, H., Patimah, I., Rahmat, A., & Abed, Y. (2016). Effect of long chain omega-3 polyunsaturated fatty acids on inflammation and metabolic markers in hypertensive and/or diabetic obese adults: a randomized controlled trial. *Food & nutrition research, 60*(1), 29268.

Emdin, C. A., Anderson, S. G., Woodward, M., & Rahimi, K. (2015). Usual blood pressure and risk of new-onset diabetes: evidence from 4.1 million adults and a meta-analysis of prospective studies. *Journal of the American College of Cardiology, 66*(14), 1552-1562.

Emerging Risk Factors Collaboration. (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet (London, England), 375*(9733), 2215-2222. doi:10.1016/S0140-6736(10)60484-9

Emerging Risk Factors Collaboration. (2011). Diabetes mellitus, fasting glucose, and risk of cause-specific death. *The New England journal of medicine, 364*(9), 829-841. doi:10.1056/NEJMoa1008862

Emerging Risk Factors Collaboration. (2012). Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet (London, England), 379*(9822), 1205-1213. doi:10.1016/S0140-6736(11)61931-4

Endrighi, R., Hamer, M., & Steptoe, A. (2016). Post-menopausal Women Exhibit Greater Interleukin-6 Responses to Mental Stress Than Older Men. *Ann Behav Med, 50*(4), 564-571. doi:10.1007/s12160-016-9783-y

English Longitudinal Study of Ageing. (2019). Retrieved from https://www.elsa-project.ac.uk/

Engum, A. (2007). The role of depression and anxiety in onset of diabetes in a large population-based study. *J Psychosom Res, 62*(1), 31-38. doi:10.1016/j.jpsychores.2006.07.009

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., & Cawthon, R. M. (2004). Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences of the United States of America, 101*(49), 17312-17315. doi:10.1073/pnas.0407162101

Espeland, M. A., Carmichael, O., Yasar, S., Hugenschmidt, C., Hazzard, W., Hayden, K. M., . . . Mielke, M. M. (2018). Sex-related differences in the prevalence of cognitive impairment among overweight and obese adults with type 2 diabetes. *Alzheimers Dement, 14*(9), 1184-1192. doi:10.1016/j.jalz.2018.05.015

Esser, N., Legrand-Poels, S., Piette, J., Scheen, A. J., & Paquot, N. (2014). Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes research and clinical practice, 105*(2), 141-150.

Esser, N., Paquot, N., & Scheen, A. J. (2015). Anti-inflammatory agents to treat or prevent type 2 diabetes, metabolic syndrome and cardiovascular disease. *Expert opinion on investigational drugs, 24*(3), 283-307. doi:10.1517/13543784.2015.974804

Facchini, F. S., Hollenbeck, C. B., Jeppesen, J., Chen, Y.-D. I., & Reaven, G. (1992). Insulin resistance and cigarette smoking. *The Lancet, 339*(8802), 1128-1130.

Farvid, M. S., Qi, L., Hu, F. B., Kawachi, I., Okereke, O. I., Kubzansky, L. D., & Willett, W. C. (2014). Phobic anxiety symptom scores and incidence of type 2 diabetes in US men and women. *Brain Behav Immun, 36*, 176-182. doi:10.1016/j.bbi.2013.10.025

Faulenbach, M., Uthoff, H., Schwegler, K., Spinas, G. A., Schmid, C., & Wiesli, P. (2012). Effect of psychological stress on glucose control in patients with Type 2 diabetes. *Diabetic medicine : a journal of the British Diabetic Association, 29*(1), 128-131. doi:10.1111/j.1464-5491.2011.03431.x

Feig, E. H., Healy, B. C., Celano, C. M., Nikrahan, G. R., Moskowitz, J. T., & Huffman, J. C. (2019). Positive psychology interventions in patients with medical illness: What predicts improvement in psychological state? *International Journal of Wellbeing, 9*(2).

Feller, S., Teucher, B., Kaaks, R., Boeing, H., & Vigl, M. (2013). Life satisfaction and risk of chronic diseases in the European prospective investigation into cancer and nutrition (EPIC)-Germany study. *PLoS One, 8*(8), e73462. doi:10.1371/journal.pone.0073462

Fisher, L., Mullan, J. T., Arean, P., Glasgow, R. E., Hessler, D., & Masharani, U. (2010). Diabetes distress but not clinical depression or depressive symptoms is associated with glycemic control in both cross-sectional and longitudinal analyses. *Diabetes Care, 33*(1), 23-28. doi:10.2337/dc09-1238

Fisher, L., Skaff, M., Mullan, J., Arean, P., Glasgow, R., & Masharani, U. (2008). A longitudinal study of affective and anxiety disorders, depressive affect and diabetes distress in adults with type 2 diabetes. *Diabetic Medicine, 25*(9), 1096-1101.

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiological reviews, 93*(1), 137-188. doi:10.1152/physrev.00045.2011

Fossati, C., Torre, G., Borrione, P., Giombini, A., Fagnani, F., Turchetta, M., . . . Pigozzi, F. (2020). Biohumoral Indicators Influenced by Physical Activity in the Elderly. *J Clin Med, 9*(4). doi:10.3390/jcm9041115

Fowler, M. J. (2008). Microvascular and macrovascular complications of diabetes. *Clinical diabetes, 26*(2), 77-82.

Fox, C. S., Golden, S. H., Anderson, C., Bray, G. A., Burke, L. E., de Boer, I. H., . . . American Diabetes, A. (2015). Update on Prevention of Cardiovascular Disease in Adults With Type 2 Diabetes Mellitus in Light of Recent Evidence: A Scientific Statement From the American Heart Association and the American Diabetes Association. *Diabetes Care, 38*(9), 1777-1803. doi:10.2337/dci15-0012

Fransson, L., Franzén, S., Rosengren, V., Wolbert, P., Sjöholm, Å., & Ortsäter, H. (2013). b-cell adaptation in a mouse model of glucocorticoid-induced metabolic syndrome. *Journal of endocrinology, 219*, 231-241.

Franzini, L., Ardigò, D., Cavalot, F., Miccoli, R., Rivellese, A. A., Trovati, M., . . . Vaccaro, O. (2013). Women show worse control of type 2 diabetes and cardiovascular disease risk factors than men: results from the MIND.IT Study Group of the Italian Society of Diabetology. *Nutrition, metabolism, and cardiovascular diseases : NMCD, 23*(3), 235-241. doi:10.1016/j.numecd.2011.12.003

Frati, A. C., Iniestra, F., & Ariza, C. R. (1996). Acute effect of cigarette smoking on glucose tolerance and other cardiovascular risk factors. *Diabetes Care, 19*(2), 112-118.

Frawley, A. (2015). Happiness research: A review of critiques. *Sociology Compass, 9*(1), 62-77.

Friedman, E. M., Hayney, M., Love, G. D., Singer, B. H., & Ryff, C. D. (2007). Plasma interleukin-6 and soluble IL-6 receptors are associated with psychological

well-being in aging women. *Health Psychol, 26*(3), 305-313. doi:10.1037/0278-6133.26.3.305

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): facts and future directions. *International Journal of Psychophysiology, 72*(1), 67-73.

Fritz, M. S., & Mackinnon, D. P. (2007). Required sample size to detect the mediated effect. *Psychol Sci, 18*(3), 233-239. doi:10.1111/j.1467-9280.2007.01882.x

Garvin, P., Nilsson, E., Ernerudh, J., & Kristenson, M. (2016). The joint subclinical elevation of CRP and IL-6 is associated with lower health-related quality of life in comparison with no elevation or elevation of only one of the biomarkers. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation, 25*(1), 213-221. doi:10.1007/s11136-015-1068-6

Ge, Z., Ben, Q., Qian, J., Wang, Y., & Li, Y. (2011). Diabetes mellitus and risk of gastric cancer: a systematic review and meta-analysis of observational studies. *European journal of gastroenterology & hepatology, 23*(12), 1127-1135. doi:10.1097/MEG.0b013e32834b8d73

Geiger, B. B., & MacKerron, G. (2016). Can alcohol make you happy? A subjective wellbeing approach. *Soc Sci Med, 156*, 184-191. doi:10.1016/j.socscimed.2016.03.034

George, L. S., & Park, C. L. (2016). Meaning in life as comprehension, purpose, and mattering: Toward integration and new research questions. *Review of General Psychology, 20*(3), 205-220.

Gerin, W. (2010). Laboratory stress testing methodology. In A. Steptoe (Ed.), *Handbook of Behavioral Medicine* (pp. 633-648). New York, NY: Springer.

Giltay, E. J., Kamphuis, M. H., Kalmijn, S., Zitman, F. G., & Kromhout, D. (2006). Dispositional optimism and the risk of cardiovascular death: the Zutphen

Elderly Study. *Arch Intern Med, 166*(4), 431-436. doi:10.1001/archinte.166.4.431

Girard, D., Tardif, J.-C., Boisclair Demarble, J., & D'Antono, B. (2016). Trait Hostility and Acute Inflammatory Responses to Stress in the Laboratory. *PLoS One, 11*(6), e0156329-e0156329. doi:10.1371/journal.pone.0156329

Glick, D., Barth, S., & Macleod, K. F. (2010). Autophagy: cellular and molecular mechanisms. *J Pathol, 221*(1), 3-12. doi:10.1002/path.2697

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Borden, W. B., . . . Turner, M. B. (2013). Executive summary: heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation, 127*(1), 143-152. doi:10.1161/CIR.0b013e318282ab8f

Golden, S. H., Williams, J. E., Ford, D. E., Yeh, H. C., Sanford, C. P., Nieto, F. J., & Brancati, F. L. (2006). Anger temperament is modestly associated with the risk of type 2 diabetes mellitus: the Atheroslcerosis Risk in Communities Study. *Psychoneuroendocrinology, 31*(3), 325-332. doi:10.1016/j.psyneuen.2005.08.008

Gong, S., Miao, Y.-L., Jiao, G.-Z., Sun, M.-J., Li, H., Lin, J., . . . Tan, J.-H. (2015). Dynamics and correlation of serum cortisol and corticosterone under different physiological or stressful conditions in mice. *PLoS One, 10*(2).

Gonzalez, J. S., Vileikyte, L., Ulbrecht, J. S., Rubin, R. R., Garrow, A. P., Delgado, C., . . . Peyrot, M. (2010). Depression predicts first but not recurrent diabetic foot ulcers. *Diabetologia, 53*(10), 2241-2248. doi:10.1007/s00125-010-1821-x

Gore, M. O., Patel, M. J., Kosiborod, M., Parsons, L. S., Khera, A., de Lemos, J. A., . . . National Registry of Myocardial Infarction, I. (2012). Diabetes mellitus and trends in hospital survival after myocardial infarction, 1994 to 2006: data from the national registry of myocardial infarction. *Circulation. Cardiovascular quality and outcomes, 5*(6), 791-797. doi:10.1161/CIRCOUTCOMES.112.965491

Gotink, R. A., Chu, P., Busschbach, J. J., Benson, H., Fricchione, G. L., & Hunink, M. G. (2015). Standardised mindfulness-based interventions in healthcare: an overview of systematic reviews and meta-analyses of RCTs. *PLoS One, 10*(4), e0124344. doi:10.1371/journal.pone.0124344

Gouliopoulos, N., Kalogeropoulos, C., Lavaris, A., Rouvas, A., Asproudis, I., Garmpi, A., . . . Moschos, M. (2018). Association of serum inflammatory markers and diabetic retinopathy: a review of literature. *Eur Rev Med Pharmacol Sci, 22*(21), 7113-7128.

Grant, N., Hamer, M., & Steptoe, A. (2009). Social isolation and stress-related cardiovascular, lipid, and cortisol responses. *Annals of behavioral medicine : a publication of the Society of Behavioral Medicine, 37*(1), 29-37. doi:10.1007/s12160-009-9081-z

Gregg, E. W., Sattar, N., & Ali, M. K. (2016). The changing face of diabetes complications. *The lancet. Diabetes & endocrinology, 4*(6), 537-547. doi:10.1016/S2213-8587(16)30010-9

Grigsby, A. B., Anderson, R. J., Freedland, K. E., Clouse, R. E., & Lustman, P. J. (2002). Prevalence of anxiety in adults with diabetes: a systematic review. *Journal of psychosomatic research, 53*(6), 1053-1060. doi:10.1016/s0022-3999(02)00417-8

Gruenewald, T. L., Kemeny, M. E., Aziz, N., & Fahey, J. L. (2004). Acute threat to the social self: Shame, social self-esteem, and cortisol activity. *Psychosomatic Medicine*, *66*(6), 915-924. doi:10.1097/01.psy.0000143639.61693.ef

Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., & Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes research and clinical practice, 103*(2), 137-149. doi:10.1016/j.diabres.2013.11.002

Gubbels Bupp, M. R. (2015). Sex, the aging immune system, and chronic disease. *Cell Immunol, 294*(2), 102-110. doi:10.1016/j.cellimm.2015.02.002

Guh, D. P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C. L., & Anis, A. H. (2009). The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health, 9*, 88. doi:10.1186/1471-2458-9-88

Haapanen, N., Miilunpalo, S., Pasanen, M., Oja, P., & Vuori, I. (1997). Agreement between questionnaire data and medical records of chronic diseases in middle-aged and elderly Finnish men and women. *Am J Epidemiol, 145*(8), 762-769. doi:10.1093/aje/145.8.762

Hackett, R. A. (2016). *The role of psychosocial wellbeing and biological stress processes in linking type II diabetes and cardiovascular disease* (Doctoral dissertation, University College London, London, United Kingdom). Retrieved from https://discovery.ucl.ac.uk/id/eprint/1528822/

Hackett, R. A., & Steptoe, A. (2017). Type 2 diabetes mellitus and psychological stress - a modifiable risk factor. *Nat Rev Endocrinol, 13*(9), 547-560. doi:10.1038/nrendo.2017.64

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*(11), 1801-1809. doi:10.1016/j.psyneuen.2012.03.016

Hackett, R. A., Kivimäki, M., Kumari, M., & Steptoe, A. (2016). Diurnal cortisol patterns, future diabetes, and impaired glucose metabolism in the Whitehall II cohort study. *The Journal of Clinical Endocrinology & Metabolism, 101*(2), 619-625.

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. *Psychosom Med, 77*(4), 458-466. doi:10.1097/psy.0000000000000172

Hackett, R. A., Poole, L., Hunt, E., Panagi, L., & Steptoe, A. (2019). Loneliness and biological responses to acute stress in people with Type 2 diabetes. *Psychophysiology, 56*(6), e13341. doi:10.1111/psyp.13341

Hackett, R. A., Steptoe, A., & Kumari, M. (2014). Association of diurnal patterns in salivary cortisol with type 2 diabetes in the Whitehall II study. *The Journal of Clinical Endocrinology & Metabolism, 99*(12), 4625-4631.

Hafez, D., Heisler, M., Choi, H., Ankuda, C. K., Winkelman, T., & Kullgren, J. T. (2018). Association between purpose in life and glucose control among older adults. *Annals of Behavioral Medicine, 52*(4), 309-318.

Hamer, M., & Steptoe, A. (2007). Association between physical fitness, parasympathetic control, and proinflammatory responses to mental stress. *Psychosomatic medicine, 69*(7), 660-666.

Hamer, M., Endrighi, R., Venuraju, S. M., Lahiri, A., & Steptoe, A. (2012). Cortisol responses to mental stress and the progression of coronary artery calcification in healthy men and women. *PLoS One, 7*(2), e31356. doi:10.1371/journal.pone.0031356

Hamer, M., Hackett, R. A., Bostock, S., Lazzarino, A. I., Carvalho, L. A., & Steptoe, A. (2014). Objectively assessed physical activity, adiposity, and inflammatory markers in people with type 2 diabetes. *BMJ Open Diabetes Res Care, 2*(1), e000030. doi:10.1136/bmjdrc-2014-000030

Hamer, M., O'Donnell, K., Lahiri, A., & Steptoe, A. (2010). Salivary cortisol responses to mental stress are associated with coronary artery calcification in healthy men and women. *Eur Heart J, 31*(4), 424-429. doi:10.1093/eurheartj/ehp386

Hamer, M., Williams, E., Vuonovirta, R., Giacobazzi, P., Gibson, E. L., & Steptoe, A. (2006). The effects of effort-reward imbalance on inflammatory and cardiovascular responses to mental stress. *Psychosomatic medicine, 68*(3), 408-413. doi:10.1097/01.psy.0000221227.02975.a0

Hanson, J. L., Nacewicz, B. M., Sutterer, M. J., Cayo, A. A., Schaefer, S. M., Rudolph, K. D., . . . Davidson, R. J. (2015). Behavioral problems after early life stress: contributions of the hippocampus and amygdala. *Biological psychiatry, 77*(4), 314-323. doi:10.1016/j.biopsych.2014.04.020

Hartweg, J., Farmer, A. J., Holman, R. R., & Neil, A. (2009). Potential impact of omega-3 treatment on cardiovascular disease in type 2 diabetes. *Current opinion in lipidology, 20*(1), 30-38.

Heinrich, P. C., Castell, J. V., & Andus, T. (1990). Interleukin-6 and the acute phase response. *Biochem J, 265*(3), 621-636. doi:10.1042/bj2650621

Hellhammer, D. H., Wüst, S., & Kudielka, B. M. (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology, 34*(2), 163-171. doi:10.1016/j.psyneuen.2008.10.026

Hemmingsen, B., Gimenez-Perez, G., Mauricio, D., Roqué, I. F. M., Metzendorf, M. I., & Richter, B. (2017). Diet, physical activity or both for prevention or delay of type 2 diabetes mellitus and its associated complications in people at increased risk of developing type 2 diabetes mellitus. *Cochrane Database Syst Rev, 12*(12), Cd003054. doi:10.1002/14651858.CD003054.pub4

Hendriks, T., Schotanus-Dijkstra, M., Hassankhan, A., de Jong, J., & Bohlmeijer, E. (2019). The efficacy of multi-component positive psychology interventions: A systematic review and meta-analysis of randomized controlled trials. *Journal of happiness studies*, 1-34.

Herder, C., Baumert, J., Thorand, B., Martin, S., Lowel, H., Kolb, H., & Koenig, W. (2006). Chemokines and incident coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. *Arterioscler Thromb Vasc Biol, 26*(9), 2147-2152. doi:10.1161/01.Atv.0000235691.84430.86

Hermanns, N., Schmitt, A., Gahr, A., Herder, C., Nowotny, B., Roden, M., . . . Kulzer, B. (2015). The effect of a Diabetes-Specific Cognitive Behavioral Treatment Program (DIAMOS) for patients with diabetes and subclinical depression:

results of a randomized controlled trial. *Diabetes Care, 38*(4), 551-560. doi:10.2337/dc14-1416

Hernandez, R., Bassett, S. M., Boughton, S. W., Schuette, S. A., Shiu, E. W., & Moskowitz, J. T. (2018). Psychological well-being and physical health: Associations, mechanisms, and future directions. *Emotion Review, 10*(1), 18-29.

Hex, N., Bartlett, C., Wright, D., Taylor, M., & Varley, D. (2012). Estimating the current and future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs. *Diabetic medicine : a journal of the British Diabetic Association, 29*(7), 855-862. doi:10.1111/j.1464-5491.2012.03698.x

Hirosaki, M., Ishimoto, Y., Kasahara, Y., Konno, A., Kimura, Y., Fukutomi, E., . . . Matsubayashi, K. (2013). Positive affect as a predictor of lower risk of functional decline in community-dwelling elderly in Japan. *Geriatr Gerontol Int, 13*(4), 1051-1058. doi:10.1111/ggi.12008

Hjemdahl, P. (1993). Plasma catecholamines--analytical challenges and physiological limitations. *Baillieres Clin Endocrinol Metab, 7*(2), 307-353. doi:10.1016/s0950-351x(05)80179-x

Hlatky, M. A., Boothroyd, D. B., Bravata, D. M., Boersma, E., Booth, J., Brooks, M. M., . . . Pocock, S. J. (2009). Coronary artery bypass surgery compared with percutaneous coronary interventions for multivessel disease: a collaborative analysis of individual patient data from ten randomised trials. *Lancet (London, England), 373*(9670), 1190-1197. doi:10.1016/S0140-6736(09)60552-3

Hobson, C. J., Kamen, J., Szostek, J., Nethercut, C. M., Tiedmann, J. W., & Wojnarowicz, S. (1998). Stressful life events: A revision and update of the Social Readjustment Rating Scale. *International journal of stress management, 5*(1), 1-23.

Hofmann, M., Köhler, B., Leichsenring, F., & Kruse, J. (2013). Depression as a risk factor for mortality in individuals with diabetes: a meta-analysis of prospective studies. *PLoS One, 8*(11), e79809-e79809. doi:10.1371/journal.pone.0079809

Holmes, T. H., & Rahe, R. H. (1967). The Social Readjustment Rating Scale. *Journal of psychosomatic research, 11*(2), 213-218. doi:10.1016/0022-3999(67)90010-4

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature, 444*(7121), 860-867.

Howells, L., Musaddaq, B., McKay, A. J., & Majeed, A. (2016). Clinical impact of lifestyle interventions for the prevention of diabetes: an overview of systematic reviews. *BMJ Open, 6*(12), e013806. doi:10.1136/bmjopen-2016-013806

Howren, M. B., Lamkin, D. M., & Suls, J. (2009). Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med, 71*(2), 171-186. doi:10.1097/PSY.0b013e3181907c1b

Hu, F. B., Meigs, J. B., Li, T. Y., Rifai, N., & Manson, J. E. (2004). Inflammatory markers and risk of developing type 2 diabetes in women. *Diabetes, 53*(3), 693-700. doi:10.2337/diabetes.53.3.693

Huang, H., Yan, P., Shan, Z., Chen, S., Li, M., Luo, C., . . . Liu, L. (2015). Adverse childhood experiences and risk of type 2 diabetes: A systematic review and meta-analysis. *Metabolism, 64*(11), 1408-1418. doi:10.1016/j.metabol.2015.08.019

Huang, W., Ren, H., Ben, Q., Cai, Q., Zhu, W., & Li, Z. (2012). Risk of esophageal cancer in diabetes mellitus: a meta-analysis of observational studies. *Cancer causes & control : CCC, 23*(2), 263-272. doi:10.1007/s10552-011-9874-9

Huffman, J. C., DuBois, C. M., Millstein, R. A., Celano, C. M., & Wexler, D. (2015). Positive Psychological Interventions for Patients with Type 2 Diabetes:

Rationale, Theoretical Model, and Intervention Development. *J Diabetes Res, 2015*, 428349. doi:10.1155/2015/428349

Hurwitz, E. L., & Morgenstern, H. (2001). Immediate and longterm effects of immune stimulation: hypothesis linking the immune response to subsequent physical and psychological wellbeing. *Med Hypotheses, 56*(6), 620-624. doi:10.1054/mehy.2000.1217

Husain, M. I., Strawbridge, R., Stokes, P. R., & Young, A. H. (2017). Anti-inflammatory treatments for mood disorders: Systematic review and meta-analysis. *Journal of Psychopharmacology, 31*(9), 1137-1148.

Hyde, M., Wiggins, R. D., Higgs, P., & Blane, D. B. (2003). A measure of quality of life in early old age: the theory, development and properties of a needs satisfaction model (CASP-19). *Aging Ment Health, 7*(3), 186-194. doi:10.1080/1360786031000101157

Ikeda, A., Schwartz, J., Peters, J. L., Fang, S., Spiro, A., 3rd, Sparrow, D., . . . Kubzansky, L. D. (2011). Optimism in relation to inflammation and endothelial dysfunction in older men: the VA Normative Aging Study. *Psychosom Med, 73*(8), 664-671. doi:10.1097/PSY.0b013e3182312497

Imperatore, G., Boyle, J. P., Thompson, T. J., Case, D., Dabelea, D., Hamman, R. F., . . . Group, S. f. D. i. Y. S. (2012). Projections of type 1 and type 2 diabetes burden in the U.S. population aged <20 years through 2050: dynamic modeling of incidence, mortality, and population growth. *Diabetes Care, 35*(12), 2515-2520. doi:10.2337/dc12-0669

International Diabetes Federation. *IDF Diabetes Atlas* (9th ed.). Brussels, Belgium: International Diabetes Federation, 2019. Retrieved from http://www.diabetesatlas.org

Inzucchi, S. E., Bergenstal, R. M., Buse, J. B., Diamant, M., Ferrannini, E., Nauck, M., . . . Matthews, D. R. (2012). Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes

Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care, 35*(6), 1364-1379. doi:10.2337/dc12-0413

Iob, E., Kirschbaum, C., & Steptoe, A. (2020). Persistent depressive symptoms, HPA-axis hyperactivity, and inflammation: the role of cognitive-affective and somatic symptoms. *Molecular Psychiatry, 25*(5), 1130-1140.

Iversen, M. M., Tell, G. S., Espehaug, B., Midthjell, K., Graue, M., Rokne, B., . . . Østbye, T. (2015). Is depression a risk factor for diabetic foot ulcers? 11-years follow-up of the Nord-Trøndelag Health Study (HUNT). *Journal of diabetes and its complications, 29*(1), 20-25. doi:10.1016/j.jdiacomp.2014.09.006

Jaffiol, C., Thomas, F., Bean, K., Jego, B., & Danchin, N. (2013). Impact of socioeconomic status on diabetes and cardiovascular risk factors: results of a large French survey. *Diabetes Metab, 39*(1), 56-62. doi:10.1016/j.diabet.2012.09.002

Jager, A., van Hinsbergh, V. W., Kostense, P. J., Emeis, J. J., Yudkin, J. S., Nijpels, G., . . . Stehouwer, C. D. (1999). von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondiabetic subjects: the Hoorn Study. *Arterioscler Thromb Vasc Biol, 19*(12), 3071-3078. doi:10.1161/01.atv.19.12.3071

Jamrozik, E., Hyde, Z., Alfonso, H., Flicker, L., Almeida, O., Yeap, B., . . . Jamrozik, K. (2014). Validity of self-reported versus hospital-coded diagnosis of stroke: a cross-sectional and longitudinal study. *Cerebrovasc Dis, 37*(4), 256-262. doi:10.1159/000358583

Jannoo, Z., Wah, Y. B., Lazim, A. M., & Hassali, M. A. (2017). Examining diabetes distress, medication adherence, diabetes self-care activities, diabetes-specific quality of life and health-related quality of life among type 2 diabetes mellitus patients. *Journal of clinical & translational endocrinology, 9*, 48-54. doi:10.1016/j.jcte.2017.07.003

Jansen, A. S., Van Nguyen, X., Karpitskiy, V., Mettenleiter, T. C., & Loewy, A. D. (1995). Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. *Science (New York, N.Y.), 270*(5236), 644-646.

Janzon, L., Berntorp, K., Hanson, M., Lindell, S.-E., & Trell, E. (1983). Glucose tolerance and smoking: a population study of oral and intravenous glucose tolerance tests in middle-aged men. *Diabetologia, 25*(2), 86-88.

Jenkins, C. D., Stanton, B. A., Niemcryk, S. J., & Rose, R. M. (1988). A scale for the estimation of sleep problems in clinical research. *J Clin Epidemiol, 41*(4), 313-321. doi:10.1016/0895-4356(88)90138-2

Jessop, D. S., & Turner-Cobb, J. M. (2008). Measurement and meaning of salivary cortisol: A focus on health and disease in children. *Stress*, *11*(1), 1-14. doi:10.1080/10253890701365527

Jones, A., McMillan, M. R., Jones, R. W., Kowalik, G. T., Steeden, J. A., Deanfield, J. E., . . . Muthurangu, V. (2012). Adiposity is associated with blunted cardiovascular, neuroendocrine and cognitive responses to acute mental stress. *PLoS One, 7*(6), e39143. doi:10.1371/journal.pone.0039143

Jorge, M. L. M. P., de Oliveira, V. N., Resende, N. M., Paraiso, L. F., Calixto, A., Diniz, A. L. D., . . . Espindola, F. S. (2011). The effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with type 2 diabetes mellitus. *Metabolism, 60*(9), 1244-1252.

Julien, E., Senécal, C., & Guay, F. (2009). Longitudinal relations among perceived autonomy support from health care practitioners, motivation, coping strategies and dietary compliance in a sample of adults with type 2 diabetes. *Journal of health psychology, 14*(3), 457-470.

Kabir, M., Skurnik, G., Naour, N., Pechtner, V., Meugnier, E., Rome, S., . . . Clément, K. (2007). Treatment for 2 mo with n− 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. *The American journal of clinical nutrition, 86*(6), 1670-1679.

Kang, H. (2013). The prevention and handling of the missing data. *Korean J Anesthesiol, 64*(5), 402-406. doi:10.4097/kjae.2013.64.5.402

Kanner, A. D., Coyne, J. C., Schaefer, C., & Lazarus, R. S. (1981). Comparison of two modes of stress measurement: daily hassles and uplifts versus major life events. *Journal of behavioral medicine, 4*(1), 1-39. doi:10.1007/bf00844845

Karatsoreos, I. N., & McEwen, B. S. (2010). Stress and Allostasis. In A. Steptoe (Ed.), *Handbook of Behavioral Medicine: Methods and Applications* (pp. 649-658). New York, NY: Springer New York.

Karatsoreos, I. N., Bhagat, S. M., Bowles, N. P., Weil, Z. M., Pfaff, D. W., & McEwen, B. S. (2010). Endocrine and physiological changes in response to chronic corticosterone: a potential model of the metabolic syndrome in mouse. *Endocrinology, 151*(5), 2117-2127.

Kashdan, T. B., Biswas-Diener, R., & King, L. A. (2008). Reconsidering happiness: The costs of distinguishing between hedonics and eudaimonia. *The Journal of Positive Psychology, 3*(4), 219-233.

Kasińska, M. A., & Drzewoski, J. (2015). Effectiveness of probiotics in type 2 diabetes: a meta-analysis. *Pol Arch Med Wewn, 125*(11), 803-813.

Kautzky-Willer, A., Harreiter, J., & Pacini, G. (2016). Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. *Endocr Rev, 37*(3), 278-316. doi:10.1210/er.2015-1137

Kautzky-Willer, A., Harreiter, J., Abrahamian, H., Weitgasser, R., Fasching, P., Hoppichler, F., & Lechleitner, M. (2019). [Sex and gender-specific aspects in

prediabetes and diabetes mellitus-clinical recommendations (Update 2019)]. *Wien Klin Wochenschr, 131*(Suppl 1), 221-228. doi:10.1007/s00508-018-1421-1

Kavanagh, A., Bentley, R. J., Turrell, G., Shaw, J., Dunstan, D., & Subramanian, S. (2010). Socioeconomic position, gender, health behaviours and biomarkers of cardiovascular disease and diabetes. *Social science & medicine, 71*(6), 1150-1160.

Kehoe, R., Wu, S. Y., Leske, M. C., & Chylack, L. T., Jr. (1994). Comparing self-reported and physician-reported medical history. *Am J Epidemiol, 139*(8), 813-818. doi:10.1093/oxfordjournals.aje.a117078

Kelley, T. L. (1935). An unbiased correlation ratio measure. *Proc Natl Acad Sci U S A*, *21*(9), 554-559. doi:10.1073/pnas.21.9.554

Kelly-Hughes, D. H., Wetherell, M. A., & Smith, M. A. (2014). Type D personality and cardiovascular reactivity to an ecologically valid multitasking stressor. *Psychology & health, 29*(10), 1156-1175.

Keyes, C. L. (2005). Mental illness and/or mental health? Investigating axioms of the complete state model of health. *J Consult Clin Psychol, 73*(3), 539-548. doi:10.1037/0022-006x.73.3.539

Kim, E. S., Delaney, S. W., & Kubzansky, L. D. (2019). Sense of Purpose in Life and Cardiovascular Disease: Underlying Mechanisms and Future Directions. *Curr Cardiol Rep, 21*(11), 135. doi:10.1007/s11886-019-1222-9

Kim, E. S., Kubzansky, L. D., Soo, J., & Boehm, J. K. (2017). Maintaining Healthy Behavior: a Prospective Study of Psychological Well-Being and Physical Activity. *Ann Behav Med, 51*(3), 337-347. doi:10.1007/s12160-016-9856-y

Kim, E. S., Strecher, V. J., & Ryff, C. D. (2014). Purpose in life and use of preventive health care services. *Proc Natl Acad Sci U S A, 111*(46), 16331-16336. doi:10.1073/pnas.1414826111

Kim, E. S., Sun, J. K., Park, N., & Peterson, C. (2013). Purpose in life and reduced incidence of stroke in older adults: 'The Health and Retirement Study'. *J Psychosom Res, 74*(5), 427-432. doi:10.1016/j.jpsychores.2013.01.01

Kirschbaum, C., Pirke, & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1-2), 76-81. doi:10.1159/000119004

Kirschbaum, C., Prüssner, J. C., Stone, A. A., Federenko, I., Gaab, J., Lintz, D., . . . Hellhammer, D. H. (1995). Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. *Psychosomatic Medicine*, *57*(5), 468-474. doi:10.1097/00006842-199509000-00009

Kitamura, M. (2008). Endoplasmic reticulum stress and unfolded protein response in renal pathophysiology: Janus faces. *American Journal of Physiology-Renal Physiology, 295*(2), F323-F334.

Kitraki, E., Kremmyda, O., Youlatos, D., Alexis, M. N., & Kittas, C. (2004). Gender-dependent alterations in corticosteroid receptor status and spatial performance following 21 days of restraint stress. *Neuroscience, 125*(1), 47-55.

Kivimäki, M., Virtanen, M., Kawachi, I., Nyberg, S. T., Alfredsson, L., Batty, G. D., . . . Jokela, M. (2015). Long working hours, socioeconomic status, and the risk of incident type 2 diabetes: a meta-analysis of published and unpublished data from 222 120 individuals. *The lancet. Diabetes & endocrinology, 3*(1), 27-34. doi:10.1016/S2213-8587(14)70178-0

Knowles, J. W., & Reaven, G. (2016). Usual blood pressure and new-onset diabetes risk: evidence from 4.1 million adults and a meta-analysis. *Journal of the American College of Cardiology, 67*(13), 1656-1657.

Kobberling, J. T. H. (1982). *Emperical risk figures of first degree relatives of non-insulin dependent diabetes*. London: Academic Press.

Kohlgruber, A., & Lynch, L. (2015). Adipose tissue inflammation in the pathogenesis of type 2 diabetes. *Current diabetes reports, 15*(11), 92.

Koizumi, M., Ito, H., Kaneko, Y., & Motohashi, Y. (2008). Effect of having a sense of purpose in life on the risk of death from cardiovascular diseases. *J Epidemiol, 18*(5), 191-196. doi:10.2188/jea.je2007388

Koppes, L. L. J., Dekker, J. M., Hendriks, H. F. J., Bouter, L. M., & Heine, R. J. (2005). Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. *Diabetes Care, 28*(3), 719-725. doi:10.2337/diacare.28.3.719

Kubzansky, L. D., & Thurston, R. C. (2007). Emotional vitality and incident coronary heart disease: benefits of healthy psychological functioning. *Arch Gen Psychiatry, 64*(12), 1393-1401. doi:10.1001/archpsyc.64.12.1393

Kuchulakanti, P. K., Chu, W. W., Torguson, R., Ohlmann, P., Rha, S.-W., Clavijo, L. C., . . . Waksman, R. (2006). Correlates and long-term outcomes of angiographically proven stent thrombosis with sirolimus- and paclitaxel-eluting stents. *Circulation, 113*(8), 1108-1113. doi:10.1161/CIRCULATIONAHA.105.600155

Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum C. (2004). HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. *Psychoneuroendocrinology*, *29*(1), 83-98. doi:10.1016/s0306-4530(02)00146-4

Kudielka, B. M., Hellhammer, D. H., & Wüst, S. (2009). Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology, 34*(1), 2-18. doi:10.1016/j.psyneuen.2008.10.004

Kuh, D., Ben-Shlomo, Y., Lynch, J., Hallqvist, J., & Power, C. (2003). Life course epidemiology. *Journal of epidemiology and community health, 57*(10), 778-783. doi:10.1136/jech.57.10.778

Kumari, M., Head, J., & Marmot, M. (2004). Prospective study of social and other risk factors for incidence of type 2 diabetes in the Whitehall II study. *Arch Intern Med, 164*(17), 1873-1880. doi:10.1001/archinte.164.17.1873

Kumari, M., Shipley, M., Stafford, M., & Kivimaki, M. (2011). Association of diurnal patterns in salivary cortisol with all-cause and cardiovascular mortality: findings from the Whitehall II study. *The Journal of Clinical Endocrinology & Metabolism, 96*(5), 1478-1485.

Kuo, C. L., Pilling, L. C., Kuchel, G. A., Ferrucci, L., & Melzer, D. (2019). Telomere length and aging-related outcomes in humans: A Mendelian randomization study in 261,000 older participants. *Aging Cell, 18*(6), e13017. doi:10.1111/acel.13017

Lacroix, A., Feelders, R. A., Stratakis, C. A., & Nieman, L. K. (2015). Cushing's syndrome. *The Lancet, 386*(9996), 913-927.

Lamers, S. M., Bolier, L., Westerhof, G. J., Smit, F., & Bohlmeijer, E. T. (2012). The impact of emotional well-being on long-term recovery and survival in physical illness: a meta-analysis. *Journal of behavioral medicine, 35*(5), 538-547. doi:10.1007/s10865-011-9379-8

Lampinen, P., Heikkinen, R. L., Kauppinen, M., & Heikkinen, E. (2006). Activity as a predictor of mental well-being among older adults. *Aging Ment Health, 10*(5), 454-466. doi:10.1080/13607860600640962

Landau, Z., Raz, I., Wainstein, J., Bar-Dayan, Y., & Cahn, A. (2017). The role of insulin pump therapy for type 2 diabetes mellitus. *Diabetes/metabolism research and reviews, 33*(1), e2822.

Landman, G. W., van Hateren, K. J., Kleefstra, N., Groenier, K. H., Gans, R. O., & Bilo, H. J. (2010). Health-related quality of life and mortality in a general and elderly population of patients with type 2 diabetes (ZODIAC-18). *Diabetes Care, 33*(11), 2378-2382. doi:10.2337/dc10-0979

Larsen, J. T., Hershfield, H. E., Stastny, B. J., & Hester, N. (2017). On the relationship between positive and negative affect: Their correlation and their co-occurrence. *Emotion (Washington, D.C.), 17*(2), 323-336. doi:10.1037/emo0000231

Larsson, S. C., & Wolk, A. (2011). Diabetes mellitus and incidence of kidney cancer: a meta-analysis of cohort studies. *Diabetologia, 54*(5), 1013-1018. doi:10.1007/s00125-011-2051-6

Lawrence, E. M., Rogers, R. G., & Wadsworth, T. (2015). Happiness and longevity in the United States. *Soc Sci Med, 145*, 115-119. doi:10.1016/j.socscimed.2015.09.020

Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal and coping.* New York, NY: Springer Publishing Company.

Lazzarino, A. I., Hamer, M., Stamatakis, E., & Steptoe, A. (2013). The combined association of psychological distress and socioeconomic status with all-cause mortality: a national cohort study. *JAMA internal medicine, 173*(1), 22-27. doi:10.1001/2013.jamainternmed.951

Lederbogen, F., Hummel, J., Fademrecht, C., Krumm, B., Kühner, C., Deuschle, M., . . . Lutz, H. (2011). Flattened circadian cortisol rhythm in type 2 diabetes. *Experimental and clinical endocrinology & diabetes, 119*(09), 573-575.

Levenstein, S., Prantera, C., Varvo, V., Scribano, M. L., Berto, E., Luzi, C., & Andreoli, A. (1993). Development of the Perceived Stress Questionnaire: a new tool for psychosomatic research. *Journal of Psychosomatic Research, 37*(1), 19-32. doi:10.1016/0022-3999(93)90120-5

Levine, S. (1970). The pituitary-adrenal system and the developing brain. *Progress in brain research, 32*, 79-85. doi:10.1016/S0079-6123(08)61521-6

Li, L., Chan, S. L., Mo, F., Hui, E. P., Koh, J., Chan, A. K., . . . Yeo, W. (2019). Status of inflammation in relation to health related quality of life in hepatocellular carcinoma patients. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation, 28*(9), 2597-2607. doi:10.1007/s11136-019-02190-0

Libby, P., Ridker, P. M., & Hansson, G. K. (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature, 473*(7347), 317-325. doi:10.1038/nature10146

Lin, D. Y., Fleming, T. R., & De Gruttola, V. (1997). Estimating the proportion of treatment effect explained by a surrogate marker. *Stat Med, 16*(13), 1515-1527. doi:10.1002/(sici)1097-0258(19970715)16:13<1515::aid-sim572>3.0.co;2-1

Liu, B., Maekawa, T., Yoshida, K., Ly, N. H., Inoue, K., Hasegawa, A., . . . Ishii, S. (2019). Telomere shortening by transgenerational transmission of TNF-α-induced TERRA via ATF7. *Nucleic Acids Res, 47*(1), 283-298. doi:10.1093/nar/gky1149

Liu, Y., Ma, C., Li, P., Ma, C., He, S., Ping, F., . . . Li, Y. (2020). Leukocyte Telomere Length Independently Predicts 3-Year Diabetes Risk in a Longitudinal Study of Chinese Population. *Oxid Med Cell Longev, 2020*, 9256107. doi:10.1155/2020/9256107

Lockwood, K. G., Marsland, A. L., Cohen, S., & Gianaros, P. J. (2016). Sex differences in the association between stressor-evoked interleukin-6 reactivity and C-reactive protein. *Brain Behav Immun, 58*, 173-180. doi:10.1016/j.bbi.2016.07.001

Lovallo, W. R., Farag, N. H., Sorocco, K. H., Cohoon, A. J., & Vincent, A. S. (2012). Lifetime adversity leads to blunted stress axis reactivity: studies from the

Oklahoma Family Health Patterns Project. *Biological psychiatry, 71*(4), 344-349.

Lowe, G., Woodward, M., Hillis, G., Rumley, A., Li, Q., Harrap, S., . . . Chalmers, J. (2014). Circulating inflammatory markers and the risk of vascular complications and mortality in people with type 2 diabetes and cardiovascular disease or risk factors: the ADVANCE study. *Diabetes, 63*(3), 1115-1123. doi:10.2337/db12-1625

Lu, F.-P., Lin, K.-P., & Kuo, H.-K. (2009). Diabetes and the risk of multi-system aging phenotypes: a systematic review and meta-analysis. *PLoS One, 4*(1), e4144-e4144. doi:10.1371/journal.pone.0004144

Luby, J. L., Barch, D., Whalen, D., Tillman, R., & Belden, A. (2017). Association Between Early Life Adversity and Risk for Poor Emotional and Physical Health in Adolescence: A Putative Mechanistic Neurodevelopmental Pathway. *JAMA pediatrics, 171*(12), 1168-1175. doi:10.1001/jamapediatrics.2017.3009

Lucas, R. E., & Gohm, C. L. (2000). Age and sex differences in subjective wellbeing across cultures. In E. Diener, & E. M. Suh (Eds.), *Culture and Subjective Well-Being* (pp. 291-317). London: The MIT Press.

Lucas, R. M., & McMichael, A. J. (2005). Association or causation: evaluating links between" environment and disease". *Bulletin of the World Health Organization, 83*, 792-795.

Lund, T. D., Munson, D., Haldy, M., & Handa, R. J. (2004). Androgen inhibits, while oestrogen enhances, restraint-induced activation of neuropeptide neurones in the paraventricular nucleus of the hypothalamus. *Journal of neuroendocrinology, 16*(3), 272-278.

MacDonald, M. R., Petrie, M. C., Varyani, F., Ostergren, J., Michelson, E. L., Young, J. B., . . . Investigators, C. (2008). Impact of diabetes on outcomes in patients

with low and preserved ejection fraction heart failure: an analysis of the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) programme. *European heart journal, 29*(11), 1377-1385. doi:10.1093/eurheartj/ehn153

MacKinnon, D. P. (2008). In *Computer Intensive Methods for Mediation Analysis Introduction to Statistical Mediation Analysis* (pp. 333-334). New York, NY: Taylor & Francis Group.

Maier, S. F., & Watkins, L. R. (1998). Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychological review, 105*(1), 83.

Majer, M., Welberg, L. A., Capuron, L., Pagnoni, G., Raison, C. L., & Miller, A. H. (2008). IFN-alpha-induced motor slowing is associated with increased depression and fatigue in patients with chronic hepatitis C. *Brain Behav Immun, 22*(6), 870-880. doi:10.1016/j.bbi.2007.12.009

Malik, U. R., Makower, D. F., & Wadler, S. (2001). Interferon-mediated fatigue. *Cancer, 92*(6 Suppl), 1664-1668. doi:10.1002/1097-0142(20010915)92:6+<1664::aid-cncr1494>3.0.co;2-9

Maric-Bilkan, C. (2017). Sex differences in micro- and macro-vascular complications of diabetes mellitus. *Clin Sci (Lond), 131*(9), 833-846. doi:10.1042/cs20160998

Markle-Reid, M., Ploeg, J., Fraser, K. D., Fisher, K. A., Bartholomew, A., Griffith, L. E., . . . Upshur, R. (2018). Community program improves quality of life and self-management in older adults with diabetes mellitus and comorbidity. *Journal of the American Geriatrics Society, 66*(2), 263-273.

Marsland, A. L., Walsh, C., Lockwood, K., & John-Henderson, N. A. (2017). The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. *Brain Behav Immun, 64*, 208-219. doi:10.1016/j.bbi.2017.01.011

Martela, F., & Steger, M. F. (2016). The three meanings of meaning in life: Distinguishing coherence, purpose, and significance. *The Journal of Positive Psychology, 11*(5), 531-545.

Martin-Maria, N., Miret, M., Caballero, F. F., Rico-Uribe, L. A., Steptoe, A., Chatterji, S., & Ayuso-Mateos, J. L. (2017). The Impact of Subjective Well-being on Mortality: A Meta-Analysis of Longitudinal Studies in the General Population. *Psychosom Med, 79*(5), 565-575. doi:10.1097/psy.0000000000000444

Marvar, P. J., Vinh, A., Thabet, S., Lob, H. E., Geem, D., Ressler, K. J., & Harrison, D. G. (2012). T lymphocytes and vascular inflammation contribute to stress-dependent hypertension. *Biological psychiatry, 71*(9), 774-782.

Massey, C. N., Feig, E. H., Duque-Serrano, L., Wexler, D., Moskowitz, J. T., & Huffman, J. C. (2019). Well-being interventions for individuals with diabetes: A systematic review. *Diabetes Res Clin Pract, 147*, 118-133. doi:10.1016/j.diabres.2018.11.014

Matoba, K., Takeda, Y., Nagai, Y., Kawanami, D., Utsunomiya, K., & Nishimura, R. (2019). Unraveling the role of inflammation in the pathogenesis of diabetic kidney disease. *International journal of molecular sciences, 20*(14), 3393.

Mattei, J., Demissie, S., Falcon, L. M., Ordovas, J. M., & Tucker, K. (2010). Allostatic load is associated with chronic conditions in the Boston Puerto Rican Health Study. *Soc Sci Med, 70*(12), 1988-1996. doi:10.1016/j.socscimed.2010.02.024

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *The New England journal of medicine, 338*(3), 171-179. doi:10.1056/NEJM199801153380307

McInnes, I. B. (2017). Cytokines. In G. S. Firestein, R. C. Budd, S. E. Gabriel, I. B. McInnes, & J. R. O'Dell (Eds.), *Kelley and Firestein's Textbook of Rheumatology* (10th ed, pp. 396-407). Elsevier.

McInnis, C. M., Thoma, M. V., Gianferante, D., Hanlin, L., Chen, X., Breines, J. G., . . . Rohleder, N. (2014). Measures of adiposity predict interleukin-6 responses to repeated psychosocial stress. *Brain Behav Immun, 42*, 33-40. doi:10.1016/j.bbi.2014.07.018

McInnis, C. M., Wang, D., Gianferante, D., Hanlin, L., Chen, X., Thoma, M. V., & Rohleder, N. (2015). Response and habituation of pro- and anti-inflammatory gene expression to repeated acute stress. *Brain, behavior, and immunity, 46*, 237-248. doi:10.1016/j.bbi.2015.02.006

McLaughlin, K. A., Lane, R. D., & Bush, N. R. (2016). Introduction to the Special Issue of Psychosomatic Medicine: Mechanisms Linking Early-Life Adversity to Physical Health. *Psychosomatic medicine, 78*(9), 976-978. doi:10.1097/PSY.0000000000000420

Melo, L. C., Dativo-Medeiros, J., Menezes-Silva, C. E., Barbosa, F. T., de Sousa-Rodrigues, C. F., & Rabelo, L. A. (2017). Physical Exercise on Inflammatory Markers in Type 2 Diabetes Patients: A Systematic Review of Randomized Controlled Trials. *Oxid Med Cell Longev, 2017*, 8523728. doi:10.1155/2017/8523728

Merril, R. M., Timmreck. T. C. (2006). *Introduction to Epidemiology* (4th ed.). Mississauga, Ontario, CA: Jones and Bartlett Publishers.

Mezuk, B., Eaton, W. W., Albrecht, S., & Golden, S. H. (2008). Depression and type 2 diabetes over the lifespan: a meta-analysis. *Diabetes Care, 31*(12), 2383-2390. doi:10.2337/dc08-0985

Miller, A. H., & Raison, C. L. (2016). The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nature reviews. Immunology, 16*(1), 22-34. doi:10.1038/nri.2015.5

Mindell, J., Biddulph, J. P., Hirani, V., Stamatakis, E., Craig, R., Nunn, S., & Shelton, N. (2012). Cohort profile: the health survey for England. *Int J Epidemiol, 41*(6), 1585-1593. doi:10.1093/ije/dyr199

Mohamed, A. F., Zhang, J., Johnson, F. R., Lomon, I. D., Malvolti, E., Townsend, R., . . . Parhofer, K. G. (2013). Avoidance of weight gain is important for oral type 2 diabetes treatments in Sweden and Germany: patient preferences. *Diabetes Metab, 39*(5), 397-403. doi:10.1016/j.diabet.2013.06.001

Mohler, E. R., Ballantyne, C. M., Davidson, M. H., Hanefeld, M., Ruilope, L. M., Johnson, J. L., . . . Investigators, D. (2008). The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study. *Journal of the American College of Cardiology, 51*(17), 1632-1641.

Morioka, I., Ishii, N., Miyai, N., Yamamoto, H., Minami, Y., Wang, T., & Miyashita, K. (2005). An occupational health study on workers exposed to a cold environment in a cold storage warehouse. In *Elsevier Ergonomics Book Series* (Vol. 3, pp. 199-204): Elsevier.

Moskowitz, J. T., Epel, E. S., & Acree, M. (2008). Positive affect uniquely predicts lower risk of mortality in people with diabetes. *Health Psychol, 27*(1s), S73-82. doi:10.1037/0278-6133.27.1.S73

Mujcic, R., & J. Oswald, A. (2016). Evolution of well-being and happiness after increases in consumption of fruit and vegetables. *American Journal of Public Health, 106*(8), 1504-1510.

Muth, N. D. (2009). What are the guidelines for percentage of body fat loss. *American Council on Exercise (ACE). Ask the Expert Blog*.

Natali, A., Toschi, E., Baldeweg, S., Ciociaro, D., Favilla, S., Saccà, L., & Ferrannini, E. (2006). Clustering of insulin resistance with vascular dysfunction and low-grade inflammation in type 2 diabetes. *Diabetes, 55*(4), 1133-1140.

National Health System Choices UK. (2018). *Menopause overview*. Retrieved from https://www.nhs.uk/conditions/menopause/

National Institute for Health and Care Excellence guidelines. (2019). Retrieved from https://www.nice.org.uk/

Newman, G. C., Bang, H., Hussain, S. I., & Toole, J. F. (2007). Association of diabetes, homocysteine, and HDL with cognition and disability after stroke. *Neurology, 69*(22), 2054-2062. doi:10.1212/01.wnl.0000280457.29680.9c

Newport, D. J., Stowe, Z. N., & Nemeroff, C. B. (2002). Parental depression: animal models of an adverse life event. *The American journal of psychiatry, 159*(8), 1265-1283. doi:10.1176/appi.ajp.159.8.1265

Newton, T. L., Fernandez-Botran, R., Miller, J. J., & Burns, V. E. (2014). Interleukin-6 and soluble interleukin-6 receptor levels in posttraumatic stress disorder: associations with lifetime diagnostic status and psychological context. *Biol Psychol, 99*, 150-159. doi:10.1016/j.biopsycho.2014.03.009

Nicolaides, N. C., Kyratzi, E., Lamprokostopoulou, A., Chrousos, G. P., & Charmandari, E. (2015). Stress, the stress system and the role of glucocorticoids. *Neuroimmunomodulation, 22*(1-2), 6-19. doi:10.1159/000362736

Nicolucci, A., Kovacs Burns, K., Holt, R. I. G., Comaschi, M., Hermanns, N., Ishii, H., . . . Group, D. S. (2013). Diabetes Attitudes, Wishes and Needs second study (DAWN2™): cross-national benchmarking of diabetes-related psychosocial outcomes for people with diabetes. *Diabetic medicine : a journal of the British Diabetic Association, 30*(7), 767-777. doi:10.1111/dme.12245

Niswender, K. D., & Schwartz, M. W. (2003). Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Frontiers in neuroendocrinology, 24*(1), 1-10. doi:10.1016/s0091-3022(02)00105-x

Niu, J., & Kolattukudy, P. E. (2009). Role of MCP-1 in cardiovascular disease: molecular mechanisms and clinical implications. *Clin Sci (Lond), 117*(3), 95-109. doi:10.1042/cs20080581

Nouwen, A., Winkley, K., Twisk, J., Lloyd, C. E., Peyrot, M., Ismail, K., . . . European Depression in Diabetes Research, C. (2010). Type 2 diabetes mellitus as a risk factor for the onset of depression: a systematic review and meta-analysis. *Diabetologia, 53*(12), 2480-2486. doi:10.1007/s00125-010-1874-x

Novais, A., Monteiro, S., Roque, S., Correia-Neves, M., & Sousa, N. (2017). How age, sex and genotype shape the stress response. *Neurobiol Stress, 6*, 44-56. doi:10.1016/j.ynstr.2016.11.004

Novak, M., Mucsi, I., Rhee, C. M., Streja, E., Lu, J. L., Kalantar-Zadeh, K., . . . Kovesdy, C. P. (2016). Increased Risk of Incident Chronic Kidney Disease, Cardiovascular Disease, and Mortality in Patients With Diabetes With Comorbid Depression. *Diabetes Care, 39*(11), 1940-1947. doi:10.2337/dc16-0048

Nowotny, B., Cavka, M., Herder, C., Löffler, H., Poschen, U., Joksimovic, L., . . . Kruse, J. (2010). Effects of acute psychological stress on glucose metabolism and subclinical inflammation in patients with post-traumatic stress disorder. *Horm Metab Res, 42*(10), 746-753. doi:10.1055/s-0030-1261924

Nyberg, S. T., Fransson, E. I., Heikkilä, K., Ahola, K., Alfredsson, L., Bjorner, J. B., . . . Consortium, I. P.-W. (2014). Job strain as a risk factor for type 2 diabetes: a pooled analysis of 124,808 men and women. *Diabetes Care, 37*(8), 2268-2275. doi:10.2337/dc13-2936

O'Connor, M. F., Irwin, M. R., & Wellisch, D. K. (2009). When grief heats up: pro-inflammatory cytokines predict regional brain activation. *Neuroimage, 47*(3), 891-896. doi:10.1016/j.neuroimage.2009.05.049

O'Keeffe, A. G., Geneletti, S., Baio, G., Sharples, L. D., Nazareth, I., & Petersen, I. (2014). Regression discontinuity designs: an approach to the evaluation of treatment efficacy in primary care using observational data. *Bmj, 349*, g5293. doi:10.1136/bmj.g5293

O'Toole, M. S., Bovbjerg, D. H., Renna, M. E., Lekander, M., Mennin, D. S., & Zachariae, R. (2018). Effects of psychological interventions on systemic levels

of inflammatory biomarkers in humans: A systematic review and meta-analysis. *Brain Behav Immun, 74*, 68-78. doi:10.1016/j.bbi.2018.04.005

Oberbach, A., Lehmann, S., Kirsch, K., Krist, J., Sonnabend, M., Linke, A., . . . Kovacs, P. (2008). Long-term exercise training decreases interleukin-6 (IL-6) serum levels in subjects with impaired glucose tolerance: effect of the -174G/C variant in IL-6 gene. *Eur J Endocrinol, 159*(2), 129-136. doi:10.1530/eje-08-0220

Ogden, C. L., Carroll, M. D., Kit, B. K., & Flegal, K. M. (2014). Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA, 311*(8), 806-814. doi:10.1001/jama.2014.732

Okely, J. A., & Gale, C. R. (2016). Well-Being and Chronic Disease Incidence: The English Longitudinal Study of Ageing. *Psychosom Med, 78*(3), 335-344. doi:10.1097/psy.0000000000000279

Okura, Y., Urban, L. H., Mahoney, D. W., Jacobsen, S. J., & Rodeheffer, R. J. (2004). Agreement between self-report questionnaires and medical record data was substantial for diabetes, hypertension, myocardial infarction and stroke but not for heart failure. *J Clin Epidemiol, 57*(10), 1096-1103. doi:10.1016/j.jclinepi.2004.04.005

Ostir, G. V., Markides, K. S., Black, S. A., & Goodwin, J. S. (2000). Emotional well-being predicts subsequent functional independence and survival. *J Am Geriatr Soc, 48*(5), 473-478. doi:10.1111/j.1532-5415.2000.tb04991.x

Out, D., Hall, R. J., Granger, D. A., Page, G. G., & Woods, S. J. (2012). Assessing salivary C-reactive protein: longitudinal associations with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence. *Brain Behav Immun, 26*(4), 543-551. doi:10.1016/j.bbi.2012.01.019

Pace, T. W. W., Negi, L. T., Adame, D. D., Cole, S. P., Sivilli, T. I., Brown, T. D., . . . Raison, C. L. (2009). Effect of compassion meditation on neuroendocrine,

innate immune and behavioral responses to psychosocial stress. *Psychoneuroendocrinology, 34*(1), 87-98. doi:10.1016/j.psyneuen.2008.08.011

Padgett, D. A., & Glaser, R. (2003). How stress influences the immune response. *Trends in immunology, 24*(8), 444-448.

Paganini-Hill, A., & Chao, A. (1993). Accuracy of recall of hip fracture, heart attack, and cancer: a comparison of postal survey data and medical records. *Am J Epidemiol, 138*(2), 101-106. doi:10.1093/oxfordjournals.aje.a116832

Panagi, L., Poole, L., Hackett, R. A., & Steptoe, A. (2019a). Happiness and Inflammatory Responses to Acute Stress in People With Type 2 Diabetes. *Ann Behav Med, 53*(4), 309-320. doi:10.1093/abm/kay039

Panagi, L., Poole, L., Hackett, R. A., & Steptoe, A. (2019b). Sex differences in interleukin-6 stress responses in people with Type 2 diabetes. *Psychophysiology, 56*(6), e13334. doi:10.1111/psyp.13334

Papanicolaou, D. A., Wilder, R. L., Manolagas, S. C., & Chrousos, G. P. (1998). The pathophysiologic roles of interleukin-6 in human disease. *Annals of internal medicine, 128*(2), 127-137. doi:10.7326/0003-4819-128-2-199801150-00009

Pariante, C. M. (2017). Why are depressed patients inflamed? A reflection on 20 years of research on depression, glucocorticoid resistance and inflammation. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology, 27*(6), 554-559. doi:10.1016/j.euroneuro.2017.04.001

Park-Lee, E., Fredman, L., Hochberg, M., & Faulkner, K. (2009). Positive affect and incidence of frailty in elderly women caregivers and noncaregivers: results of Caregiver-Study of Osteoporotic Fractures. *J Am Geriatr Soc, 57*(4), 627-633. doi:10.1111/j.1532-5415.2009.02183.x

Park, M., Katon, W. J., & Wolf, F. M. (2013). Depression and risk of mortality in individuals with diabetes: a meta-analysis and systematic review. *General hospital psychiatry, 35*(3), 217-225. doi:10.1016/j.genhosppsych.2013.01.006

Pascoe, M. C., Thompson, D. R., & Ski, C. F. (2017). Yoga, mindfulness-based stress reduction and stress-related physiological measures: A meta-analysis. *Psychoneuroendocrinology, 86*, 152-168. doi:10.1016/j.psyneuen.2017.08.008

Patino, C. M., & Ferreira, J. C. (2018). Inclusion and exclusion criteria in research studies: definitions and why they matter. *J Bras Pneumol, 44*(2), 84. doi:10.1590/s1806-37562018000000088

Pearlin, L. I., Lieberman, M. A., Menaghan, E. G., & Mullan, J. T. (1981). The stress process. *J Health Soc Behav, 22*(4), 337-356.

Pedersen, B. K. (2012). Muscular interleukin-6 and its role as an energy sensor. *Med Sci Sports Exerc, 44*(3), 392-396. doi:10.1249/MSS.0b013e31822f94ac

Penckofer, S., Byrn, M., Adams, W., Emanuele, M. A., Mumby, P., Kouba, J., & Wallis, D. E. (2017). Vitamin D supplementation improves mood in women with type 2 diabetes. *Journal of diabetes research, 2017*.

Peters, S. A., Huxley, R. R., & Woodward, M. (2014a). Diabetes as a risk factor for stroke in women compared with men: a systematic review and meta-analysis of 64 cohorts, including 775,385 individuals and 12,539 strokes. *Lancet, 383*(9933), 1973-1980. doi:10.1016/s0140-6736(14)60040-4

Peters, S. A., Huxley, R. R., & Woodward, M. (2014b). Diabetes as risk factor for incident coronary heart disease in women compared with men: a systematic review and meta-analysis of 64 cohorts including 858,507 individuals and 28,203 coronary events. *Diabetologia, 57*(8), 1542-1551. doi:10.1007/s00125-014-3260-6

Peters, S. A. E., Huxley, R. R., Sattar, N., & Woodward, M. (2015). Sex Differences in the Excess Risk of Cardiovascular Diseases Associated with Type 2 Diabetes: Potential Explanations and Clinical Implications. *Current cardiovascular risk reports, 9*(7), 36-36. doi:10.1007/s12170-015-0462-5

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. *Physiological reviews, 98*(4), 2133-2223.

Peterson, J. C., Charlson, M. E., Hoffman, Z., Wells, M. T., Wong, S. C., Hollenberg, J. P., . . . Allegrante, J. P. (2012). A randomized controlled trial of positive-affect induction to promote physical activity after percutaneous coronary intervention. *Arch Intern Med, 172*(4), 329-336. doi:10.1001/archinternmed.2011.1311

Phillips, A. C. (2011). Blunted cardiovascular reactivity relates to depression, obesity, and self-reported health. *Biol Psychol, 86*(2), 106-113. doi:10.1016/j.biopsycho.2010.03.016

Phillips, C. M., & Perry, I. J. (2013). Does inflammation determine metabolic health status in obese and nonobese adults? *The Journal of Clinical Endocrinology & Metabolism, 98*(10), E1610-E1619.

Phillips, G., Renton, A., Moore, D. G., Bottomley, C., Schmidt, E., Lais, S., . . . Frostick, C. (2012). The Well London program-a cluster randomized trial of community engagement for improving health behaviors and mental wellbeing: baseline survey results. *Trials, 13*(1), 105.

Picard, M., Juster, R. P., & McEwen, B. S. (2014). Mitochondrial allostatic load puts the 'gluc' back in glucocorticoids. *Nat Rev Endocrinol, 10*(5), 303-310. doi:10.1038/nrendo.2014.22

Picard, M., & McEwen, B. S. (2018). Psychological Stress and Mitochondria: A Conceptual Framework. *Psychosomatic medicine, 80*(2), 126-140. doi:10.1097/PSY.0000000000000544

Piciu, A. M., Johar, H., Lukaschek, K., Thorand, B., & Ladwig, K. H. (2018). Life satisfaction is a protective factor against the onset of Type 2 diabetes in men but not in women: findings from the MONICA/KORA cohort study. *Diabetic medicine : a journal of the British Diabetic Association, 35*(3), 323-331. doi:10.1111/dme.13574

Pickup, J., Mattock, M., Chusney, G., & Burt, D. (1997). NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia, 40*(11), 1286.

Pickup, J. C. (2004). Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care, 27*(3), 813-823. doi:10.2337/diacare.27.3.813

Pinos, H., Collado, P., Rodríguez-Zafra, M., Rodríguez, C., Segovia, S., & Guillamón, A. (2001). The development of sex differences in the locus coeruleus of the rat. *Brain Res Bull, 56*(1), 73-78. doi:10.1016/s0361-9230(01)00540-8

Pinquart, M. (2018). Parenting stress in caregivers of children with chronic physical condition-A meta-analysis. *Stress and health : journal of the International Society for the Investigation of Stress, 34*(2), 197-207. doi:10.1002/smi.2780

Pinquart, M., & Sörensen, S. (2003). Differences between caregivers and noncaregivers in psychological health and physical health: a meta-analysis. *Psychology and aging, 18*(2), 250-267. doi:10.1037/0882-7974.18.2.250

Polsky, S., & Akturk, H. K. (2017). Alcohol consumption, diabetes risk, and cardiovascular disease within diabetes. *Current diabetes reports, 17*(12), 136.

Poole, L., Hackett, R. A., Panagi, L., & Steptoe, A. (2019). Subjective wellbeing as a determinant of glycated hemoglobin in older adults: longitudinal findings from the English Longitudinal Study of Ageing. *Psychol Med*, 1-9. doi:10.1017/s0033291719001879

Poole, L., Kidd, T., Leigh, E., Ronaldson, A., Jahangiri, M., & Steptoe, A. (2015). Psychological distress and intensive care unit stay after cardiac surgery: The role of illness concern. *Health psychology : official journal of the Division of Health Psychology, American Psychological Association, 34*(3), 283-287. doi:10.1037/hea0000183

Poulsen, P., Grunnet, L. G., Pilgaard, K., Storgaard, H., Alibegovic, A., Sonne, M. P., . . . Vaag, A. (2009). Increased risk of type 2 diabetes in elderly twins. *Diabetes, 58*(6), 1350-1355. doi:10.2337/db08-1714

Poulsen, P., Kyvik, K. O., Vaag, A., & Beck-Nielsen, H. (1999). Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance--a population-based twin study. *Diabetologia, 42*(2), 139-145. doi:10.1007/s001250051131

Prather, A. A., Carroll, J. E., Fury, J. M., McDade, K. K., Ross, D., & Marsland, A. L. (2009). Gender differences in stimulated cytokine production following acute psychological stress. *Brain, behavior, and immunity, 23*(5), 622-628. doi:10.1016/j.bbi.2008.11.004

Preacher, K. J., & Hayes, A. F. (2004). SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav Res Methods Instrum Comput, 36*(4), 717-731. doi:10.3758/bf03206553

Preacher, K. J., & Hayes, A. F. (2008). Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods, 40*(3), 879-891. doi:10.3758/brm.40.3.879

Prospective Studies Collaboration and Asia Pacific Cohort Studies Collaboration. (2018). Sex-specific relevance of diabetes to occlusive vascular and other mortality: a collaborative meta-analysis of individual data from 980 793 adults from 68 prospective studies. *The lancet. Diabetes & endocrinology, 6*(7), 538-546. doi:10.1016/S2213-8587(18)30079-2

Puig-Perez, S., Hackett, R., Salvador, A., & Steptoe, A. (2017). Optimism moderates psychophysiological responses to stress in older people with Type 2 diabetes. *Psychophysiology, 54*(4), 536-543.

Rådholm, K., Wiréhn, A. B., Chalmers, J., & Östgren, C. J. (2016). Use of antidiabetic and antidepressant drugs is associated with increased risk of myocardial infarction: a nationwide register study. *Diabetic medicine : a journal of the British Diabetic Association, 33*(2), 218-223. doi:10.1111/dme.12822

Radloff, L.S. (1977). The CES-D Scale. A Self-Report Depression Scale for Research in the General Population. *Appl Psychol Meas, 1*(3), 385-401. doi:10.1177/014662167700100306

Raina, P., Torrance-Rynard, V., Wong, M., & Woodward, C. (2002). Agreement between self-reported and routinely collected health-care utilization data among seniors. *Health Serv Res, 37*(3), 751-774. doi:10.1111/1475-6773.00047

Rajagopalan, S., Brook, R., Rubenfire, M., Pitt, E., Young, E., & Pitt, B. (2001). Abnormal brachial artery flow-mediated vasodilation in young adults with major depression. *Am J Cardiol, 88*(2), 196-198, a197. doi:10.1016/s0002-9149(01)01623-x

Raparelli, V., Morano, S., Franconi, F., Lenzi, A., & Basili, S. (2017). Sex Differences in Type-2 Diabetes: Implications for Cardiovascular Risk Management. *Curr Pharm Des, 23*(10), 1471-1476. doi:10.2174/1381612823666170130153704

Rasmussen, H. N., Scheier, M. F., & Greenhouse, J. B. (2009). Optimism and physical health: a meta-analytic review. *Ann Behav Med, 37*(3), 239-256. doi:10.1007/s12160-009-9111-x

Raygan, F., Ostadmohammadi, V., Bahmani, F., & Asemi, Z. (2018). The effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic status in type 2 diabetic patients with coronary heart disease:

a randomized, double-blind, placebo-controlled trial. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 84*, 50-55.

Rohleder, N., Schommer, N. C., Hellhammer, D. H., Engel, R., & Kirschbaum, C. (2001). Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosom Med, 63*(6), 966-972. doi:10.1097/00006842-200111000-00016

Rohleder, N., Wolf, J. M., Herpfer, I., Fiebich, B. L., Kirschbaum, C., & Lieb, K. (2006). No response of plasma substance P, but delayed increase of interleukin-1 receptor antagonist to acute psychosocial stress. *Life Sci, 78*(26), 3082-3089. doi:10.1016/j.lfs.2005.12.016

Rose, S., & Laan, M. J. (2009). Why match? Investigating matched case-control study designs with causal effect estimation. *Int J Biostat, 5*(1), Article 1. doi:10.2202/1557-4679.1127

Rosenberg, M. (1979). *Conceiving the self*. New York, NY: Basic Books.

Rosenblat, J. D., Cha, D. S., Mansur, R. B., & McIntyre, R. S. (2014). Inflamed moods: a review of the interactions between inflammation and mood disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 53*, 23-34.

Rosenblat, J. D., Cha, D. S., Mansur, R. B., & McIntyre, R. S. (2014). Inflamed moods: a review of the interactions between inflammation and mood disorders. *Prog Neuropsychopharmacol Biol Psychiatry, 53*, 23-34. doi:10.1016/j.pnpbp.2014.01.013

Rotella, F., & Mannucci, E. (2013). Diabetes mellitus as a risk factor for depression. A meta-analysis of longitudinal studies. *Diabetes research and clinical practice, 99*(2), 98-104. doi:10.1016/j.diabres.2012.11.022

Russell, D., Peplau, L. A., & Cutrona, C. E. (1980). The revised UCLA Loneliness Scale: concurrent and discriminant validity evidence. *J Pers Soc Psychol, 39*(3), 472-480. doi:10.1037//0022-3514.39.3.472

Ryan, R. M., & Deci, E. L. (2001). On happiness and human potentials: a review of research on hedonic and eudaimonic well-being. *Annu Rev Psychol, 52*, 141-166. doi:10.1146/annurev.psych.52.1.141

Ryff, C. (1989). Happiness is everything, or is it?. Explorations on the meaning of psychological well-being. *J Pers Soc Psychol*, *57*(6), 1069-1081.

Saito, I., Folsom, A. R., Brancati, F. L., Duncan, B. B., Chambless, L. E., & McGovern, P. G. (2000). Nontraditional risk factors for coronary heart disease incidence among persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Annals of internal medicine, 133*(2), 81-91.

Salpea, K. D., Talmud, P. J., Cooper, J. A., Maubaret, C. G., Stephens, J. W., Abelak, K., & Humphries, S. E. (2010). Association of telomere length with type 2 diabetes, oxidative stress and UCP2 gene variation. *Atherosclerosis, 209*(1), 42-50. doi:10.1016/j.atherosclerosis.2009.09.070

Sanghera, D. K., & Blackett, P. R. (2012). Type 2 Diabetes Genetics: Beyond GWAS. *Journal of diabetes & metabolism, 3*(198), 6948. doi:10.4172/2155-6156.1000198

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*(1), 55-89. doi:10.1210/edrv.21.1.0389.v

Satoh, N., Shimatsu, A., Kotani, K., Sakane, N., Yamada, K., Suganami, T., . . . Ogawa, Y. (2007). Purified eicosapentaenoic acid reduces small dense LDL, remnant lipoprotein particles, and C-reactive protein in metabolic syndrome. *Diabetes Care, 30*(1), 144-146.

Scheier, M. F., Carver, C. S., & Bridges, M. W. (1994). Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the Life Orientation Test. *J Pers Soc Psychol, 67*(6), 1063-1078. doi:10.1037//0022-3514.67.6.1063

Schellenberg, E. S., Dryden, D. M., Vandermeer, B., Ha, C., & Korownyk, C. (2013). Lifestyle interventions for patients with and at risk for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med, 159*(8), 543-551. doi:10.7326/0003-4819-159-8-201310150-00007

Scherrer, J. F., Garfield, L. D., Chrusciel, T., Hauptman, P. J., Carney, R. M., Freedland, K. E., . . . Lustman, P. J. (2011). Increased risk of myocardial infarction in depressed patients with type 2 diabetes. *Diabetes Care, 34*(8), 1729-1734. doi:10.2337/dc11-0031

Schoormans, D., Radonic, T., de Witte, P., Groenink, M., Azim, D., Lutter, R., . . . Zwinderman, A. H. (2012). Mental quality of life is related to a cytokine genetic pathway. *PLoS One, 7*(9), e45126. doi:10.1371/journal.pone.0045126

Schunk, M., Reitmeir, P., Schipf, S., Volzke, H., Meisinger, C., Ladwig, K. H., . . . Holle, R. (2015). Health-related quality of life in women and men with type 2 diabetes: a comparison across treatment groups. *J Diabetes Complications, 29*(2), 203-211. doi:10.1016/j.jdiacomp.2014.11.010

Schwartz, G. G., Il'yasova, D., & Ivanova, A. (2003). Urinary cadmium, impaired fasting glucose, and diabetes in the NHANES III. *Diabetes Care, 26*(2), 468-470.

Scragg, R., & Metcalf, P. (2004). Do triglycerides explain the U-shaped relation between alcohol and diabetes risk?: Results from a cross-sectional survey of alcohol and plasma glucose. *Diabetes research and clinical practice, 66*(2), 147-156.

Seale, J., Wood, S., Atkinson, H., Bate, E., Lightman, S., Ingram, C., . . . Harbuz, M. (2004). Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats. *Journal of neuroendocrinology, 16*(6), 516-524.

Seale, J., Wood, S., Atkinson, H., Harbuz, M., & Lightman, S. (2004). Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone

pulse profile and stress-induced hypothalamic-pituitary-adrenal axis activity of male and female rats. *Journal of neuroendocrinology, 16*(12), 989-998.

Segerstrom, S. C., & Miller, G. E. (2004). Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychological bulletin, 130*(4), 601-630. doi:10.1037/0033-2909.130.4.601

Seghieri, G., Policardo, L., Anichini, R., Franconi, F., Campesi, I., Cherchi, S., & Tonolo, G. (2017). The Effect of Sex and Gender on Diabetic Complications. *Curr Diabetes Rev, 13*(2), 148-160. doi:10.2174/1573399812666160517115756

Seidell, J. C. (1997). Time trends in obesity: an epidemiological perspective. *Hormone and Metabolic Research, 29*(04), 155-158.

Seidl, H., Meisinger, C., Kirchberger, I., Burkhardt, K., Kuch, B., & Holle, R. (2016). Validity of self-reported hospital admissions in clinical trials depends on recall period length and individual characteristics. *J Eval Clin Pract, 22*(3), 446-454. doi:10.1111/jep.12506

Seligman, M. E. P. (2011). *Flourish: A New Understanding of Happiness and Wellbeing*. London: Nicholas Brealey Publishing.

Selye, H. (1950). Stress and the general adaptation syndrome. *British medical journal, 1*(4667), 1383-1392. doi:10.1136/bmj.1.4667.1383

Shah, A. D., Langenberg, C., Rapsomaniki, E., Denaxas, S., Pujades-Rodriguez, M., Gale, C. P., . . . Hemingway, H. (2015). Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1· 9 million people. *The lancet Diabetes & endocrinology, 3*(2), 105-113.

Shahab, L., & West, R. (2012). Differences in happiness between smokers, ex-smokers and never smokers: cross-sectional findings from a national household survey. *Drug Alcohol Depend, 121*(1-2), 38-44. doi:10.1016/j.drugalcdep.2011.08.011

Shalev, A., Liberzon, I., & Marmar, C. (2017). Post-Traumatic Stress Disorder. *The New England journal of medicine, 376*(25), 2459-2469. doi:10.1056/NEJMra1612499

Sheikh, S., Sinha, A. D., & Agarwal, R. (2011). Home blood pressure monitoring: how good a predictor of long-term risk? *Curr Hypertens Rep, 13*(3), 192-199. doi:10.1007/s11906-011-0193-z

Shephard, R. J. (2002). Cytokine responses to physical activity, with particular reference to IL-6: sources, actions, and clinical implications. *Crit Rev Immunol, 22*(3), 165-182.

Shibayama, T., Sato, E., Nishigaki, M., Ochiai, R., & Kazuma, K. (2011). Influence of dietary therapy appraisal on future perceived control of type 2 diabetes. *J Health Psychol, 16*(8), 1141-1150. doi:10.1177/1359105311401770

Shields, G. S., Spahr, C. M., & Slavich, G. M. (2020). Psychosocial interventions and immune system function: a systematic review and meta-analysis of randomized clinical trials. *JAMA Psychiatry*.

Shiffman, S., Stone, A. A., & Hufford, M. R. (2008). Ecological momentary assessment. *Annu Rev Clin Psychol, 4*, 1-32. doi:10.1146/annurev.clinpsy.3.022806.091415

Shimokata, H., Muller, D. C., & Andres, R. (1989). Studies in the distribution of body fat: III. Effects of cigarette smoking. *JAMA, 261*(8), 1169-1173.

Shirai, K., Iso, H., Ohira, T., Ikeda, A., Noda, H., Honjo, K., . . . Tsugane, S. (2009). Perceived level of life enjoyment and risks of cardiovascular disease incidence and mortality: the Japan public health center-based study. *Circulation, 120*(11), 956-963. doi:10.1161/circulationaha.108.834176

Shirom, A., Toker, S., Melamed, S., Berliner, S., & Shapira, I. (2012). Life and Job Satisfaction as Predictors of the Incidence of Diabetes. *Applied psychology. Health and well-being, 4*(1), 31-48. doi:10.1111/j.1758-0854.2011.01054.x

Shoelson, S. E., Lee, J., & Goldfine, A. B. (2006). Inflammation and insulin resistance. *The Journal of clinical investigation, 116*(7), 1793-1801.

Short, M. E., Goetzel, R. Z., Pei, X., Tabrizi, M. J., Ozminkowski, R. J., Gibson, T. B., . . . Wilson, M. G. (2009). How accurate are self-reports? Analysis of self-reported health care utilization and absence when compared with administrative data. *J Occup Environ Med, 51*(7), 786-796. doi:10.1097/JOM.0b013e3181a86671

Siddiqui, M. A., Khan, M. F., & Carline, T. E. (2013). Gender differences in living with diabetes mellitus. *Mater Sociomed, 25*(2), 140-142. doi:10.5455/msm.2013.25.140-142

Sieu, N., Katon, W., Lin, E. H. B., Russo, J., Ludman, E., & Ciechanowski, P. (2011). Depression and incident diabetic retinopathy: a prospective cohort study. *General hospital psychiatry, 33*(5), 429-435. doi:10.1016/j.genhosppsych.2011.05.021

Simon, J. A., Seeley, D. G., Lipschutz, R. C., Vittinghoff, E., & Browner, W. S. (1997). The relation of smoking to waist-to-hip ratio and diabetes mellitus among elderly women. *Preventive medicine, 26*(5), 639-644.

Simpson, C. F., Boyd, C. M., Carlson, M. C., Griswold, M. E., Guralnik, J. M., & Fried, L. P. (2004). Agreement between self-report of disease diagnoses and medical record validation in disabled older women: factors that modify agreement. *Journal of the American Geriatrics Society, 52*(1), 123-127.

Sin, N. L., & Lyubomirsky, S. (2009). Enhancing well-being and alleviating depressive symptoms with positive psychology interventions: a practice-friendly meta-analysis. *J Clin Psychol, 65*(5), 467-487. doi:10.1002/jclp.20593

Sin, N. L., Graham-Engeland, J. E., & Almeida, D. M. (2015). Daily positive events and inflammation: findings from the National Study of Daily Experiences. *Brain Behav Immun, 43*, 130-138. doi:10.1016/j.bbi.2014.07.015

Sin, N. L., Graham-Engeland, J. E., Ong, A. D., & Almeida, D. M. (2015). Affective reactivity to daily stressors is associated with elevated inflammation. *Health Psychol, 34*(12), 1154-1165. doi:10.1037/hea0000240

Sjögren, E., Leanderson, P., Kristenson, M., & Ernerudh, J. (2006). Interleukin-6 levels in relation to psychosocial factors: studies on serum, saliva, and in vitro production by blood mononuclear cells. *Brain Behav Immun, 20*(3), 270-278. doi:10.1016/j.bbi.2005.08.001

Slavish, D. C., Graham-Engeland, J. E., Smyth, J. M., & Engeland, C. G. (2015). Salivary markers of inflammation in response to acute stress. *Brain, behavior, and immunity, 44*, 253-269. doi:10.1016/j.bbi.2014.08.008

Slavish, D. C., Jones, D. R., Smyth, J. M., Engeland, C. G., Song, S., McCormick, N. M., & Graham-Engeland, J. E. (2019). Positive and Negative Affect and Salivary Markers of Inflammation Among Young Adults. *Int J Behav Med*. doi:10.1007/s12529-019-09795-2

Smith, K. J., Béland, M., Clyde, M., Gariépy, G., Pagé, V., Badawi, G., . . . Schmitz, N. (2013). Association of diabetes with anxiety: a systematic review and meta-analysis. *Journal of psychosomatic research, 74*(2), 89-99. doi:10.1016/j.jpsychores.2012.11.013

Smith, M. A., Thompson, A., Hall, L. J., Allen, S. F., & Wetherell, M. A. (2018). The physical and psychological health benefits of positive emotional writing: Investigating the moderating role of Type D (distressed) personality. *British journal of health psychology, 23*(4), 857-871.

Soczynska, J. K., Kennedy, S. H., Goldstein, B. I., Lachowski, A., Woldeyohannes, H. O., & McIntyre, R. S. (2009). The effect of tumor necrosis factor antagonists on mood and mental health-associated quality of life: novel hypothesis-driven treatments for bipolar depression? *Neurotoxicology, 30*(4), 497-521. doi:10.1016/j.neuro.2009.03.004

Soinio, M., Marniemi, J., Laakso, M., Lehto, S., & Rönnemaa, T. (2006). High-sensitivity C-reactive protein and coronary heart disease mortality in patients with type 2 diabetes: a 7-year follow-up study. *Diabetes Care, 29*(2), 329-333.

Song, J. W., & Chung, K. C. (2010). Observational studies: cohort and case-control studies. *Plast Reconstr Surg, 126*(6), 2234-2242. doi:10.1097/PRS.0b013e3181f44abc

Spranger, J., Kroke, A., Möhlig, M., Hoffmann, K., Bergmann, M. M., Ristow, M., . . . Pfeiffer, A. F. (2003). Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes, 52*(3), 812-817.

Sproston, K., & Mindell, J. (2004). *Health Survery for England: Methodology and documentation* (Vol 2). Leeds: The Information Centre.

Steensberg, A., Fischer, C. P., Keller, C., Møller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American journal of physiology. Endocrinology and metabolism, 285*(2), E433-E437. doi:10.1152/ajpendo.00074.2003

Steffick, D.E. (2000). *Documentation of Affective Functioning Measures in the Health and Retirement Study*. Ann Arbor, MI: University of Michigan Survey Research Center. HRS Health Work Group. Retrieved from https://hrs.isr.umich.edu/publications/biblio/5411

Stellar, J. E., John-Henderson, N., Anderson, C. L., Gordon, A. M., McNeil, G. D., & Keltner, D. (2015). Positive affect and markers of inflammation: discrete positive emotions predict lower levels of inflammatory cytokines. *Emotion, 15*(2), 129-133. doi:10.1037/emo0000033

Steptoe, A. (2000). Stress, social support and cardiovascular activity over the working day. *International Journal of Psychophysiology, 37*(3), 299-308. doi:10.1016/s0167-8760(00)00109-4

Steptoe, A. (2016). Diabetes: Stress resilience and risk of type 2 diabetes mellitus. *Nature reviews. Endocrinology, 12*(4), 189-190. doi:10.1038/nrendo.2016.17

Steptoe, A. (2019a). Happiness and Health. *Annu Rev Public Health, 40*, 339-359. doi:10.1146/annurev-publhealth-040218-044150

Steptoe, A. (2019b). Investing in Happiness: The Gerontological Perspective. *Gerontology, 65*(6), 634-639. doi:10.1159/000501124

Steptoe, A., & Fancourt, D. (2019). Leading a meaningful life at older ages and its relationship with social engagement, prosperity, health, biology, and time use. *Proc Natl Acad Sci U S A, 116*(4), 1207-1212. doi:10.1073/pnas.1814723116

Steptoe, A., & Poole, L. (2010). Use of Biological Measures in Behavioral Medicine. In A. Steptoe (Ed.), *Handbook of Behavioral Medicine: Methods and Applications* (pp. 619-632). New York, NY: Springer New York.

Steptoe, A., & Wardle, J. (2005). Positive affect and biological function in everyday life. *Neurobiology of Aging, 26*(1), 108-112.

Steptoe, A., & Wardle, J. (2011). Positive affect measured using ecological momentary assessment and survival in older men and women. *Proc Natl Acad Sci U S A, 108*(45), 18244-18248. doi:10.1073/pnas.1110892108

Steptoe, A., & Wardle, J. (2012). Enjoying life and living longer. *Arch Intern Med, 172*(3), 273-275. doi:10.1001/archinternmed.2011.1028

Steptoe, A., Breeze, E., Banks, J., & Nazroo, J. (2013). Cohort profile: the English longitudinal study of ageing. *Int J Epidemiol, 42*(6), 1640-1648. doi:10.1093/ije/dys168

Steptoe, A., de Oliveira, C., Demakakos, P., & Zaninotto, P. (2014). Enjoyment of life and declining physical function at older ages: a longitudinal cohort study. *Cmaj, 186*(4), E150-156. doi:10.1503/cmaj.131155

Steptoe, A., Deaton, A., & Stone, A. A. (2015). Subjective wellbeing, health, and ageing. *Lancet, 385*(9968), 640-648. doi:10.1016/s0140-6736(13)61489-0

Steptoe, A., Dockray, S., & Wardle, J. (2009). Positive affect and psychobiological processes relevant to health. *J Pers, 77*(6), 1747-1776. doi:10.1111/j.1467-6494.2009.00599.x

Steptoe, A., Feldman, P. J., Kunz, S., Owen, N., Willemsen, G., & Marmot, M. (2002). Stress responsivity and socioeconomic status: a mechanism for increased cardiovascular disease risk? *Eur Heart J, 23*(22), 1757-1763. doi:10.1053/euhj.2001.3233

Steptoe, A., Hackett, R. A., Lazzarino, A. I., Bostock, S., La Marca, R., Carvalho, L. A., & Hamer, M. (2014). Disruption of multisystem responses to stress in type 2 diabetes: investigating the dynamics of allostatic load. *Proc Natl Acad Sci U S A, 111*(44), 15693-15698. doi:10.1073/pnas.1410401111

Steptoe, A., Hamer, M., & Chida, Y. (2007). The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav Immun, 21*(7), 901-912. doi:10.1016/j.bbi.2007.03.011

Steptoe, A., Kivimaki, M., Lowe, G., Rumley, A., & Hamer, M. (2016). Blood Pressure and Fibrinogen Responses to Mental Stress as Predictors of Incident Hypertension over an 8-Year Period. *Ann Behav Med, 50*(6), 898-906. doi:10.1007/s12160-016-9817-5

Steptoe, A., Kunz-Ebrecht, S., Owen, N., Feldman, P. J., Willemsen, G., Kirschbaum, C., & Marmot, M. (2003). Socioeconomic status and stress-related biological responses over the working day. *Psychosomatic medicine, 65*(3), 461-470. doi:10.1097/01.psy.0000035717.78650.a1

Steptoe, A., O'Donnell, K., Badrick, E., Kumari, M., & Marmot, M. (2008). Neuroendocrine and inflammatory factors associated with positive affect in healthy men and women: the Whitehall II study. *Am J Epidemiol, 167*(1), 96-102. doi:10.1093/aje/kwm252

Steptoe, A., Owen, N., Kunz-Ebrecht, S., & Mohamed-Ali, V. (2002). Inflammatory cytokines, socioeconomic status, and acute stress responsivity. *Brain Behav Immun, 16*(6), 774-784.

Steptoe, A., Wardle, J., & Marmot, M. (2005). Positive affect and health-related neuroendocrine, cardiovascular, and inflammatory processes. *Proc Natl Acad Sci U S A, 102*(18), 6508-6512. doi:10.1073/pnas.0409174102

Steptoe, A., Willemsen, G., Owen, N., Flower, L., & Mohamed-Ali, V. (2001). Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels. *Clin Sci (Lond), 101*(2), 185-192.

Stevens, J. (2002). *Applied multivariate statistics for the social sciences* (4th ed.). Mahwah, N.J: Erlbaum.

Stone, A. A., & Mackie, C. (2013). *Subjective Well-Being: Measuring Happiness, Suffering, and Other Dimensions of Experience*. Washington (DC): National Academies Press.

Stringhini, S., Batty, G. D., Bovet, P., Shipley, M. J., Marmot, M. G., Kumari, M., . . . Kivimaki, M. (2013). Association of lifecourse socioeconomic status with chronic inflammation and type 2 diabetes risk: the Whitehall II prospective cohort study. *PLoS Med, 10*(7), e1001479. doi:10.1371/journal.pmed.1001479

Suarez, E. C., Krishnan, R. R., & Lewis, J. G. (2003). The relation of severity of depressive symptoms to monocyte-associated proinflammatory cytokines and chemokines in apparently healthy men. *Psychosom Med, 65*(3), 362-368. doi:10.1097/01.psy.0000035719.79068.2b

Sullivan, S., Hammadah, M., Wilmot, K., Ramadan, R., Pearce, B. D., Shah, A., . . . Vaccarino, V. (2018). Young Women With Coronary Artery Disease Exhibit Higher Concentrations of Interleukin-6 at Baseline and in Response to Mental Stress. *Journal of the American Heart Association, 7*(23), e010329-e010329. doi:10.1161/JAHA.118.010329

Swerdlow, D. I., Holmes, M. V., Kuchenbaecker, K. B., Engmann, J. E., Shah, T., Sofat, R., . . . Casas, J. P. (2012). The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet, 379*(9822), 1214-1224. doi:10.1016/s0140-6736(12)60110-x

Szyf, M., Weaver, I. C. G., Champagne, F. A., Diorio, J., & Meaney, M. J. (2005). Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Frontiers in neuroendocrinology, 26*(3-4), 139-162. doi:10.1016/j.yfrne.2005.10.002

Tabák, A. G., Herder, C., Rathmann, W., Brunner, E. J., & Kivimäki, M. (2012). Prediabetes: a high-risk state for diabetes development. *Lancet (London, England), 379*(9833), 2279-2290. doi:10.1016/S0140-6736(12)60283-9

Tabassum, F., Kumari, M., Rumley, A., Lowe, G., Power, C., & Strachan, D. P. (2008). Effects of socioeconomic position on inflammatory and hemostatic markers: a life-course analysis in the 1958 British birth cohort. *American journal of epidemiology, 167*(11), 1332-1341.

Takkinen, S., Suutama, T., & Ruoppila, I. (2001). More meaning by exercising? Physical activity as a predictor of a sense of meaning in life and of self-rated health and functioning in old age. *Journal of Aging and Physical Activity*.

Tang, W., Pankow, J. S., Carr, J. J., Tracy, R. P., Bielinski, S. J., North, K. E., . . . Arnett, D. K. (2007). Association of sICAM-1 and MCP-1 with coronary artery calcification in families enriched for coronary heart disease or hypertension: the NHLBI Family Heart Study. *BMC Cardiovasc Disord, 7*, 30. doi:10.1186/1471-2261-7-30

Tankó, L. B., & Christiansen, C. (2004). An update on the antiestrogenic effect of smoking: a literature review with implications for researchers and practitioners. *Menopause, 11*(1), 104-109.

Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A., & Updegraff, J. A. (2000). Biobehavioral responses to stress in females: tend-and-befriend,

not fight-or-flight. *Psychological review, 107*(3), 411-429. doi:10.1037/0033-295x.107.3.411

Telford, R. D. (2007). Low physical activity and obesity: causes of chronic disease or simply predictors? *Medicine & Science in Sports & Exercise, 39*(8), 1233-1240.

Thayer, J. F., & Sternberg, E. (2006). Beyond heart rate variability: vagal regulation of allostatic systems. *Annals of the New York Academy of Sciences, 1088*(1), 361-372.

Thoma, M. V., Kirschbaum, C., Wolf, J. M., & Rohleder, N. (2012). Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biological Psychology, 91*(3), 342-348. doi:10.1016/j.biopsycho.2012.07.008

Thomas, J., Jones, G., Scarinci, I., & Brantley, P. (2003). A descriptive and comparative study of the prevalence of depressive and anxiety disorders in low-income adults with type 2 diabetes and other chronic illnesses. *Diabetes Care, 26*(8), 2311-2317.

Tilg, H., & Moschen, A. R. (2008). Inflammatory mechanisms in the regulation of insulin resistance. *Molecular medicine, 14*(3-4), 222-231.

Tilg, H., Trehu, E., Atkins, M. B., Dinarello, C. A., & Mier, J. W. (1994). Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *Blood, 83*(1), 113-118. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8274730

Tillin, T., Hughes, A. D., Godsland, I. F., Whincup, P., Forouhi, N. G., Welsh, P., . . . Chaturvedi, N. (2013). Insulin resistance and truncal obesity as important determinants of the greater incidence of diabetes in Indian Asians and African Caribbeans compared with Europeans: the Southall And Brent REvisited (SABRE) cohort. *Diabetes Care, 36*(2), 383-393. doi:10.2337/dc12-0544

Tindle, H. A., Duncan, M. S., Liu, S., Kuller, L. H., Fugate Woods, N., Rapp, S. R., . . . Freiberg, M. S. (2018). Optimism, pessimism, cynical hostility, and biomarkers of metabolic function in the Women's Health Initiative. *Journal of diabetes, 10*(6), 512-523. doi:10.1111/1753-0407.12584

Ting, R. Z. W., Lau, E. S. H., Ozaki, R., Lau, W. W. Y., Kong, A. P. S., Luk, A. O. Y., . . . So, W.-y. (2013). High risk for cardiovascular disease in Chinese type 2 diabetic patients with major depression--a 7-year prospective analysis of the Hong Kong Diabetes Registry. *Journal of affective disorders, 149*(1-3), 129-135. doi:10.1016/j.jad.2013.01.012

Tobias, M. (2011). Global control of diabetes: information for action. *Lancet (London, England), 378*(9785), 3-4. doi:10.1016/S0140-6736(11)60604-1

Tomczak, M., & Tomczak, E. (2014). The need to report effect size estimates revisited. An overview of some recommended measures of effect size.

Tran, B. X., Nguyen, L. H., Pham, N. M., Vu, H. T. T., Nguyen, H. T., Phan, D. H., . . . Latkin, C. A. (2020). Global Mapping of Interventions to Improve Quality of Life of People with Diabetes in 1990–2018. *International journal of environmental research and public health, 17*(5), 1597.

Tretli, S., Lund-Larsen, P. G., & Foss, O. P. (1982). Reliability of questionnaire information on cardiovascular disease and diabetes: cardiovascular disease study in Finnmark county. *J Epidemiol Community Health, 36*(4), 269-273. doi:10.1136/jech.36.4.269

Tsenkova, V. K., Karlamangla, A. S., & Ryff, C. D. (2016). Parental History of Diabetes, Positive Affect, and Diabetes Risk in Adults: Findings from MIDUS. *Ann Behav Med, 50*(6), 836-843. doi:10.1007/s12160-016-9810-z

Tsenkova, V. K., Love, G. D., Singer, B. H., & Ryff, C. D. (2007). Socioeconomic status and psychological well-being predict cross-time change in glycosylated hemoglobin in older women without diabetes. *Psychosom Med, 69*(8), 777-784. doi:10.1097/PSY.0b013e318157466f

Tuomi, T., Santoro, N., Caprio, S., Cai, M., Weng, J., & Groop, L. (2014). The many faces of diabetes: a disease with increasing heterogeneity. *Lancet (London, England), 383*(9922), 1084-1094. doi:10.1016/S0140-6736(13)62219-9

Unwin, N., Watson, W., Harland, J., White, M., Bhopal, R., Stephenson, S., . . . Alberti, K. (1995). Social class differences in the prevalence of glucose intolerance. *Diabetic medicine : a journal of the British Diabetic Association, 12*, S31.

van Dam, R. M., Rimm, E. B., Willett, W. C., Stampfer, M. J., & Hu, F. B. (2002). Dietary patterns and risk for type 2 diabetes mellitus in US men. *Annals of internal medicine, 136*(3), 201-209.

van Minkelen, R., Wettinger, S. B., de Visser, M. C., Vos, H. L., Reitsma, P. H., Rosendaal, F. R., . . . Doggen, C. J. (2009). Haplotypes of the interleukin-1 receptor antagonist gene, interleukin-1 receptor antagonist mRNA levels and the risk of myocardial infarction. *Atherosclerosis, 203*(1), 201-205. doi:10.1016/j.atherosclerosis.2008.06.029

van Son, J., Nyklíček, I., Pop, V. J., Blonk, M. C., Erdtsieck, R. J., & Pouwer, F. (2014). Mindfulness-based cognitive therapy for people with diabetes and emotional problems: long-term follow-up findings from the DiaMind randomized controlled trial. *Journal of psychosomatic research, 77*(1), 81-84.

van Wijk, I., Kappelle, L. J., van Gijn, J., Koudstaal, P. J., Franke, C. L., Vermeulen, M., . . . Li, L. A. C. s. g. (2005). Long-term survival and vascular event risk after transient ischaemic attack or minor ischaemic stroke: a cohort study. *Lancet (London, England), 365*(9477), 2098-2104. doi:10.1016/S0140-6736(05)66734-7

Vancampfort, D., Rosenbaum, S., Ward, P. B., Steel, Z., Lederman, O., Lamwaka, A. V., . . . Stubbs, B. (2016). Type 2 Diabetes Among People With Posttraumatic Stress Disorder: Systematic Review and Meta-Analysis. *Psychosom Med, 78*(4), 465-473. doi:10.1097/psy.0000000000000297

VanderWeele, T. J. (2017). On the promotion of human flourishing. *Proceedings of the National Academy of Sciences of the United States of America, 114*(31), 8148-8156. doi:10.1073/pnas.1702996114

Vedhara, K., Miles, J., Wetherell, M., Dawe, K., Searle, A., Tallon, D., . . . Drake, N. (2010). Coping style and depression influence the healing of diabetic foot ulcers: observational and mechanistic evidence. *Diabetologia, 53*(8), 1590-1598.

Vgontzas, A. N., Bixler, E. O., Lin, H. M., Prolo, P., Trakada, G., & Chrousos, G. P. (2005). IL-6 and its circadian secretion in humans. *Neuroimmunomodulation, 12*(3), 131-140. doi:10.1159/000084844

Vgontzas, A. N., & Chrousos, G. P. (2002). Sleep, the hypothalamic-pituitary-adrenal axis, and cytokines: multiple interactions and disturbances in sleep disorders. *Endocrinology and metabolism clinics of North America, 31*(1), 15-36. doi:10.1016/s0889-8529(01)00005-6

Viau, V., Bingham, B., Davis, J., Lee, P., & Wong, M. (2005). Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. *Endocrinology, 146*(1), 137-146.

Viau, V., & Meaney, M. J. (1996). The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. *Journal of neuroscience, 16*(5), 1866-1876.

Voellmin, A., Winzeler, K., Hug, E., Wilhelm, F. H., Schaefer, V., Gaab, J., . . . Bader, K. (2015). Blunted endocrine and cardiovascular reactivity in young healthy women reporting a history of childhood adversity. *Psychoneuroendocrinology, 51*, 58-67.

von Känel, R., Kudielka, B. M., Preckel, D., Hanebuth, D., & Fischer, J. E. (2006). Delayed response and lack of habituation in plasma interleukin-6 to acute

mental stress in men. *Brain, behavior, and immunity, 20*(1), 40-48. doi:10.1016/j.bbi.2005.03.013

Vozarova, B., Weyer, C., Lindsay, R. S., Pratley, R. E., Bogardus, C., & Tataranni, P. A. (2002). High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes, 51*(2), 455-461.

Wang, X., Bao, W., Liu, J., Ouyang, Y.-Y., Wang, D., Rong, S., . . . Liu, L.-G. (2013). Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care, 36*(1), 166-175. doi:10.2337/dc12-0702

Wang, Y., O'Neil, A., Jiao, Y., Wang, L., Huang, J., Lan, Y., . . . Yu, C. (2019). Sex differences in the association between diabetes and risk of cardiovascular disease, cancer, and all-cause and cause-specific mortality: a systematic review and meta-analysis of 5,162,654 participants. *BMC medicine, 17*(1), 136-136. doi:10.1186/s12916-019-1355-0

Wang, Z., Mandel, H., Levingston, C. A., & Young, M. R. I. (2016). An exploratory approach demonstrating immune skewing and a loss of coordination among cytokines in plasma and saliva of Veterans with combat-related PTSD. *Hum Immunol, 77*(8), 652-657. doi:10.1016/j.humimm.2016.05.018

Wang, Z., Rowley, K., Best, J., McDermott, R., Taylor, M., & O'Dea, K. (2007). Hemostatic factors in Australian Aboriginal and Torres Strait islander populations. *Metabolism, 56*(5), 629-635.

Wannamethee, S., Shaper, A., Perry, I., & Alberti, K. (2002). Alcohol consumption and the incidence of type II diabetes. *Journal of Epidemiology & Community Health, 56*(7), 542-548.

Ware, J. E., Jr., & Sherbourne, C. D. (1992). The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care, 30*(6), 473-483.

Wareham, N., Ness, E., Byrne, C., Cox, B., Day, N., & Hales, C. (1996). Cigarette smoking is not associated with hyperinsulinemia: evidence against a causal relationship between smoking and insulin resistance. *Metabolism, 45*(12), 1551-1556.

Wellen, K. E., & Hotamisligil, G. S. (2005). Inflammation, stress, and diabetes. *The Journal of clinical investigation, 115*(5), 1111-1119.

Wetherell, M. A., & Carter, K. (2014). The multitasking framework: The effects of increasing workload on acute psychobiological stress reactivity. *Stress and Health, 30*(2), 103-109.

Wetherell, M. A., Craw, O., Smith, K., & Smith, M. A. (2017). Psychobiological responses to critically evaluated multitasking. *Neurobiology of stress, 7*, 68-73.

Whiting, D. R., Guariguata, L., Weil, C., & Shaw, J. (2011). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes research and clinical practice, 94*(3), 311-321. doi:10.1016/j.diabres.2011.10.029

Wichit, N., Mnatzaganian, G., Courtney, M., Schulz, P., & Johnson, M. (2017). Randomized controlled trial of a family-oriented self-management program to improve self-efficacy, glycemic control and quality of life among Thai individuals with Type 2 diabetes. *Diabetes research and clinical practice, 123*, 37-48.

Willeit, P., Raschenberger, J., Heydon, E. E., Tsimikas, S., Haun, M., Mayr, A., . . . Kiechl, S. (2014). Leucocyte telomere length and risk of type 2 diabetes mellitus: new prospective cohort study and literature-based meta-analysis. *PLoS One, 9*(11), e112483. doi:10.1371/journal.pone.0112483

Willi, C., Bodenmann, P., Ghali, W. A., Faris, P. D., & Cornuz, J. (2007). Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA, 298*(22), 2654-2664. doi:10.1001/jama.298.22.2654

Williams, E. D., Magliano, D. J., Tapp, R. J., Oldenburg, B. F., & Shaw, J. E. (2013). Psychosocial stress predicts abnormal glucose metabolism: the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. *Ann Behav Med, 46*(1), 62-72. doi:10.1007/s12160-013-9473-y

Williams, L. H., Rutter, C. M., Katon, W. J., Reiber, G. E., Ciechanowski, P., Heckbert, S. R., . . . Von Korff, M. (2010). Depression and incident diabetic foot ulcers: a prospective cohort study. *The American journal of medicine, 123*(8), 748-754.e743. doi:10.1016/j.amjmed.2010.01.023

Windham, G. C., Mitchell, P., Anderson, M., & Lasley, B. L. (2005). Cigarette smoking and effects on hormone function in premenopausal women. *Environmental health perspectives, 113*(10), 1285-1290.

Windle, G., Hughes, D., Linck, P., Russell, I., & Woods, B. (2010). Is exercise effective in promoting mental well-being in older age? A systematic review. *Aging Ment Health, 14*(6), 652-669. doi:10.1080/13607861003713232

Winston, G. J., Barr, R. G., Carrasquillo, O., Bertoni, A. G., & Shea, S. (2009). Sex and racial/ethnic differences in cardiovascular disease risk factor treatment and control among individuals with diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care, 32*(8), 1467-1469. doi:10.2337/dc09-0260

Winters, S. J. (2001). Evaluation of testicular function. In K. L. Becker (Ed.), *Principles and practice of endocrinology and metabolism* (pp. 1115-1124). Philadelphia, PA: Lippincott Williams & Wilkins.

Woodman, R. J., Mori, T. A., Burke, V., Puddey, I. B., Barden, A., Watts, G. F., & Beilin, L. J. (2003). Effects of purified eicosapentaenoic acid and docosahexaenoic

acid on platelet, fibrinolytic and vascular function in hypertensive type 2 diabetic patients. *Atherosclerosis, 166*(1), 85-93.

Woods-Giscombe, C. L., Gaylord, S. A., Li, Y., Brintz, C. E., Bangdiwala, S. I., Buse, J. B., . . . Faurot, K. (2019). A Mixed-Methods, Randomized Clinical Trial to Examine Feasibility of a Mindfulness-Based Stress Management and Diabetes Risk Reduction Intervention for African Americans with Prediabetes. *Evid Based Complement Alternat Med, 2019*, 3962623. doi:10.1155/2019/3962623

World Health Organization. (2008). *Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation*. Retreived from https://apps.who.int/iris/bitstream/handle/10665/44583/9789241501491_eng.pdf;jsessionid=8E1BB533DFA8554252010E3C7C3FAF26?sequence=1

World Health Organization. (2011). Guidelines Approved by the Guidelines Review Committee. In *Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation*. Geneva: World Health Organization.

World Health Organization. (2016a). *Global report on diabetes*. Retrieved from http://www.who.int/diabetes/global-report/en/

World Health Organization. (2016b). *Diabetes country profiles, 2016*. Retrieved from https://www.who.int/diabetes/country-profiles/diabetes_profiles_explanatory_notes.pdf

World Health Organization. (2020a). *Diabetes*. Retrieved from https://www.who.int/health-topics/diabetes

World Health Organization. (2020b). *Obesity and overweight*. Retreived from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Wu, Y., Ding, Y., Tanaka, Y., & Zhang, W. (2014). Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *International journal of medical sciences, 11*(11), 1185-1200. doi:10.7150/ijms.10001

Yadlapati, S., & Efthimiou, P. (2016). Impact of IL-1 inhibition on fatigue associated with autoinflammatory syndromes. *Mod Rheumatol, 26*(1), 3-8. doi:10.3109/14397595.2015.1069459

Yi, J. P., Vitaliano, P. P., Smith, R. E., Yi, J. C., & Weinger, K. (2008). The role of resilience on psychological adjustment and physical health in patients with diabetes. *Br J Health Psychol, 13*(Pt 2), 311-325. doi:10.1348/135910707x186994

Younce, C. W., & Kolattukudy, P. E. (2010). MCP-1 causes cardiomyoblast death via autophagy resulting from ER stress caused by oxidative stress generated by inducing a novel zinc-finger protein, MCPIP. *Biochem J, 426*(1), 43-53. doi:10.1042/bj20090976

Younge, J. O., Wery, M. F., Gotink, R. A., Utens, E. M., Michels, M., Rizopoulos, D., . . . Roos-Hesselink, J. W. (2015). Web-Based Mindfulness Intervention in Heart Disease: A Randomized Controlled Trial. *PLoS One, 10*(12), e0143843. doi:10.1371/journal.pone.0143843

Yudkin, J. S., Stehouwer, C., Emeis, J., & Coppack, S. (1999). C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arteriosclerosis, thrombosis, and vascular biology, 19*(4), 972-978.

Zaninotto, P., & Steptoe, A. (2019). Association Between Subjective Well-being and Living Longer Without Disability or Illness. *JAMA Netw Open, 2*(7), e196870. doi:10.1001/jamanetworkopen.2019.6870

Zaninotto, P., Wardle, J., & Steptoe, A. (2016). Sustained enjoyment of life and mortality at older ages: analysis of the English Longitudinal Study of Ageing. *Bmj, 355*, i6267. doi:10.1136/bmj.i6267

Zhang, J., Niaura, R., Dyer, J. R., Shen, B.-J., Todaro, J. F., McCaffery, J. M., . . . Ward, K. D. (2006). Hostility and urine norepinephrine interact to predict insulin

resistance: the VA Normative Aging Study. *Psychosomatic medicine, 68*(5), 718-726.

Zhang, X., Norris, S. L., Chowdhury, F. M., Gregg, E. W., & Zhang, P. (2007). The effects of interventions on health-related quality of life among persons with diabetes: a systematic review. *Medical care*, 820-834.

Zhao, J., Zhu, Y., Lin, J., Matsuguchi, T., Blackburn, E., Zhang, Y., . . . Howard, B. V. (2014). Short leukocyte telomere length predicts risk of diabetes in american indians: the strong heart family study. *Diabetes, 63*(1), 354-362. doi:10.2337/db13-0744

Zhu, Y., Liu, X., Ding, X., Wang, F., & Geng, X. (2019). Telomere and its role in the aging pathways: telomere shortening, cell senescence and mitochondria dysfunction. *Biogerontology, 20*(1), 1-16. doi:10.1007/s10522-018-9769-1

Zole, E., & Ranka, R. (2018). Mitochondria, its DNA and telomeres in ageing and human population. *Biogerontology, 19*(3-4), 189-208. doi:10.1007/s10522-018-9748-6

Peer-reviewed publications

1. **Panagi**, L., Hackett, R.A., Steptoe, A., & Poole, L. (under review). Enjoyment of life predicts incident type 2 diabetes over 12 years of follow-up: findings from the English Longitudinal Study of Ageing. *Journal of Epidemiology and Community Health*. [Impact factor 3.872]
2. Bawa, H., Poole, L., Cooke, D., **Panagi**, **L**., Steptoe, A., & Hackett, R. A. (under review). Diabetes-related distress and daily cortisol output in people with type 2 diabetes. *Diabetes Research and Clinical Practice*. [Impact factor 2.722]
3. Poole, L., Hackett, R. A., **Panagi**, **L**., & Steptoe, A. (2019). Subjective wellbeing as a determinant of glycated hemoglobin in older adults: Longitudinal findings from the English Longitudinal Study of Ageing. *Psychological Medicine*, 1-9. doi:10.1017/s0033291719001879. [Impact factor 5.641]
4. **Panagi, L**., Poole, L., Hackett, R. A., & Steptoe, A. (2019). Sex differences in IL-6 stress responses in people with type 2 diabetes. *Psychophysiology*, *56*(6), e13334. doi:10.1111/psyp.13334. [Impact factor 3.378]
5. Hackett, R.A., Poole, L., Hunt, E., **Panagi**, **L**., & Steptoe, A. (2019). Loneliness and biological responses to acute stress in people with type 2 diabetes. *Psychophysiology*, *56*(6), e13341. doi: 10.1111/psyp.13341. [Impact factor 3.378]
6. **Panagi**, **L**., Poole, L., Hackett, R. A., & Steptoe, A. (2019). Happiness and inflammatory responses to acute stress in people with type 2 diabetes. *Annals of Behavioral Medicine, 53*(4), 309-320. doi:10.1093/abm/kay039. [Impact factor 3.575]

Conference presentations

1. Enjoyment of life predicts incident type 2 diabetes over 12 years of follow-up: Findings from the English Longitudinal Study of Ageing. Abstract accepted for oral presentation at the 78th Annual Scientific Meeting of the American Psychosomatic Society, Long Beach, California (March 2020)[90].
2. Sex differences in IL-6 stress responses in people with type 2 diabetes. Oral presentation at the 77th Annual Scientific Meeting of the American Psychosomatic Society, Vancouver, Canada (March 2019).
3. Happiness and inflammatory responses to acute stress in people with type 2 diabetes. Poster presentation at the 7th International Conference on Interpersonal Acceptance and Rejection, Athens, Greece (May 2018).
4. Happiness and inflammatory responses to acute stress in people with type 2 diabetes. Abstract accepted for oral presentation at the 76th Annual Scientific Meeting of the American Psychosomatic Society, Louisville, Kentucky (March 2018)[91].

[90] This Meeting was cancelled due to coronavirus situation.
[91] This presentation was not given due to medical reasons that did not allow me to travel to the US.

Appendices

Appendix A. Table of effect sizes (Sullivan et al., 2018).

Table 4. Unadjusted and Adjusted Inflammatory Response of IL-6 (pg/mL) by Sex at Specified Values of Age (40, 50, 60, 70, and 80 Years) Among Patients With CAD, MIPS and MIMS2 Combined Cohorts

	Outcome: Inflammatory Response*		
	Women	Men	
	Ratio (95% CI)	Ratio (95% CI)	*P* Value
Model 1			
Age=40 y	1.83 (1.64, 2.04)	1.52 (1.39, 1.66)	0.01
Age=50 y	1.61 (1.50, 1.73)	1.42 (1.34, 1.50)	0.01
Age=60 y	1.42 (1.34, 1.50)	1.32 (1.27, 1.37)	0.05
Age=70 y	1.25 (1.14, 1.36)	1.24 (1.18, 1.30)	0.86
Age=80 y	1.10 (0.96, 1.25)	1.16 (1.06, 1.25)	0.51
Model 2			
Age=40 y	1.85 (1.66, 2.07)	1.53 (1.39, 1.68)	0.01
Age=50 y	1.62 (1.51, 1.75)	1.42 (1.34, 1.51)	0.01
Age=60 y	1.42 (1.34, 1.51)	1.33 (1.28, 1.38)	0.05
Age=70 y	1.25 (1.14, 1.36)	1.23 (1.17, 1.30)	0.83
Age=80 y	1.09 (0.96, 1.25)	1.15 (1.06, 1.25)	0.54
Model 3			
Age=40 y	1.85 (1.65, 2.07)	1.53 (1.39, 1.68)	0.01
Age=50 y	1.62 (1.51, 1.74)	1.42 (1.34, 1.51)	0.01
Age=60 y	1.43 (1.35, 1.52)	1.33 (1.28, 1.38)	0.04
Age=70 y	1.26 (1.15, 1.37)	1.23 (1.17, 1.30)	0.73
Age=80 y	1.11 (0.97, 1.27)	1.15 (1.06, 1.25)	0.63
Model 4			
Age=40 y	1.85 (1.66, 2.07)	1.53 (1.39, 1.68)	0.01
Age=50 y	1.63 (1.52, 1.75)	1.42 (1.34, 1.51)	0.01
Age=60 y	1.43 (1.35, 1.52)	1.33 (1.28, 1.38)	0.04
Age=70 y	1.26 (1.15, 1.38)	1.24 (1.17, 1.30)	0.75
Age=80 y	1.11 (0.97, 1.27)	1.15 (1.06, 1.26)	0.61

Model 1 adjusted for: age, sex, time, age×sex, age×time, sex×time, and age×sex×time. Model 2 adjusted for: model 1 covariates+race, education, diabetes mellitus, hypertension, body mass index (continuous), lifetime history of depression, smoking status, aspirin, beta blocker, statins, angiotensin-converting enzyme inhibitors, antidepressants, previous myocardial infarction, and heart failure. Model 3 adjusted for: model 2 covariates+summed rest score. Model 4 adjusted for model 3 covariates+plate effect and data source. CAD indicates coronary artery disease; CI, confidence interval; IL-6, interleukin-6; MIMS2, Myocardial Infarction and Mental Stress Study 2; MIPS, Mental Stress Ischemia Prognosis Study.

*A natural log transformation was used for biomarker values in analyses. Inflammatory response calculated as: exp($\log_e$(Post stress values)−$\log_e$(resting values))=exp($\log_e$(Post stress value/resting value)) and can be interpreted as a ratio.

Appendix B. Chapter 3 Supplementary Tables.

Appendix B1.

Table S1

Cox proportional hazards regression on enjoyment of life at 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 after adjustment for sociodemographic variables (N = 4134)

	HR	95% CI	*p* value
CASP enjoyment of life [score]	0.92	0.87 to 0.97	0.003
Age [years]	1.01	1.00 to 1.03	0.060
Sex [ref. cat.: men]	0.61	0.48 to 0.77	< 0.001
Financial wealth [£]			
Quintile 1 [ref. cat.]	1		
Quintile 2	0.59	0.41 to 0.87	0.007
Quintile 3	0.66	0.47 to 0.92	0.014
Quintile 4	0.52	0.37 to 0.73	< 0.001
Quintile 5	0.33	0.23 to 0.48	< 0.001
Ethnicity [ref. cat.: White]	2.05	0.91 to 4.60	0.083
Marital/cohabiting status [ref. cat.: married or cohabiting]	0.87	0.66 to 1.15	0.331

Note. CASP = Control Autonomy Self-realisation Pleasure scale; CI = confidence interval; HR = hazard ratio; N = number; ref. cat. = reference category.

Appendix B2.

Table S2

Cox proportional hazards regression on enjoyment of life at 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 after adjusting for behavioural variables (N = 4134)

	HR	95% CI	*p* value
CASP enjoyment of life [score]	0.92	0.87 to 0.97	0.004
Physical activity per week			
Light or none [ref. cat.]	1		
Moderate or vigorous 1 day	0.73	0.52 to 1.02	0.067
Moderate or vigorous >1 days	0.77	0.58 to 1.02	0.070
Smoking status [ref. cat.: smokers]	0.67	0.50 to 0.90	0.007
Alcohol consumption per week [ref. cat.: < 5 days]	0.80	0.60 to 1.07	0.131
BMI [kg/m^2]	1.11	1.09 to 1.13	<0.001

Note. CASP = Control Autonomy Self-realisation Pleasure scale; CI = confidence interval; HR = hazard ratio; N = number; ref. cat. = reference category.

Appendix B3.

Table S3

Cox proportional hazards regression on enjoyment of life at 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 after adjustment for clinical variables (N = 4134)

	HR	95% CI	*p* value
CASP enjoyment of life [score]	0.91	0.86 to 0.97	0.003
Hypertension [ref. cat.: no]	1.65	1.31 to 2.08	<0.001
CHD [ref. cat.: no]	1.39	0.99 to 1.95	0.059
HbA1c [%]	2.74	2.52 to 2.97	<0.001

Note. CASP = Control Autonomy Self-realisation Pleasure scale; CI = confidence interval; CHD = coronary heart disease; HbA1c = glycated hemoglobin; HR = hazard ratio; N = number; ref. cat. = reference category.

Appendix B4.

Table S4

Cox proportional hazards regression on purpose in life at 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 after adjustment for sociodemographic variables (N = 4134)

	HR	95% CI	*p* value
CASP purpose in life [score]	0.90	0.77 to 1.04	0.154
Age [years]	1.01	1.00 to 1.03	0.057
Sex [ref. cat.: men]	0.60	0.48 to 0.75	<0.001
Financial wealth [£]			
Quintile 1 [ref. cat.]	1		
Quintile 2	0.58	0.34 to 0.85	0.005
Quintile 3	0.63	0.45 to 0.89	0.006
Quintile 4	0.50	0.36 to 0.70	<0.001
Quintile 5	0.31	0.22 to 0.45	<0.001
Ethnicity [ref. cat.: White]	2.03	0.90 to 4.56	0.086
Marital/cohabiting status [ref. cat.: married or cohabiting]	0.89	0.67 to 1.17	0.391

Note. CASP = Control Autonomy Self-realisation Pleasure scale; CI = confidence interval; HR = hazard ratio; N = number; ref. cat. = reference category.

Appendix B5.

Table S5

Cox proportional hazards regression on purpose in life at 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 after adjusting for behavioural variables (N = 4134)

	HR	95% CI	*p* value
CASP purpose in life [score]	0.87	0.75 to 1.01	0.066
Physical activity per week			
Light or none [ref. cat.]	1		
Moderate or vigorous 1 day	0.71	0.51 to 1.00	0.047
Moderate or vigorous > 1 days	0.74	0.56 to 0.99	0.040
Smoking status [ref. cat.: smokers]	0.65	0.48 to 0.87	0.004
Alcohol consumption per week [ref. cat.: < 5 days]	0.79	0.59 to 1.06	0.111
BMI [kg/m^2]	1.11	1.09 to 1.13	<0.001

Note. BMI = body mass index; CASP = Control Autonomy Self-realisation Pleasure scale; CI = confidence interval; HR = hazard ratio; kg/m^2 = kilograms per square metre; N = number; ref. cat. = reference category.

Appendix B6.

Table S6

Cox proportional hazards regression on purpose in life at 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 after adjusting for clinical variables (N = 4134)

	HR	95% CI	*p* value
CASP purpose in life [score]	0.93	0.80 to 1.09	0.389
Hypertension [ref. cat.: no]	1.69	1.34 to 2.12	<0.001
CHD [ref. cat.: no]	1.41	1.01 to 1.98	0.046
HbA1c [%]	2.75	2.53 to 2.99	<0.001

Note. CASP = Control Autonomy Self-realisation Pleasure scale; CHD = coronary heart disease; CI = confidence interval; HbA1c = glycated hemoglobin; HR = hazard ratio; N = number; ref. cat. = reference category.

Appendix B7.

Table S7

Cox proportional hazards regression on purpose in life at 2004/5 predicting type 2 diabetes incidence from 2006/7 to 2016/7 after adjusting for sociodemographic, behavioural, and clinical variables (N = 4134)

	HR	95% CI	*p* value
CASP purpose in life [score]	0.92	0.79 to 1.08	0.322
Age [years]	1.01	1.00 to 1.03	0.064
Sex [ref. cat.: men]	0.63	0.50 to 0.80	<0.001
Financial wealth [£			
Quintile 1 [ref. cat.]	1		
Quintile 2	1.00	0.66 to 1.50	0.993
Quintile 3	1.12	0.78 to 1.61	0.556
Quintile 4	1.00	0.66 to 1.39	0.810
Quintile 5	0.67	0.44 to 1.01	0.057
Ethnicity [ref. cat.: White]	1.87	0.83 to 4.21	0.134
Marital/cohabiting status [ref. cat.: married or cohabiting]	0.76	0.57 to 1.01	0.061
Physical activity per week			
Light or none [ref. cat.]	1		
Moderate or vigorous 1 day	0.93	0.65 to 1.33	0.697
Moderate or vigorous >1 days	0.94	0.69 to 1.28	0.701
Smoking status [ref. cat.: smokers]	0.92	0.66 to 1.27	0.594
Alcohol consumption per week [ref. cat.: < 5 days]	0.85	0.63 to 1.14	0.273
BMI [kg/m^2]	1.11	1.08 to 1.13	<0.001
Hypertension [ref. cat.: no]	1.37	1.08 to 1.73	0.010
CHD [ref. cat.: no]	1.12	0.79 to 1.59	0.512
HbA1c [%]	2.56	2.31 to 2.83	<0.001

Note. BMI = body mass index; CASP = Control Autonomy Self-realisation Pleasure scale; CHD = coronary heart disease; CI = confidence interval; HbA1c = glycated hemoglobin; HR = hazard ratio; kg/m^2 = kilograms per square metre; N = number; ref. cat. = reference category.

Appendix C. The Diabetes Follow-up Study documents.

Appendix C1. The Diabetes Follow-up Study Participant Information Sheet.

Study IRAS ID: 226142

Diabetes Follow-Up Study

Participant information sheet

- We would like to invite you to take part in a follow-up study. Before you decide whether you wish to take part, it is important that you understand the purpose of the study and what it involves.
- Please read through the following information and discuss it with others if you wish.
- If anything is unclear or if you would like more information please do not hesitate to contact Ms Laura Panagi either by phone (020 7679 1723) or e-mail (laura.panagi.16@ucl.ac.uk).

What is the purpose of the study?

We are interested in understanding all the changes that happen to the body in people with Type 2 Diabetes. In the first study you took part in, we looked at how you felt when we gave you some stressful tasks in our laboratory. We think that how your body copes with stress may have a long-term impact on your health. Therefore, we are now interested in looking at how the things we found out about you in the first study are linked with how you are currently doing. For example, we know that people with diabetes sometimes go on to have other health problems such as heart disease or problems with their eyes or feet. We would like to find out how you have been getting on since the earlier study you took part in with us and how your mood and health has been since then. This research will help us work out all the different things that are involved in the long-term health of people with diabetes and may inform new ways of improving patient care.

Why have I been invited?

You have been invited as you took part in our earlier study entitled "The Psychobiology of Social Position: The Diabetes Study". All participants from that initial study are invited to participate in this follow-up study. The study is being conducted by researchers at the Department of Behavioural Science and Health of University College London (UCL). The study team are Professor Andrew Steptoe (study supervisor), Dr Lydia Poole (study supervisor), Dr Ruth Hackett (chief investigator), and Ms Laura Panagi (student researcher and co-investigator).

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you are interested in taking part then you will be given this information sheet to keep. You will also have the opportunity to ask questions and discuss the study in more detail. Even after agreeing to take part, you will be free to withdraw at any time without giving a reason. A decision not to take part or withdraw from the study will not affect your medical treatment in any way.

What does it involve?

The study involves the completion of a questionnaire booklet. The questions in the booklet will be about your current health, well-being, lifestyle and feelings. Our aim is to link these data with your previous data collected in our earlier study. This will be carried out through statistical analyses and will help us identify the causes of the long-term health in people with diabetes. You will need approximately 35 minutes to complete the booklet, and we will ask you to return it to us using the freepost envelope provided. Once we receive your booklet, we will post you a £20 Marks and Spencer voucher as a token of our thanks. With your permission, we will ask your General Practitioner (GP) to provide us information about your health from your medical notes. The things your GP will provide include information about your latest blood pressure and blood test results. We will also ask them to confirm whether you have any other illnesses such as if you have suffered a heart attack or stroke. All this

information will be held in the strictest of confidence and any information you and your GP provide will be anonymised so that you are not able to be identified.

What should I do if I would like to take part?

If you have any questions about the study, please contact Ms Laura Panagi either by phone (020 7679 1723) or e-mail (laura.panagi.16@ucl.ac.uk). You will have the opportunity to ask questions and discuss the study in more detail. If you are interested in taking part, please read and sign the consent form enclosed with this letter and post it back to us using the freepost envelope provided. Please also complete the contact information form so we can make sure we have the most up-to-date details for you. Please return the consent form and contact information forms to us within two weeks from receiving this letter. After having received your consent, we will post you the questionnaire booklet to fill in at home.

What are the possible disadvantages of taking part?

This is not a treatment study and so the likelihood of any harm is minimal. However, we understand you will have to spend some time answering the questionnaire booklet for us. Therefore, we are offering a £20 voucher from Marks and Spencer to every participant as a token of our thanks. This will be posted to you after receiving the completed questionnaire booklet from you.

What are the possible benefits of taking part?

Although there may be no direct benefits to you personally, by taking part you will contribute to the scientific knowledge of factors relating to the long-term health of people with diabetes. The information we get from this study may also help us to treat future patients.

Will my GP be aware of my participation in the study?

Yes. With your permission, we will contact your GP to notify them of your participation in this follow-up study and we will ask them to provide us information about your health. Specifically, we will ask your GP to provide us your latest test results on 1)

blood pressure, 2) glycated hemoglobin (HbA1c), 3) fasting glucose levels, 4) cholesterol levels and 5) triglycerides levels, as well as information on 6) any health conditions such as whether or not you have been told you have had a heart attack and stroke since we last saw you. The research team at UCL will collect this information about you for this research study from your GP. This information will include your name, NHS number, home address and telephone details and health information, which is regarded as a special category of information. We will use this information to understand how your health has been recently. Finally, if we have any concerns about any of the results you provide us in your questionnaire booklet, we will notify your GP so they can offer their help to you.

Will my responses to the questionnaire booklet and my medical data be kept confidential?

We want to emphasise that all responses obtained will be strictly confidential and will only be used for research purposes. To ensure the information is kept secure, your questionnaire responses and medical data will be kept separately from personally identifiable information in a secure system, ensuring that all information remains strictly anonymous. These data cannot be linked back to you as it will be coded with your participant number and not your name. Your personal data will be kept in a secure system at UCL designed for handling identifiable data which has been certified to the ISO27001 information security standard and conforms to the NHS Information Governance Toolkit. Your medical records will not hold any of your results from this research.

The research team at UCL will collect information from you for this research study in accordance with our instructions. UCL will use your name, home address and telephone details to contact you about the research study, and make sure that relevant information about the study is recorded to oversee the quality of the study. Individuals from UCL and regulatory organisations may look at your medical and research records where they are relevant to your participation in this study to check the accuracy of the research study. Your GP will pass relevant information from your medical records to the research team at UCL regarding your current health (your latest

test results on blood pressure, HbA1c, fasting glucose levels, cholesterol levels and triglycerides levels, as well as information on any health conditions since we last saw you). The only people at UCL who will have access to information that identifies you will be people who need to contact you regarding your participation in this study or to audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details. With your consent, UCL will keep identifiable information from this study for up to 20 years after the study has finished.

What will happen to the results of the research study?

The results will be statistically analysed and findings will be made known to the medical community and published in scientific journals. This is so the results can be shared with other doctors and researchers. You will not be identified in any publication. You will be personally informed about the results of this study by a letter posted to your address.

What if I don't want to carry on with the study?

You will be free to terminate your participation in the study 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. Please notify Ms Laura Panagi in case of study withdrawal either via phone or e-mail.

What if something goes wrong?

We do not expect you to suffer any adverse effects from this questionnaire study. However, if you have a concern about any aspect of this study, you should ask to speak to the study researchers in the first instance. If you remain unhappy and wish to complain formally, you can do this through UCL complaints procedures by emailing research-incidents@ucl.ac.uk. UCL insurance and indemnity cover is being provided.

Data Privacy Notice

Notice: UCL is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. UCL will keep identifiable information about you for 20 years after the study has finished. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. You can find out more about how we use your information by contacting UCL's Data Protection Office at data-protection@ucl.ac.uk or at www.ucl.ac.uk/legal-services/privacy/participants-health-and-care-research-privacy-notice.

Who has reviewed the study?

This study has been reviewed and given favourable opinion by the East of Scotland Research Ethics Service.

Who is organising and funding the research?

The research is sponsored and organised by UCL and funded by the Anastasios G. Leventis Foundation. No conflicts of interest exist.

What should I do now?

If you are interested in taking part in the study, please read and sign the consent form and complete the contact details form enclosed with this information sheet. Please post these back to us using the freepost envelope provided - there is no need to use a stamp - within two weeks from receiving this letter. After having received your consent, we will post you the booklet to fill in at home. If you are not able to complete the consent form at the moment, but would like us to contact you to have a chat about the study, please return this contact details form by itself in the freepost envelope so

we can give you a call to answer any questions you might have about the study. Or, if you prefer, you can contact Ms Laura Panagi directly either by phone (020 7679 1723) or e-mail (laura.panagi.16@ucl.ac.uk) with any queries regarding any aspect of the study. If you would prefer not to take part, you do not need to do anything. A reminder letter will be sent to you in two weeks' time.

Co-investigator (contact person)
Ms Laura Panagi
Tel: 020 7679 1723
E-mail: laura.panagi.16 @ucl.ac.uk

Study Supervisor
Professor Andrew Steptoe
Tel: 02076791804
E-mail:
a.steptoe@ucl.ac.uk

Address: UCL Department of Behavioural Science & Health, Psychobiology Group, 1-19 Torrington Place, London WC1E 6BT

Thank you for taking the time to read this information sheet

Appendix C2. The Diabetes Follow-up Study consent form.

Study IRAS ID: 226142

Diabetes Follow-Up Study

CONSENT FORM FOR ADULTS WITH TYPE 2 DIABETES IN RESEARCH STUDIES

Please complete this form after you have read the Participant Information Sheet

Title of Study: Diabetes Follow-up Study

Address: Research Department of Behavioural Science and Health, University College London, 1-19 Torrington Place, London WC1E 6BT

Chief Investigator: Dr Ruth Hackett (tel.: 0207 679 1688, e-mail: ruth.hackett.09@ucl.ac.uk)

Co-investigator: Ms Laura Panagi (tel.: 020 7679 1723, e-mail: laura.panagi.16@ucl.ac.uk)

Principal Investigator/Primary Supervisor: Professor Andrew Steptoe (a.steptoe@ucl.ac.uk)

Secondary Supervisor: Dr Lydia Poole (lydia.poole@ucl.ac.uk)

UCL Data Protection Officer: Mr Spenser Crouch (s.crouch@ucl.ac.uk)

This study has been approved by the UCL Research Ethics Committee.

Project ID number: 226142

Consent form instructions

Thank you for considering taking part in this research. If you have any questions arising from the Information Sheet or this consent form, please contact the research team before you decide whether or not to join in. The individuals organising the research are responsible for explaining the project to you before you agree to take part. Please contact Ms Laura Panagi either via phone (020 7679 1723) or via e-mail (laura.panagi.16@ucl.ac.uk) with any questions or queries.

What is this form?

This form is the consent form. If you would like to take part in our study, we ask you to complete this form so that we have written proof of your willingness to take part. Participation in this study is completely voluntary.

What do I need to do with this form?

If you would like to take part, please read each of the statements on the consent form carefully. If you are happy with each of the statements, please put your initials in the box to indicate that you agree. At the bottom of the page is a space for you to sign and date the form.

Who should I return this form to?

Please return this form to us using the freepost envelope provided within two weeks' time. Please include the completed contact details form in the envelope.

What happens next?

When we have received your form through the post, we will also sign the form before making a photocopy which we will then post back to you for your records. Along with this photocopy we will also post you the questionnaire booklet for you to complete, at home, in your own time.

What if I am not able to complete the consent form at the moment?

If you are not able to complete the consent form at the moment, but would like to us to contact you to have a chat about the study, please return the contact details form by itself in the freepost envelope so we can give you a call to answer any questions you might have about the study. Or, if you prefer, you can contact Ms Laura Panagi directly either by phone (020 7679 1723) or e-mail (laura.panagi.16@ucl.ac.uk) with any queries regarding any aspect of the study.

ID _____

Diabetes Follow-Up Study

Consent Form

I confirm that I understand that by initialling each box below I am consenting to this element of the study. I understand that if I do not initial a box it means that I DO NOT consent to that part of the study. I understand that by not giving consent for any one element that I may be deemed ineligible for the study.

		Initials
1.	I confirm that I have read and understood the Information Sheet for the above study. I have had an opportunity to consider the information and what will be expected of me. I have also had the opportunity to ask questions which have been answered to my satisfaction, and I would like to take part in the study.	
2.	I understand that I will be able to withdraw my data at any time without giving a reason.	
3.	I consent to the processing of my personal information (name, surname, home address) for the purposes explained to me. I understand that such information will be handled in accordance with all applicable data protection legislation.	
4.	I understand that all personal information will remain strictly confidential and that all efforts will be made to ensure I cannot be identified. Personal data will be kept in a secure system at UCL, with access being limited to Dr. Ruth Hackett and Ms Laura Panagi. I understand that my data gathered in this study will be kept separately from personally identifiable information in a secure system. It will not be possible to identify me in any publications.	

5.	I understand that my information may be subject to review by responsible individuals from the University for monitoring and audit purposes.	
6.	I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason and without the care I receive or being affected. I understand that if I decide to withdraw, any personal data I have provided up to that point will be deleted unless I agree otherwise.	
7.	I understand that support will be available to me should I wish to complain either informally or formally about any aspects of the study.	
8.	I understand the benefits of participating.	
9.	I understand that the data will not be made available to any commercial organisations but is solely the responsibility of the researchers undertaking this study.	
10	I understand that I will be compensated £20 for my participation in the study.	
11	I understand that my GP will be notified of my participation in this study.	
12	I understand that my GP will be contacted for information about 1) my latest blood pressure results, 2) my latest diabetes-related blood test results, and 3) any diagnoses of long-standing illness.	
13	I agree that my GP may be contacted if any unexpected results are found in relation to my health.	
14	I am aware of who I should contact if I wish to lodge a complaint.	

15	I voluntarily agree to take part in this study.	
16	I understand that other authenticated researchers may have access to my anonymous questionnaire and health records for research purposes in the future. These data will be kept separately from personally identifiable information; Thus, identification will be impossible.	

If you would like your contact details to be retained so that you can be contacted in the future by UCL researchers who would like to invite you to participate in follow up studies to this project, or in future studies of a similar nature, please tick the appropriate box below.

	Yes, I would be happy to be contacted in this way	
	No, I would not like to be contacted	

_____ _____ _____

Name of participant Date Signature

_____ _____ _____

Name of researcher Date Signature

Appendix C3. Parts of the questionnaire booklet of the Diabetes Follow-up Study.

Section 2 - This section is about your health

1a. When were you diagnosed with type 2 diabetes? Please indicate the **year of diagnosis**.

2a. Have you been **admitted to hospital** (including both day cases and overnight stays) in the last seven (7) years?

Yes 1

No 2 — **Go to question 3a**

2b. *Please enter number of times (please provide details below)*

	Please enter reason for admission	*Please enter month of admission*	*Please enter year of admission*
Admission 1			
Admission 2			
Admission 3			
Admission 4			

Admission 5			

If more than five admissions, please use the comments page at the end of the questionnaire.

■ Using the list below, please indicate which **medical conditions** (if any) you have. If you have a medical condition please indicate if and when you were **diagnosed by a doctor**.

Medical condition	***Please indicate yes or no.*** ***Yes*** *= I have the condition.* ***No*** *= I do not have the condition.*	*Was this diagnosed by a doctor?* ***Please tick*** ✓ ***Yes*** ***No***		*Please enter* ***month and year of diagnosis*** *(if diagnosed by a doctor)*
Example *Heart attack*	*Yes*	✓		*June, 2016*
High blood pressure (hypertension)				
Angina or long-term heart problems				
Heart attack (myocardial infarction)				
Stroke/brain haemorrhage/blood clot in the brain				
Peripheral arterial disease (e.g. claudication)				
Kidney disease (nephropathy)				
Nerve damage (neuropathy; e.g. foot problems including pain, ulcer, infections, gangrene, amputation)				

Medical condition	***Please indicate yes or no**.* ***Yes** = I have the condition.* ***No** = I do not have the condition.*	*Was this diagnosed by a doctor?* ***Please tick ✓*** ***Yes***	***No***	*Please enter **month and year of diagnosis** (if diagnosed by a doctor)*
Glaucoma				
Cataract				
Retinopathy				
Blindness or severe visual impairment				
Foot problems (e.g. foot ulcers, amputation)				
Alzheimer's disease or dementia				
Depression				
Anxiety				
Another long-term mental health problem (please specify)				

Please list any other physical or mental longstanding illnesses, diseases or medical conditions (e.g. cancer/ type of cancer, inflammatory diseases such as rheumatoid arthritis, fibromyalgia, Irritable Bowel Syndrome, osteoarthritis, lung disease) that you have been diagnosed with and for which you have sought treatment.

Longstanding means anything that **has troubled you over a period of time** or is likely to affect you over a period of time.

Name of the longstanding illness, disease or medical condition	*Was this diagnosed by a doctor?* ***Please tick*** ✓ ***Yes***	***No***	*Please enter* ***month and year*** *of diagnosis (if diagnosed by a doctor), e.g. June, 2016*

9a. In general would you say your health is:

(Please tick one)

Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

10a. Compared with one year ago, how would you rate your health in general now?

(Please tick one)

Much better now than one year ago	1
Somewhat better now than one year ago	2
About the same as one year ago	3
Somewhat worse than one year ago	4
Much worse than one year ago	5

11.

The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

(Please tick one box for each question)

		Yes, limited a lot	Yes, limited a little	No, not limited at all
11a.	**Vigorous** activities, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
11b.	**Moderate** activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	☐ 1	☐ 2	☐ 3
11c.	Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
11d.	Climbing **several** flights of stairs	☐ 1	☐ 2	☐ 3
11e.	Climbing **one** flight of stairs	☐ 1	☐ 2	☐ 3
11f.	Bending, kneeling or stooping	☐ 1	☐ 2	☐ 3
11g.	Walking **more than one mile**	☐ 1	☐ 2	☐ 3
11h.	Walking **half a mile**	☐ 1	☐ 2	☐ 3
11i.	Walking **one hundred yards**	☐ 1	☐ 2	☐ 3
11j.	Bathing and dressing yourself	☐ 1	☐ 2	☐ 3

12. During the past four weeks have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

(Please tick one answer for each question)

		Yes	No
12a.	Cut down the **amount of time** you spent on work or other activities.	☐ 1	☐ 2
12b.	**Accomplished less** than you would like.	☐ 1	☐ 2
12c.	Were limited in the **kind** of work or other activities you could do.	☐ 1	☐ 2
12d.	Had **difficulty** performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2

13. During the past four weeks have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

(Please tick one answer for each question)

		Yes	No
13a.	Cut down the **amount of time** you spent on work or other activities	☐ 1	☐ 2
13b.	**Accomplished less** than you would like	☐ 1	☐ 2
13c.	Didn't do work or other activities as **carefully** as usual	☐ 1	☐ 2

14a. During the **past four weeks** to what extent have your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?

(Please tick one)

Not at all	☐ 1
Slightly	☐ 2
Moderately	☐ 3
Quite a bit	☐ 4
Extremely	☐ 5

15a. How much **bodily** pain have you had during the **past four weeks**? ***(Please tick one)***

None	1
Very mild	2
Mild	3
Moderate	4
Severe	5
Very severe	6

16a. During the **past four weeks,** how much did **pain** interfere with your normal work (including both work outside the home and housework)? ***(Please tick one)***

Not at all	1
A little bit	2
Moderately	3
Quite a bit	4
Extremely	5

---

17. During the **past four weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting friends, relatives, etc)?

(Please tick one answer)

All of the time	Most of the time	Some of the time	A little bit of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

18. How much of the time during the **past four weeks**:

(Please tick one answer for each question)

		All of the time	Most of the time	A good bit of the time	Some of the time	A little bit of the time	None of the time
18a.	Did you feel full of life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
18b.	Have you been a very nervous person?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
18 c.	Have you felt so down in the dumps that nothing could cheer you up?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
18d.	Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
18e.	Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
18f.	Have you felt downhearted and low?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
18g.	Did you feel worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
18h.	Have you been a happy person?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
18i.	Did you feel tired?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

19. Please choose the answer that best describes how **TRUE** or **FALSE** each of the following statements is for you:

(Please tick one answer for each question)

	Definitely true	Mostly True	Don't know	Mostly false	Definitely false
19a. I seem to get sick a little easier than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
19b. I'm as healthy as anyone I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
19c. I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
19d. My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Appendix D. Chapter 8 Supplementary Table.

Table S8

Comparisons between participants who took part at follow-up (*N* = 66) *and those lost to follow-up (N = 74)*

	Participated at follow-up n (%) or mean ± SD	Lost to follow-up n (%) or mean ± SD	Effect size, *p* value
Age [years]	63.70 ± 6.65	63.72 ± 7.35	d = 0.00, p = 0.987
Sex [women]	27 (40.9)	25 (33.8)	φ = -0.074, p = 0.487
Ethnicity			V = 0.212, p = 0.098
White	58 (87.9)	54 (73.0)	
Asian	3 (4.5)	9 (12.2)	
Afro-Caribbean	2 (3.0)	8 (10.8)	
Other	3 (4.5)	3 (4.1)	
[a]Household income [£]			V = 0.209, p = 121
<£20,000	23 (36.5)	34 (48.6)	
£20,000-40,000	16 (25.4)	22 (31.4)	
£40,000-60,000	6 (9.5)	5 (7.1)	
>£60,000	18 (28.6)	9 (13.9)	
Marital status			V = 0.161, p = 0.162
Married	28 (42.4)	42 (56.8)	
Single	15 (22.7)	16 (21.6)	
Divorced, separated or widowed	23 (34.8)	16 (21.6)	
[b]Educational level			V = 0.32, p = 0.003
No qualifications	0 (0.0)	12 (16.4)	
Up to O-levels	10 (15.4)	15 (20.5)	
A-levels or ONC	6 (9.2)	8 (11.0)	
Degree or above	49 (75.4)	38 (52.1)	
[c]Smoking status [smokers]	9 (13.6)	11 (15.1)	φ = 0.020, p = 1.000
[d]Moderate or vigorous physical activity per week [hours]	5.40 ± 8.55	4.00 ± 9.09	d = 0.16, p = 0.372
[e]BMI [kg/m^2]	30.79 ± 5.31	30.71 ± 6.12	d = 0.01, p = 0.935
[e]Body fat [%]	36.26 ± 7.98	35.53 ± 9.30	d = 0.08, p = 0.625
[f]WHR	0.99 ± 0.08	0.98 ± 0.09	d = 0.12, p = 0.377
[g]HbA1c [%]	7.04 ± 1.08	7.45 ± 1.67	d = 0.29, p = 0.090

	Participated at follow-up n (%) or mean ± SD	Lost to follow-up n (%) or mean ± SD	Effect size, *p* value
[h]Oral anti-diabetic medication [yes]	52 (80.0)	57 (80.3)	φ = -0.004, *p* = 1.000
[h]Insulin or other injectable anti-diabetic medication [yes]	8 (12.3)	7 (9.9)	φ = -0.039, *p* = 0.856
[h]Anti-hypertensive medication [yes]	48 (73.8)	48 (67.6)	φ = -0.068, *p* = 0.542
[h]Cholesterol-lowering medication [yes]	59 (90.8)	47 (66.2)	φ = -0.30, *p* = 0.001
[h]Aspirin [yes]	28 (43.1)	20 (28.2)	φ = -0.156, *p* = 0.102
[h]Beta blockers [yes]	6 (9.2)	10 (14.1)	φ = 0.075, *p* = 0.541
Pre-task subjective stress [score]	1.50 ± 0.90	1.49 ± 0.88	*d* = 0.01, *p* = 0.929
[i]Depressive symptoms [score]	9.90 ± 6.66	13.62 ± 10.30	*d* = 0.43, *p* = 0.012
[j]Daily happiness [score]	2.66 ± 0.82	2.60 ± 0.92	*d* = 0.07, *p* = 0.737
[k]Pre-task IL-6 [pg/ml]	1.91 ± 1.03	2.37 ± 1.47	*d* = 0.36, *p* = 0.041
[l]Immediately post-task IL-6 [pg/ml]	1.99 ± 1.03	2.35 ± 1.41	*d* = 0.29, *p* = 0.096
[m]45 min IL-6 [pg/ml]	1.93 ± 0.95	2.63 ± 1.66	*d* = 0.52, *p* = 0.006
[n]75 min IL-6 [pg/ml]	2.02 ± 0.93	2.58 ± 1.48	*d* = 0.45, *p* = 0.021
[l]Δ immediately post-task [pg/ml]	0.02 ± 0.38	-0.03 ± 0.69	*d* = 0.09, *p* = 0.629
[m]Δ 45 min [pg/ml]	0.10 ± 0.42	0.16 ± 0.93	*d* = 0.08, *p* = 0.658
[n]Δ 75 min [pg/ml]	0.26 ± 0.66	0.20 ± 0.91	*d* = 0.08, *p* = 0.695
[o]Physical health-related quality of life [score]	72.75 ± 19.54	69.16 ± 24.43	*d* = 0.16, *p* = 0.344
[o]Mental health-related quality of life [score]	75.29 ± 15.07	68.62 ± 22.63	*d* = 0.35, *p* = 0.041
Time of testing [morning]	24 (36.4)	39 (52.7)	φ = -0.164, *p* = 0.077

Note. Differences between the two groups were tested using *t*-tests for continuous measures and chi-square tests for categorical measures. BMI = body mass index; HbA1c = glycated hemoglobin; IL-6 = interleukin-6; kg/m^2 = kilograms per square metre; N = number; n = number; min = minutes; ONC = ordinary national certificate; SD = standard deviation; WHR = waist-to-hip ratio; Δ delta (change) score. [a]n = 63, 70; [b]n = 65, 73; [c]n = 66, 73; [d]n = 65, 62; [e]n = 66, 72; [f]n = 66, 73; [g]n = 65, 69; [h]n = 65, 71; [i]n= 65, 72, [j]n = 61, 69, [k]n = 62, 68; [l]n= 59, 67; [m]n = 54, 61; [n]n = 51, 56; [o]n = 66, 73.