Adverse childhood experiences and coronary heart disease: investigation of neuroendocrine and autonomic nervous systems pathways

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A thesis submitted for the degree of Doctor of Philosophy

University College London

Declaration

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

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I would like to express my sincere appreciation to my supervisors, Professor Rebecca Hardy and Professor Andrew Steptoe. I cannot thank Rebecca more for her unconditional support for my PhD life in various aspects. Not only her advice as an expert helped me to complete my professional part of PhD journey, but also her understanding and own practice of work-life balance made me think of how I want to commence my second career life. I also would like to show my deep gratitude to Andrew. His insightful suggestions from the beginning of my PhD throughout years indicated to me a right direction of my PhD. I would not have been able to achieve this stage of my life without my supervisors' support.

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Abstract

Findings of the association of adverse childhood experiences (ACEs) with coronary heart disease (CHD) are inconsistent. Further, the underlying biological mechanism remains unclear. Accordingly, my PhD examined two hypothesised biological pathways linking ACEs and CHD: the neuroendocrine and the autonomic nervous systems.

Data were from two longitudinal observational studies, the Whitehall II study (WHII), in which I carried out an analysis on how non-response influences mortality, and the National Child Development Study (NCDS). I applied a methodology which allowed for different effect sizes for each ACE, while still being able to assess the cumulative effect of ACEs.

Cox proportional hazard models were used to examine the association between 14 retrospectively collected ACEs and incident CHD using WHII with an average follow-up of 12.9 years. No dose-response effect was observed, with a 6.0% (95% confidence interval: -13%, 1%) reduction in CHD in the absence of ACEs.

Random effects models were used to examine associations between ACEs and diurnal cortisol patterns, an indicator of the neuroendocrine system, drawn from six saliva samples in WHII, and two samples in NCDS. Increasing number of ACEs showed no association with overall cortisol secretion, or awakening response, but the cortisol on waking decreased with the flatter diurnal slope at a mean age of 65.9 (SD 6.0) in WHII. There was no association between prospectively measured ACEs and cortisol at age 44/45 years in NCDS.

Finally, the association between ACEs and heart rate variability (HRV), a marker of the autonomic nervous system, was assessed in WHII. I used random effects models to examine three measures over 10 years follow-up from age 55.3 (SD 6.0). There was no association between ACEs with HRV.

Prevention or mitigation of ACEs are unlikely to impact rates of CHD, but may have benefits for health outcomes relating to the neuroendocrine system.

Impact Statement

Coronary heart disease (CHD) is the leading cause of premature mortality and disability worldwide. A large body of research has identified a range of clinical (e.g., low high-density lipoprotein cholesterol) and behavioural (e.g., physical inactivity) risk factors in adulthood for the incidence of CHD. Psychological stress is another potential modifiable risk factor with effects on several stages of the disease process, from the underlying cause of risky behaviours, development of atherosclerosis, to a trigger of cardiac events. Given CHD represents a long-term disease process which starts in early life, it is important to understand whether psychologically stressful events in early life, namely adverse childhood experiences (ACEs), are associated with incident CHD.

Findings in my PhD are largely null with only small associations of ACEs with salivary cortisol, a marker of the neuroendocrine system, and no associations observed with CHD or heart rate variability. While my finding, that the association of ACEs with CHD is null or small, does not suggest that tackling ACEs will have a great impact on reduction in incident CHD, ACEs remain likely to have impacts on other important health outcomes such as depression, given that depression is consistently shown to be associated with the dysregulated neuroendocrine system,

which I showed was linked to increasing number of ACEs. My studies also showed differences in magnitude of effects across individual ACEs. It may indicate that particular ACEs are related to CHD and the examined systems, which should be prioritised in terms of intervention, although policies need to consider the clustering of ACEs.

This PhD highlights the importance of publishing primarily null findings, as it is possible that previous publication or citation bias may have been misleading in overstating an association where one might not exist. My findings therefore provide a balance to the previous largely positive studies. This also highlights the importance of the open science agenda, to ensure the full extent of the evidence generated is available, irrespective of the nature of the findings. Another aspect of this PhD, which may be beneficial particularly in epidemiology and social science, is the methodology. Most existing studies applied a simple sum of ACEs, the so-called ACEs score, which requires the strong assumption that each ACE has an equal effect on the outcome, which could result in under- or overestimations of the associations. The approach I used, allows each ACE to have different effect size while still calculating a cumulative effect. Although, my approach also has limitations, it provides an opportunity to think critically about a commonly used methodology.

The findings in my PhD have been disseminated mainly to a scientific community. Given that one of the main focuses of this PhD is to address methodological challenges in existing studies, academic researchers are a particularly important audience. My work has been presented at international academic conferences and published in international peer-reviewed journals. Thus, it has contributed to raising awareness of the methodological issue, as well as to disseminate the findings in the relevant community.

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I, Mifuyu Akasaki, conceived the initial idea for the paper; elaborated this with all authors; designed the study; undertook analysis; and drafted the article. Mika Kivimaki and Andrew Steptoe contributed to drafting the article. Owen Nicholas contributed to designing the study; undertaking the analysis; and drafting the article. Martin J Shipley contributed to undertaking the analysis; and drafting the article. All authors contributed to the critical revision and the final approval of the version to be published.

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I, Mifuyu Akasaki, conceived the initial idea for the paper; elaborated this with all authors; designed the study; undertook analysis; and drafted the article. Owen Nicholas contributed to undertaking the analysis, and drafting the article. Jessica Abell contributed to designing the study, and drafting the article. Carlos A. Valencia-Hernández contributed to drafting the article. Rebecca Hardy contributed to undertaking the analysis, and drafting the article. Andrew Steptoe contributed to designing the study, and drafting the article. All authors contributed to the critical revision and the final approval of the version to be published.

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Publications

The following peer-reviewed publications and conference presentations have resulted from the work presented in this thesis:

Peer-reviewed publications

Akasaki M, Nicholas O, Abell J, Valencia-Hernández CA, Hardy R, Steptoe A. Adverse childhood experiences and incident coronary heart disease: a counterfactual analysis in the Whitehall II prospective cohort study. American journal of preventive cardiology. 2021 Sep 1;7:100220.

Akasaki M, Kivimäki M, Steptoe A, Nicholas O, Shipley MJ. Association of attrition with mortality: findings from 11 waves over three decades of the Whitehall II study. J Epidemiol Community Health. 2020 Oct 1;74(10):824-30.

Oral conference presentations

Akasaki M, Hardy R, Steptoe A. 1348 Childhood adversities and diurnal patterns of salivary cortisol in adulthood: two UK-based prospective cohort studies. International Journal of Epidemiology. 2021 Sep;50(Supplement_1):dyab168-022.

Akasaki M, Batty D, Steptoe A, et al OP26 Adverse childhood experiences, population attributable risk and incremental risk of coronary heart disease: a 13 years follow-up of the Whitehall II cohort studyJ Epidemiol Community Health 2020;74:A12-A13.

Akasaki M, Kivimaki M, Steptoe A, et al OP69 Association of attrition with mortality: findings from 11 waves over three decades of the Whitehall II studyJ Epidemiol Community Health 2020;74:A33.

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Chapter 1 Introduction

1.1. Coronary heart disease

1.1.1. Coronary heart disease and its development over the life course

Coronary heart disease (CHD) is the leading cause of premature mortality and disability in the world.[1,2] CHD, also known as coronary artery disease or ischaemic heart disease, is a type of heart disease that occurs when coronary arteries are unable to deliver oxygen-rich blood to tissues, often caused by atherosclerosis which is the buildup of plaque inside the coronary arteries. A large body of research has identified a range of risk factors for the development and progression of CHD.[3] These studies usually focus on risk factors in adulthood, yet CHD has a long-term disease process, from the development of atherosclerosis, subclinical disease, to its clinical manifestations.

This natural history of CHD appears to start early in life.[4] Studies of autopsy have shown that fatty streaks, the earliest visible lesion of atherosclerosis, are observed in individuals at the ages of 15 to 19.[5] In fetuses a different distribution of fatty streaks was seen according to maternal health conditions: fetal aortas from hypercholesterolemic mothers had more lesions than those from

normocholesterolemic mothers.[6] These observations have led to research interest in childhood and adolescence to understand how CHD develops, and what factors contribute to the development of these preclinical changes throughout the life course.

Although genetic variants are implicated in development of CHD, multiple modifiable risk factors from across life have also been identified. Some researchers have shed light on the substantial role of one such modifiable risk factor, psychological stress, which can impact directly or indirectly from the stage of development through the entire period of disease progression.[7] One major relevant finding to date is that more disadvantaged socioeconomic position in childhood is related to increased risks of CHD in adulthood.[8] This association may be indirect because those who grow up in a disadvantaged circumstance are more likely to engage in health risk behaviours than those who do not.[9] Findings from physiological studies during the past decade, however, suggest an underlying mechanism: the biological embedding of psychologically stressful adverse events in childhood,[10,11] which are more likely to occur among those in more disadvantaged circumstances.[12]

In this chapter, I describe CHD in terms of disease burden, pathophysiology, and risk factors; review the current research on adverse childhood experiences in relation to the development of CHD; and hypothesise two potential biological pathways, via the neuroendocrine system and the autonomic nervous system, between adverse childhood experiences and incident CHD.

1.1.2. Disease burden

The worldwide health burden of CHD is substantial. According to the World Health Organization's estimates, CHD is the number one cause of death in the world, representing 16.0% of total mortality in 2019.[13] In England, CHD is the second leading cause of mortality, with over 51 000 deaths, accounting for 10.3% of all deaths, in 2019.[14] In addition, 7.9% of people aged 55 to 79 have been estimated to have CHD.[15] Although CHD incidence and mortality have steadily declined over the past few decades in England,[16] increasing life expectancy and advanced medicine have led to 1712.8 years per 100 000 population disability-adjusted life years (DALYs) being lost due to CHD.[17] Furthermore, multimorbidity, defined as the presence of two or more chronic medical conditions, has been increasing and is recognised as a considerable public health concern.[18,19] Among people who have cardiovascular disease (CVD), 81.1% (95% confidence interval: 78.7 to 83.8)[16,20]

have been estimated to have at least one other disease or health condition, of which 15.0% (14.3 to 16.1) have five or more health conditions.[16] The most prevalent additional condition is hypertension 28.9% (27.7 to 31.5), followed by depression 23.0% (21.3 to 26.0).[16]

There are also considerable economic costs attributable to CHD. The estimated total cost of CHD in 2015 in the UK was €9.1 billion, of which 6.9 € billion were in non-health care costs.[21] Productivity losses due to mortality accounted for 37.2% of the total cost, while the proportion of productivity losses due to morbidity was 10.6%.

1.1.3. Pathophysiology

CHD is defined as the condition where coronary arteries are unable to deliver oxygen-rich blood to tissues, generally caused by atherosclerosis. Coronary artery spasm also appears to contribute to such a blockage by interacting with atherosclerotic coronary arteries. The International Classification of Diseases (ICD) 10 classifies CHD into 6 categories: angina pectoris (I20), acute myocardial infarction (I21), subsequent myocardial infarction (I22), certain current complications following acute myocardial infarction (I23), other acute ischaemic heart disease (I24), and chronic ischaemic heart disease (I25). In this section, I describe the

physiological processes through which atherosclerosis and coronary artery spasm cause CHD.

Atherosclerosis

Atherosclerosis, a specific type of arteriosclerosis, is an inflammatory condition where plaque builds up inside the arteries. The initiation of atherosclerosis happens when endothelial cells on the surface of intima are inflamed by factors such as high blood pressure, elevated level of glucose, and low-density lipoprotein (LDL) cholesterol in blood, free fatty acids, and infection. Blood leukocyte is less likely to adhere to healthy endothelium, but once the endothelium becomes inflamed, it expresses adhesion molecules. Proinflammatory cytokines in atheroma direct adherent leukocytes to migrate into the intima and T cells join macrophages in the intima during lesion evolution. The leukocytes produce cytokines and growth factors to promote migration and proliferation of smooth muscle cells. Smooth muscle cells in the media degrade elastin and collagen. Inflammatory mediators prevent collagen synthesis and stimulate collagenases by foam cells in the intima. This makes the fibrous cap (which contains macrophages and smooth muscle cells) in the plaques thin, resulting in them being prone to rupture. When the plaque ruptures, thrombosis is caused, resulting in a narrowing of the vessel lumen.[22,23]

Coronary artery spasm

Coronary artery spasm is a sudden and temporal vasoconstriction of coronary arteries, caused by a spasm in vascular smooth muscles. The vasoconstriction can occur in both nonobstructive and atherosclerotic (obstructive) coronary arteries. Endothelial dysfunction, vascular smooth muscle cell hyperreactivity, and the autonomic nervous system appear to play a substantial role in causing coronary artery spasm, although underlying mechanisms are still uncertain.[24] Existing clinical studies have reported that approximately 50% of patients who underwent diagnostic coronary angiography for anginal symptoms had normal coronary arteries (stenosis<20%), but around 60% of those who had no narrowing of the coronary arteries showed abnormal coronary vasomotion in intracoronary acetylcholine testing, an established method to assess coronary artery spasm.[25,26] Coronary artery spasm is a distinct entity from atherosclerosis, however, a prolonged coronary artery spasm triggers thrombosis by platelet activation and fibrinopeptide A secretions.[27]

1.1.4. CHD risk factors

A large body of research has identified a range of risk factors for the development and progression of CHD. One of the first cohort studies on CHD initiated in 1940s

and 1950s, is the Framingham Heart Study,[28,29] which advanced our understanding of the natural history of, and risk factors for, CHD. The Framingham Heart Study, based in town of Framingham located in Massachusetts, USA, started as a longitudinal community-based study in 1948. The first research from the study, published in 1957, identified three risk factors for CHD; elevated blood pressure, overweight, and high cholesterol level.[30] In 1961, they also reported that male sex, older age, and left ventricular hypertrophy could predict the incidence of CHD,[31] followed by the reports on the association of cigarette smoking with CHD in 1962[32] and 1964.[33] The Framingham Heart Study further documented the links of CHD with diabetes[34] and obesity[35] in subsequent studies. These findings later led to the development of risk prediction algorithms for CVD as well as CHD.[36,37]

A substantial number of studies in different settings have replicated the findings of the Framingham Heart Study and have also identified additional non-modifiable and modifiable risk factors for CHD. Being male, being older, and having a family history of CHD are thought of as non-modifiable risk factors, while the majority of established CHD risk factors, such as hypertension, dyslipidemia, obesity, diabetes, are modifiable, and more likely to be attributable to individual's lifestyle.[38] While these adult lifestyle factors can be treated, for example, through antihypertensive

medication, identification of factors from earlier in the life course could lead to early prevention of the development of these risk factors.

Since the 1990s, evidence supporting an association of psychological stress with CHD has accumulated. Stress is difficult to define, having multiple different, but likely inter-related, dimensions, and is complex and challenging to measure. Despite such challenges, both external stressors (e.g., work stress, life events) and internal reactions to stress (e.g., depression) have been shown to be consistently related to CHD in studies across the world.[39] In the next section, I describe the history of stress research, and findings of existing studies for the association between stress and CHD.

1.2. Psychological stress and coronary heart disease

1.2.1. History of scientific research into stress

Stress is defined as a challenging experience, both emotionally and physiologically.[40] On the one hand, stress is not necessarily harmful, but would rather be beneficial in the case that an individual feels a sense of control over stress and acquires a certain accomplishment through dealing with challenges. On the other hand, stress can be detrimental when those experiences are beyond one's control and mastery, and when the stress lasts a long time, frequently recurs, or occurs unpredictably.

American physiologist Walter B Cannon began scientific research on stress in the early 20th century. The term ‘fight or flight’ was coined in his book ‘*Bodily changes in pain, hunger, fear and rage: An account of recent researches into the function of emotional excitement*.’ In this book, Cannon describes a physiological reaction in response to stressors, such as perceived harmful events.[41] Cannon also developed the concept of ‘homeostasis’ which was previously introduced by Claude Bernard as *Milieu intérieur (internal environment)*. He defined homeostasis as a physiological system to maintain and regulate the stability inside the body, shedding light on adrenaline as an important player in this system.

In the mid-twenty century, Hungarian endocrinologist Hans Selye presented a concept of stress in a slightly different way from Cannon in his book ‘*The Stress of Life*’. According to Selye’s theory, physiological changes due to stress are manifestations of adaptive reaction as well as of damage, called ‘general adaptation syndrome’. This syndrome has three stages of development; (i) alarm reaction; (ii) resistance; (iii) exhaustion. In the stage of alarm reaction, a remarkable physiological alternation in regulatory processes is manifested in blood pressure, glucose in blood, electrolyte balance, blood flow, and membrane permeability.[42] When the stress stimuli are prolonged, the second stage, resistance develops and the organism is in

the state of adaptation under the secretion of steroid hormones. Then, the organism enters the third stage, exhaustion, if the stress stimulus remains or increases. This stage is triggered by depletion of adaption efficiency following the activated physiological stress reaction and pathophysiological changes.

During the last five decades, a concept of stress response has been developed with the theory of allostasis and allostatic load by Bruce McEwen. Allostasis, originally introduced by Sterling and Eyer,[43] means achieving stability and maintenance of homeostasis by active processes of the autonomic nervous system, the hypothalamic-pituitary-adrenal (HPA) axis, cardiovascular, immune, and metabolic systems.[44] These regulatory systems work in response to each other so that the organism can respond to daily challenges. However, persistent challenges can impair the regulatory systems, termed as allostatic load, in a form of prolonged response or insufficient response. Manifestations of the harmful effects of this underactivity and overactivity have been extensively recognised.

1.2.2. Stress response

The stress response starts in the limbic system of the brain. The information that an individual receives visually or auditorily is sent to the amygdala, and then the

amygdala sends a signal to the hypothalamus. This part of the brain then communicates with the body via the autonomic nervous system regulating involuntary functions. This is followed by the activation of the HPA axis, which is the second component of the stress response system. These two systems are known to interrelate with each other.

There are two key aspects in stress response. First, one's body responds to stressors by, for example, increasing heart rate and blood pressure. These responses enable the body to adapt to the unexpected stressors as well as to daily actions such as getting up in the morning. Second, the basal state of stress response systems reflects the extent to which homeostasis in the body has been maintained, indicating effects of chronic stress on allostasis. Although these two aspects can be independent from each other, there may be interactions.[45-47] For example, people in a disadvantaged socioeconomic position showed prolonged recovery after mental stress to basal values in blood pressure and heart rate variability, compared to those in a more advantaged socioeconomic position.[48]

1.2.3. Stress, physiological ageing, and behavioural changes

Stressful events or experiences appear to accelerate ageing process.[49,50] The key organ of the stress response is the brain, which determines what is “threatening” and “stressful”, and also controls physiological and behavioural responses.[44] Some regions of the brain, such as the amygdala, hippocampus, and prefrontal cortex, respond to acute and chronic stress through releasing hormones and by structural remodeling.[44]

The hippocampus has a role in learning and memory. Its structure is plastic and vulnerable, and becomes easily damaged by repeated stress, as well as internal (e.g., stroke) and external (e.g., head trauma) stimuli.[51] One of the functions of the hippocampus is to shut off the stress response in the HPA axis, which is to be impaired when the hippocampus is damaged or atrophied, leading to a prolonged HPA response to stressors. The resulting elevation in cortisol promotes further hippocampal damage and atrophy.[40] Increased cortisol levels measured in saliva have been shown to predict reduction in hippocampal volume and in performance on hippocampus-dependent memory tasks.[52] The prefrontal cortex, which plays an important role in executive function such as our thoughts, actions, and emotions, is also considered to be very sensitive to structural changes by acute and chronic

stress exposure.[53] In animal studies, the dendritic remodeling in the prefrontal cortex is shown to require only one week to start,[54] while the hippocampus requires many weeks of stress exposure before structural changes are initiated.[55] The structural remodeling in these brain regions, called fronto-limbic regions, lead to impulsive behavioural style such as poor diet,[40,56] which is an established risk factor of CHD, as well as to dysregulation of the HPA axis and the autonomic nervous system.

1.2.4. Acute stress and cardiac events

Acute psychological stress has been increasingly recognised as a factor that can trigger cardiac events particularly among people with pre-existing CHD, or with advanced preclinical conditions such as atherosclerosis.[57]. For example, on the day after the Northridge earthquake in Los Angeles in 1994, the number of cardiac deaths within the city increased from a daily average of 4.6 to 24 among those who did not have physical trauma nor exertions.[58,59] The majority of these cardiac events occurred in individuals with pre-existing CHD.[59] A study of the Hanshin-Awaji earthquake in 1995 in Japan reported similar findings.[60] Anger may also lead to cardiac events.[61] A study in those with implantable cardioverter defibrillators reported that 15% of defibrillator firings were observed after acute episodes of anger in comparison with 3% in normal periods.[62] These observations might be explained by

increased shear stress at atherosclerotic plaque, heightened platelet activation, or by regional myocardial ischaemia distal to stenotic vessels resulting in ventricular dysrhythmias.[63,64]

Even among those without pre-existing CHD, effects of acute stress on cardiac events have been reported. A systematic review documented that 26.8% of cases of onset of apical ballooning syndrome or Takotsubo syndrome were observed after psychological stress.[65] Although Takotsubo syndrome has been gradually differentiated from CHD due to its pathophysiology, it is recognised to be a type of cardiomyopathy,[66] and is of relevance because this syndrome is a stress-related heart disease where the underlying mechanisms have yet to be elucidated.

1.2.5. Chronic stress and disease process of coronary heart disease

In comparison with acute stress, which can involve plaque rupture, subsequent thrombosis, and increased coagulability, chronic stress appears to contribute to the development of atherosclerosis.[67,68] Physiological stress response, as described in section 1.4, involves the neuroendocrine (HPA axis) and autonomic nervous systems (sympatho-adrenal axis) to maintain the homeostasis. However, when stress

persists, or exceeds individuals' adaptive capacity, the regulatory systems are impaired, resulting in prolonged response or insufficient response.

Studies on work stress have been conducted extensively during the past three decades, as work is one of the major sources of stressors, including social isolation, marital problems, caring responsibilities for children or sick spouse, in adulthood.[68-71] A large body of research has documented consistent findings showing that work stress in middle-age is related to increased risk of CHD.[57] A meta-analyses of studies examining underlying mechanisms of the association between work stress and CHD reported that work stress, characterised as effort-reward imbalance, is correlated positively with elevated cortisol,[72] and associated with higher level of inflammation, and with adverse metabolic and hemostatic function.[73] As well as higher blood pressure in people with work stress,[74] heart rate variability, an indicator of the balance between two major branches of autonomic nervous system, sympathetic and parasympathetic, has been shown to be reduced among those who reported effort-reward imbalance.[73,75] The cumulative evidence from these studies indicate that work stress in adulthood may be associated with an unfavourable physiological stress response, which may contribute to the development of atherosclerosis. However, there remains the possibility of reverse causality in this

association, because timing of assessment of work stress in the studies is often close to when biomarkers are assessed, therefore underlying health conditions may result in adverse biomarker changes and can lead to increased reported work stress (i.e., through difficulty in dealing with the demands of the workplace when ill).

1.3. Adverse childhood experiences and lifelong health

As highlighted in section 1.1.1, atherosclerosis can start developing early in life and there are consistent findings of the association between more disadvantaged childhood socioeconomic position and higher risk of CHD. These observations have provoked research interest into adverse childhood experiences (ACEs) during the past two decades. This section summarises existing research of adverse experiences in childhood.

1.3.1. Definition of adverse childhood experiences

Defining ACEs is a key challenge in studies investigating their impact on heath. Even after two decades of ACEs research, there is no standard definition.[76] It therefore remains unclear what ACEs refer to, what types of experiences are regarded as

ACEs, and what qualify as adverse experiences and what qualify as normative. However, there are some commonly used definitions of ACEs employed.

The Centers for Disease Control and Prevention (CDC) and some other studies define ACEs as traumatic events which occur before the age of 18 years.[77] One of the most well-known studies on ACEs conducted by the CDC conceptualised three main ACEs; *abuse*, *neglect*, and *household challenges*.[78] This conceptualisation is the most commonly used in ACEs research. In the CDC definition, *abuse* includes emotional, physical, and sexual abuse, while a broader definition of abuse may include *neglect*, which is a failure to meet children's basic physical and emotional needs (i.e., food, clothing, housing, attachment). *Household challenges*, or household dysfunctions, include domestic violence, parental substance use, parental physical and mental illness, parental separation, family members' deaths (e.g., siblings, parents), and incarcerations. While any kind of abuse, such as emotional, physical, and sexual, have direct effects on children, household challenges, such as witnessing parental argument, or domestic violence may influence children indirectly through being imprinted on their minds. In addition, adverse events beyond households, such as distal relationships (i.e., school peers, community) and societal or environmental events (e.g., federation collapse, natural disaster), have been

increasingly recognised as composites of ACEs,[79] although these ACEs are not included in the CDC questionnaires.

However, in practice, classification and facets of ACEs captured in studies rely on the questionnaire used, and on whether questionnaires have been developed specifically to measure what we currently term ACEs. Historical longitudinal studies, which include individuals old enough to have developed CHD, established several decades ago will not have used such questionnaires. Hence, despite the development of theories to define and classify ACEs, the way in which ACEs are operationalised across studies relating them to CHD and other health outcomes varies.

1.3.2. Prevalence

The estimated prevalence of having at least one ACE ranges from 33% to 88% in a recent systematic review,[80] and 47% in a national household survey in England.[81] The overall prevalence and distribution of individual ACEs appear to vary by subgroups of the population within countries (e.g., private insurance holders, ethnicity), and across countries (e.g., low- and middle-income countries).[80] For example, a population-based study in the USA with 214 157 participants

documented that emotional abuse was the most common event (34.4%), followed by substance abuse (27.6%) and parental separation or divorce (27.6%). On the other hand, the most common event was parental separation or divorce (45.4%) among Hispanic communities in the USA,[82] and domestic violence (47%) in Saudi Arabia.[83] These differences across subgroups and countries need to be considered when interpretating the findings of studies considering associations.

Responding to the observed high prevalence of ACEs, interest in developing policy related to preventing ACEs has increased across the world.[84] Policy and intervention programmes designed to tackle ACEs in England are currently operated locally, and there is no national policy, from prevention to support and empowerment, systematically and holistically. For instance, Bristol City Council declared its aim to develop an ACE aware, and trauma informed, city as a part of the Bristol One City Plan in 2019. They have developed a Knowledge and Skills Framework for Trauma Informed Practice to map out the essential knowledge and skills necessary for individual workers and their organisations to become ACEs and trauma informed.[85] The Birmingham Health and Wellbeing Board in Birmingham City Council also developed and initiated the ACEs Birmingham approach.[86] On the other hand, one particular type of ACE, smacking, is now completely banned under law in Scotland

and Wales as of March 2022. The ban is due to the potential detrimental short-term effects of smacking, such as injury or death, irrespective of evidence of long-term effects on health. It is not, however, yet known what the impact of these policies and initiatives on ACEs has been.

1.3.3. Findings from existing studies and remaining research questions

Mental health problems, such as depression and psychosis, and engaging in risky health behaviours are established as major consequences of having adverse experiences in early life.[87] The first study on ACEs including more than 17 000 participants in USA reported a dose-response association between increasing number of ACEs and greater risk of engaging in risky health behaviours, such as substance abuse and unsafe sex, and worse mental health in adulthood.[78,88] To date, these findings, in particular the associations with worse mental health, have been consistently replicated in several independent study samples.[89-91]

Some studies have documented that non-communicable diseases, including CHD, are also associated with ACEs.[80,90,92-95] However, the associations of ACEs with physical health, particularly CHD are inconsistent across studies: some, but not all, studies show positive associations.[96-98] This inconsistency in findings may be due, at

least in part, to differences between studies in the definition of ACEs, study design, and the measurement of both ACEs and CHD, statistical approach to analysis of ACEs, inclusion of childhood socioeconomic position as an ACE, and study population.

Study design

The majority of existing studies on ACEs are cross-sectional, where ACEs were measured at the same time as assessment of self-reported health outcomes. For example, when considering outcome, some cross-sectional studies with self-report CHD have documented associations of ACEs with doubling of, or far higher, risk of disease.[78,99,100] On the other hand, longitudinal studies with retrospectively measured ACEs, but subsequent collection of CHD diagnosis using electronic health records are more likely to report smaller magnitudes of, or even no, association.[98,101] These findings suggest that there may be bias in cross-sectional studies. Studies have found that negative mood or poor mental health at the time of recall of ACEs can result in negative recollection of childhood, while those with good functioning are more likely to recall childhood more favourably and to forget ACEs.[102] As those with CHD are more likely to have poorer mental health than those without, this differential recall could bias associations between ACEs and CHD in cross-sectional studies.

Even the longitudinal studies, however, apply retrospectively measured ACEs, which is susceptible to recall bias. It is therefore important to examine associations of prospectively measured ACEs with health outcomes in longitudinal settings, and to compare findings with studies in which retrospectively measured ACEs, to investigate whether there is difference in findings, and if so how and why. As far as I know there is no study examining an association of prospectively measured ACEs with CHD. Some cohort studies, such as the National Child Development Study, collected ACEs prospectively, but these studies are still too young to have a sufficient number of incident CHD events in order to run analyses. Among longitudinal studies with retrospectively measured ACEs, there are few with follow-up from the time when ACEs are assessed, which are also linked with central registry records for notification of CHD. Further, the existing studies used the same cohort study from Denmark.[98,101] Therefore, my study provides evidence for the association of retrospectively measured ACEs with objectively measured incident CHD based on data from the UK population.

Measurement

Unstandardised questionnaires of ACEs

One widely used questionnaires is that developed by the CDC (section 1.3.1), which conducted the first study on ACEs with approximately 17 000 participants in USA (The CDC-Kaiser Permanente Adverse Childhood Experiences Study), based on items from the Conflicts Tactics Scale (psychological and physical abuse, domestic violence),[103] Wyatt Questionnaire (sexual abuse),[104] and the Childhood Trauma Questionnaire (emotional and physical neglect).[103] The World Health Organization has also developed a questionnaire, called the Adverse Childhood Experiences International Questionnaire (ACE-IQ), with the aim to measure ACEs across countries. However, to the best of my knowledge, there is no research which has evaluated the reliability or validity of these questionnaires. In addition to these two questionnaires, other existing ACEs studies have used items from various sources. For example, the Whitehall II cohort study adopted items from the European Prospective Investigation into Cancer and Nutrition (EPIC); Health and Life Experiences Questionnaire (HLEQ);[105] the Childhood Experience of Care and Abuse (CECA) interview;[106,107] the Midlife Development in the United States (MIDUS) study;[108] with the rest designed specifically for the Whitehall II study. While birth cohort studies provide prospectively measured ACEs, these ACEs are not

comparable with those measured by recently developed questionnaires specifically to measure what we now term ACEs. Therefore, studies may provide non-comparable findings because they capture different aspects of ACEs.

Information bias in retrospective and prospective measurement of ACEs

Most existing studies, whether cross-sectional or longitudinal, of ACEs and CHD measured ACEs retrospectively in adulthood. However, retrospective ACEs are more likely to be influenced by health status (e.g., depression) at the time of assessment, which is particularly problematic in cross-sectional studies when the outcome of interest is measured at the same time. It is known that there is discrepancy between retrospective and prospective measurement of ACEs, which is more likely to be seen in more subjective ACEs, such as emotional abuse, rather than objective/factual ACEs such as parental separation.[102,109] Prospectively measured ACEs may be less influenced by recall bias due to a shorter time interval from events, but more open to report bias, particularly for sensitive events such as sexual abuse which is likely to be under-reported. It is therefore important to interpret findings within the context of either retrospectively or prospectively measured ACEs,[110] as well as possibly according to the characteristic of the ACEs (i.e., subjective or objective events). A study reported that adjustment for childhood

characteristics such as poverty attenuated the magnitude of discrepancy between retrospectively and prospectively measured childhood exposures (e.g., financial hardships).[111] This adjustment may offer a possible alternative approach to address this issue.

Discount of timing, duration, and severity of ACEs

Childhood and adolescence are considered critical or sensitive periods in life due to the rapid changes taking place.[9] The majority of studies have examined ACEs up to age 18 years, but it may be that ACEs experienced at a specific sensitive stage in childhood or adolescence may be particularly harmful. Few studies have investigated the differences in the effects on health dependent on when ACEs occurred (i.e., a specific age), or for how long they were experienced.[112] Moreover, ACEs are often analysed as a binary response, yes or no, even in cases where ACEs were originally collected on a Likert scale.[78,113] This oversimplification may disregard the severity of an ACE, and it may be that severe ACEs have a greater effect on health than those that are less severe.

Reliance on summary ACEs score

Summary ACEs scores, that is the sum of the number of ACEs reported by an individual, are commonly used in research in order to study a dose-response association, based on the theory that multiple ACEs have a cumulative impact on outcomes. The explicit assumption in the statistical models of this approach is, however, that each experience has an equal effect on the outcome with no correlation between experiences.[114] This approach can affect the precision of estimates because effect sizes could in fact differ across ACEs, and by sex,[115] and because ACEs are more likely to co-occur in an individual and will thus be correlated.[80] The nature of co-occurrence also implicates that examining a specific ACE, without taking into account other co-occurring ACEs, can lead to biased estimations. As such, a scoring scheme that recognises different effect sizes is seen as crucial.[114,116]

Inclusion of childhood socioeconomic position as ACEs

Childhood socioeconomic position (SEP) has been gradually recognised as a separate construct from ACEs,[96,97,114] although some studies have included markers of childhood SEP in a composite of ACEs. Despite its correlation with ACEs, being in a disadvantaged SEP is not necessarily equivalent to an adverse event. To identify

whether an experience is adverse or not, ‘strain’ is a key aspect. For instance, financial difficulties are regarded as an adverse event, because they lead to strain, whereas being in a more disadvantaged SEP according to income or occupation level is not always an adverse experience if there is no strain experienced. Therefore, rather than being an ACE, disadvantaged SEP is a context in which ACEs are more likely to occur,[12] meaning that childhood SEP likely confounds the association of ACEs with outcomes.

1.4. Biological pathways

A growing body of research has identified a series of consequences of ACEs ranging from dysregulation of biomarkers to clinical manifestations of diseases. Therefore, a possible pathway between ACEs and CHD is through the biological changes triggered by psychosocial stress. It has been documented that ACEs are associated with peripheral biomarkers of inflammation, cardiovascular function, and metabolism.[117-119] These biomarkers have also been shown to predict incidence of obesity, Type II diabetes, hypertension, and subsequent CHD (see section 1.4.3 for more details). Those systems are considered to interact with each other, but there appear to be two main systems related to stress, which are the neuroendocrine system and the autonomic nervous system. In the following section, I describe

inflammatory, cardiovascular and metabolic functions, the neuroendocrine and the autonomic nervous systems, and current knowledge of their associations with health outcomes and with ACEs.

1.4.1. Inflammatory, metabolic, and cardiovascular systems

Associations between markers of metabolic (e.g., high cholesterol) and cardiovascular (e.g., high blood pressure) systems with development of CHD are established. It has also been widely accepted that the inflammatory system relates to CHD.[120] The more commonly used indicators of peripheral inflammation are C-Reactive Protein (CRP), Interleukin-6 (IL-6), tumor necrosis factor-alpha, and white blood cells.[121,122] Inflammation is also known to be closely associated with procoagulatory processes.[123,124]

A meta-analysis has reported that childhood trauma, one type of ACE, is associated with slightly elevated CRP and IL-6 in adulthood, and their effect sizes differ according to the type of traumatic events.[121] The same direction of association is reported in a systematic review including a wider range of inflammatory markers.[122] There is little evidence for the association of ACEs with inflammatory markers in

youth.[93] These markers are more likely to be influenced by, or possibly partly mediated by adulthood health behaviours (e.g., smoking) or body mass index.[125-127]

ACEs (physical and emotional abuse) have been shown to be associated with obesity in adulthood, a physiological manifestation due to dysregulation of metabolic system, while there is little evidence for the association with type II diabetes according to a meta-analysis.[128] This may be because type II diabetes involves long-term disease process, meaning that its incidence is more likely to be determined by the age of study participants (i.e., younger participants are more likely to manifest obesity, a precursor of type II diabetes, but too early to have type II diabetes). This is supported by findings of a study reporting associations of ACEs (neglect and physical abuse) with slightly elevated HbA_{1c} at age of 44 to 45,[129] although not all studies found positive associations.[97] Other indicators related to metabolic system, such as high-density lipoprotein, total cholesterol, have been examined as a component of summary risk scores,[91,130,131] showing a positive direction of the association with ACEs. To date, there is no meta-analysis of the association of ACEs with cardiometabolic biomarkers, apart from obesity.[128]

Blood pressure (systolic and diastolic) is a major risk factor for cardiovascular function.[91,130,132-134] Some studies of ACEs have included blood pressure as a composite of summary biological indicators,[91,132,133] while a study examined developmental trajectories of blood pressure across age.[134] This study, with retrospectively measured ACEs, showed that those who had multiple ACEs had a faster increase in blood pressure after the age of 30 years than those who did not,[134] while another study found no evidence of an association with blood pressure in middle-age.[97]

1.4.2. Neuroendocrine system

Cortisol, which is a hormone secreted by the hypothalamic–pituitary–adrenal (HPA) axis, plays an important role in glucose production, metabolism, regulation of water balance, inflammatory responses, and immune functioning. It also has negative effects such as contributing to increased LDL cholesterol and damage to the hippocampus when the cortisol secretion deviates, which may be caused by the HPA axis dysfunction.[40]

Cortisol secretion has a circadian rhythm,[135] approximately corresponding to a 24-hour cycle of the body clock, with a peak approximately 30-45 minutes after

awakening, called the cortisol awakening response (CAR), followed by a decline towards evening. In mammals, a master pacemaker of the circadian rhythm, residing in the suprachiasmatic nuclei in the hypothalamus, is stimulated by environmental time mainly via light to the retina, which then synchronises the peripheral clock via the neuroendocrine (i.e., HPA axis) and the autonomic nervous systems.[136] The CAR appears to involve extra-pituitary pathways along with the HPA axis. Furthermore, the volume of cortisol secretion is an important indicator of health, as seen in individuals with Cushing syndrome (hypercortisolism) and Addison's disease (hypocortisolism).

Serum, salivary, and urinary cortisol are able to reflect diurnal patterns of secretion. Salivary and urinary cortisol measures are generally favoured in stress research because their values are highly correlated with those of serum unbound cortisol,[137] while avoiding the need for blood sampling.

It has been shown that cortisol secretion is associated with mental health outcomes. Systematic reviews have reported that depression is associated with increased volume of cortisol secretion during the waking period, and posttraumatic stress with

decreased secretion.[138,139] Flatter diurnal slopes have been associated with adverse psychological symptoms and disease, such as depression,[140] while evidence related to the CAR is less consistent.[138,141] Few studies have documented the association of cortisol, particularly CAR, with physical health.[142] The Whitehall II cohort study reported no association between CAR and all-cause and cardiovascular mortality,[142] whereas a flatter diurnal slope was associated with mortality and with physiological manifestations, including obesity.[140]

Findings on the association between ACEs and cortisol secretion are heterogeneous,[143] and it is challenging to compare results across studies, due to variations in the measurement of both cortisol and of ACEs.[140] A systematic review and meta-analysis concluded that there was no evidence overall that ACEs are associated with the CAR.[143,144] However, a study relating levels of CAR with severity of childhood abuse reported a *J* shaped association (i.e., moderately suppressed CAR in the non-severe abuse group and elevated CAR in the severe group) among healthy adult individuals.[145] A lower cortisol level at awakening has also been documented in those exposed to ACEs,[146-151] which might in part explain the observation of a blunted diurnal slope among this group.[118,146,147,149-152] The few

studies examining the total volume of cortisol secretion during the day in general population samples have reported no association with ACEs.[152]

Most of the existing studies are, however, limited because they examine only specific types of ACEs[122] or clusters of ACEs,[153] while studies considering the cumulative effect of multiple childhood adversities tend to apply a simple sum of the number of adversities, which does not take account of the potential different effects of each adversity. It therefore remains unclear whether an increasing number of diverse ACEs is associated with disturbed cortisol secretion. Moreover, many of the existing studies had small study samples, which means that associations cannot be estimated with adequate precision.

Sex differences in stress response have been highlighted.[154] These differences can be found in the HPA axis, such as inputs to paraventricular nucleus of the hypothalamus in which corticotropin-releasing factor neurons exist. Gonadal hormones are the main drivers of these differences, as well as genetic factors that are thought to contribute to development of sexually differentiated brain structures.[155] Therefore, exposures to stress in early life, when the brain, including regions

controlling stress regulation are developing rapidly, may lead to sex-specific dysregulation of stress response throughout the life course. Despite evidence that the association between ACEs and CVD may be stronger in women,[156] sex differences in the relation between adversities and possible mediators, such as cortisol, remains unclear.[150,157]

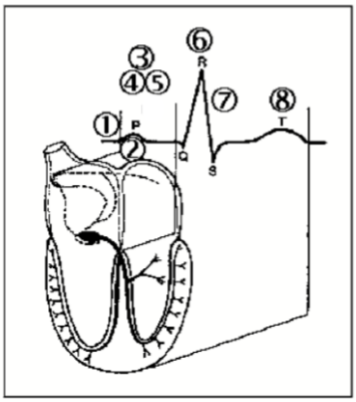
1.4.3. Autonomic nervous system

The autonomic nervous system is composed of sympathetic and parasympathetic nervous systems. The sympathetic nervous system triggers the fight-or-flight response, accelerating heartbeat and inhibiting peristalsis, while the parasympathetic nervous system functions conversely by controlling the rest-and-digest response to calm the body down. Having received the signal from the amygdala, the hypothalamus communicates with the adrenal medulla through autonomic nerves, prompting it to release catecholamines, adrenaline and noradrenaline, to the bloodstream. Adrenaline triggers physical changes, ranging from increasing blood pressure and dilating bronchi to allow greater oxygen intake, to converting glycogen to glucose in the liver to supply nutrients throughout the body. This autonomic reactivity is an instantaneous response to stressors, while the activation of the HPA axis happens in minutes[158]. If the stress remains, the hypothalamus releases

corticotropin-releasing hormone (CRH). The CRH is transported to the pituitary gland to stimulate the secretion of adrenocorticotropic hormone (ACTH), which travels to the adrenal cortex. The adrenal cortex then releases glucocorticoid hormones, primarily cortisol, keeping the body under tension. Once the stress fades away, cortisol level drops, and the parasympathetic nervous system, of which the main component is the vagus nerve, lessens the stress response. When excessive secretion of catecholamines sustains, it could lead to elevated lipid levels and free-fatty acids, as well as suppressed cellular immune functioning, hemodynamic changes (e.g., increased blood pressure), large variations in heart rhythms, and neurochemical imbalances.[159]

Observations of an association of fatal arrythmia with the disturbed autonomic nervous function, either heightened sympathetic or depressed parasympathetic activity, has led to clinical and research interest into how to quantify autonomic activity. Among various measurements of the activity,[160] heart rate variability (HRV) has become one of the most favoured markers due to its non-invasive derivation from electrocardiogram (ECG) recordings.[161] Electrocardiogram signals show PQRST complexes (Figure 1-1).The P wave represents the depolarisation of the atrium following the sinoatrial node activity; QRS wave shows the depolarisation of

the ventricle produced by the atrioventricular node; and the T wave arises from the repolarisation of the ventricle.[162]



	Physiologic Event	ECG Evidence
1.	SA node initiates impulse	Not visible
2.	Depolarization of atrial muscle	P wave
3.	Atrial contraction	Not visible
4.	Depolarization of AV node & Common Bundle	Not visible
5.	Repolarization of atrial muscle	Not visible
6.	Depolarization of ventricular muscle	QRS complex
7.	Contraction of ventricular muscle	Not visible
8.	Repolarization of ventricular muscle	T wave

Figure 1-1. PQRST complexes. Adapted from Becker DE. Fundamentals of electrocardiography interpretation. Anesthesia progress. 2006;53(2):53-64.

HRV represents variations in the interval between consecutive heart beats, generally measured in two domains: time and frequency.[161] In the time domain, the simplest measure is the standard deviation (SD) of the normal-to-normal (NN) intervals (SDNN), which represents all intervals between adjacent QRS complexes resulting from sinus node depolarisation. One of the other most commonly used markers derived from these intervals is the square root of the mean squared differences of successive NN intervals (RMSSD). Frequency domains, on the other hand, represent distribution of power across bands of the frequency spectrum. The three main components are very-low frequency (VLF), low-frequency (LF), and high-frequency (HF), although VLF obtained from short-term ECG recordings is not

considered to be a reliable marker.[161] There are other markers used to represent HRV, such as heart rate (combined effect of sympathetic and parasympathetic activity), respiratory sinus arrhythmia (parasympathetic control) and pre-ejection period (sympathetic control). [163,164]

HRV does have limitations as a marker of the autonomic nervous system. As HRV is related to sinus node function, HRV in people who have a history of cardiac conditions, such as atrial fibrillation and ischemic heart disease, may not be comparable with that in those with an intact sinus node.[165]

Distressed autonomic activity has been found to predict postinfarction mortality,[166,167] as well as to have an association with a wide range of cardiovascular conditions and outcomes in people without prior cardiovascular history.[75,168,169]

Following the first study, published in the 1970s, reporting increased risk of mortality in people who showed low HRV after acute myocardial infarction, a large number of studies have examined HRV as a potential clinical marker of subsequent cardiometabolic outcomes. A systematic review with meta-analysis has documented

that a low SDNN and LF were associated with 35% (95% confidence interval 10% to 67%) and 45% (12% to 87%), respectively, higher risk of first episodes of CVD during follow-up times ranging from 3.5 to 15 years. In contrast, the association with HF was null.[168] Similarly, decreased HRV (lower SDNN, RMSSD, HF, LF) has been associated with risk factors of CVD, including hypertension, type II diabetes, and health risk behaviours.[75,169] Thus, the disruption of autonomic activity may be a contributing factor to the development of, or a trigger of, cardiac events, and a prognostic factor of recurrent events or deaths.

Sex differences in the autonomic nervous functioning appear to exist.[170,171] Women are more likely to have a higher heart rate (smaller mean RR intervals), with less variability in the time domain, indexed by SDNN, than men.[170] For the frequency domain, LF was shown to be lower, while HF was greater in women than men in a systematic review.[170] That is, women's autonomic activity is more likely to be controlled by the vagal nerve, while men's autonomic activity is likely to be dominated by the sympathetic system.

While the HPA axis is one of the more commonly studied potential pathways linking ACEs with CVD, which is generally composed of CHD and stroke, few studies have examined the pathway via the autonomic nervous system.[172,173] A cross-sectional study of 10 260 people with a mean age of 44.3 (SD: 13.2) years documented no association between ACEs and SDNN and RMSSD, after adjusting for confounders.[172] This study used a sum of only 4 types of ACEs (childhood maltreatment) and did not include any household challenge-type ACEs. Similarly no association was observed in a longitudinal study (n=2778) which used heart rate, respiratory sinus arrhythmia, and pre-ejection period and used a score summing the number and frequency of ACEs.[173] The study sample was not population-based as it sampled from the community, primary health care, and specialised mental health care so as to include individuals with severe depression and anxiety disorders. Both studies were from the Netherlands and sex differences in associations were, either not found[172] or not addressed.[173] These studies measured HRV at a single time point in midlife when the mean age of participants was 44.3 (13.2)[172] and 41.7 (13.1).[173] It could therefore be that null findings are because the sample is not yet old enough for changes in the autonomic system to have occurred. Moreover, given that HRV is related to sinus node function, careful consideration is required as to whether to include people who have had cardiac events at baseline and over the follow-up

period. Furthermore, as the autonomic nervous system is known to function differently in men and women, it is important to consider possible sex differences in associations.

1.5. Conclusion

In conclusion, CHD is a leading cause of mortality and disability all over the world. Prevention of the development and progression of CHD is therefore crucial. There is accumulating evidence suggesting that psychological stress, a modifiable risk factor, is related to CHD. Given that CHD develops over the life course from early life, there is increasing interest in the association of ACEs, a source of stress in childhood, with CHD. Despite relatively consistent findings for the association between ACEs and CHD, biological pathways from ACEs to the development of CHD remain unclear. I hypothesised two potential biological pathways, via the neuroendocrine system and the autonomic nervous system.

Chapter 2 Aims and Objectives

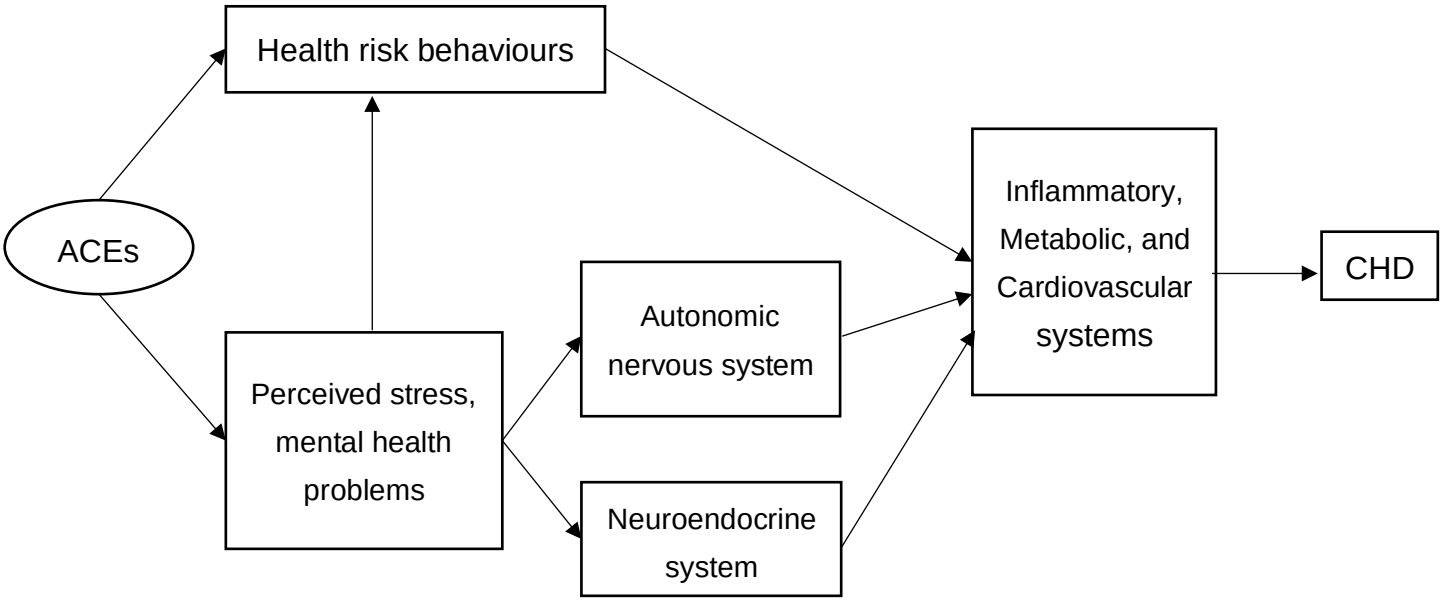
Aims

The primary research questions of this PhD are:

1. *Do adverse childhood experiences increase the risk of developing coronary heart disease later in life*
2. *What are the biological mechanisms explaining the association?*

Figure 2-1 Figure 2-1. Conceptual diagram of association between adverse childhood experiences (ACEs) and development of coronary heart disease (CHD)shows the conceptual diagram of the association between adverse childhood experiences (ACEs) and development of coronary heart disease (CHD).

Figure 2-1. Conceptual diagram of association between adverse childhood experiences (ACEs) and development of coronary heart disease (CHD)



Accordingly, the aim of this PhD is to investigate the role of the neuroendocrine and autonomic nervous systems in the association between ACEs and development of CHD.

Objectives

The PhD is composed of three main studies with one methodological study. The objectives of each study are set out below.

The objective of study 1 was:

To quantify the potential bias due to missing data in the study sample of the Whitehall II cohort study.

The objective of study 2 was:

To examine the association between ACEs, assessed retrospectively, and incident objectively ascertained CHD in the Whitehall II study.

The objectives of study 3 were:

To examine the association of ACEs with diurnal patterns of salivary cortisol as a marker of the neuroendocrine system in the Whitehall II cohort study.

To determine the same association in the National Child Development Study, which unlike the Whitehall II cohort study has prospectively measured ACEs.

The objectives of study 4 were:

To examine the association of ACEs with resting heart rate and heart rate variability as a marker of the autonomic nervous system.

To assess the association between ACEs and the developmental trajectory of these markers over a 10-year period.

Chapter 3 Methods

3.1. Introduction

In this Chapter, I describe the methodology used in my PhD, including the study populations, exposure and outcomes, exclusion criteria, follow-up in longitudinal analyses, and the general approach to statistical analysis. Further details of the methodology specific to each analysis are found in each of studies chapters: Chapter 4, 5, 6, and 7.

3.2. Study populations

I used the Whitehall II cohort study across all studies in my PhD due to the availability of relevant outcomes. ACEs in the Whitehall II cohort study were, however, measured retrospectively, which may be subject to recall and report bias. The National Child Development Study (NCDS; the 1958 British Birth Cohort Study), on the other hand, collected adverse experiences prospectively throughout childhood. I therefore used the NCDS for a comparison in the study examining an association of adversity with salivary cortisol, of which measurement is available in the NCDS.

3.2.1. Whitehall II cohort study

The Whitehall II study was established in 1985 with the aim of determining factors contributing to the social gradient in health. The participants recruited at baseline (wave 1) were a total of 10 308 (men 6895; women 3413) aged 35-55, who were non-industrial civil servants from 20 Civil Service departments in London, UK.[174] As of December 2021, there have been 12 completed waves of data collection, including wave 10 as a pilot. Data collection for wave 13 was in paused in 2020 due to the COVID-19 pandemic. The response rate has been over 65% across all waves. Table 3-1 summarises the time period of each wave, along with the response status, and the number of deaths due to all causes and to CHD. At the most recent completed wave in 2012-2013, a total of 6308 provided data and there had been 1414 deaths. As of August 2017, the date of most recent follow up for death, there were 1943 deaths, of which 285 (14.7%) were due to CHD.

Data have been collected using both self-administered questionnaires and medical examinations at odd-numbered waves, and self-administered questionnaires only at even-numbered waves. Intervals between waves are around two to five years. See appendices for a questionnaire at wave 5 when ACEs were collected, and protocols of salivary cortisol (waves 7 and 9) and heart rate variability (waves 5, 7, 9). The study is approved by the London-Harrow Research Ethics Committee, reference

number 85/0938, and the Scotland A Research Ethics Committee, reference number 16/SS/0003. Written informed consent was obtained from all participants.

Table 3-1. Response status and cumulative death (CHD, all-cause) at each wave in the Whitehall II study

Wave	Period	Participants (responders)	Attrition[a]			Cumulative CHD death (%) [d]	Cumulative all-cause death
			Cumulative Withdrawal (%)[b]	*Non-response (%)[b]*	*Total (%)[c]*		
1	1985-1988	**10 308**	-	-	-	-	-
2	1989-1990	**8132**	2127 (20.7)[e]		**2127 (20.7)**	11 (22.4)	**49**
3	1991-1994	**8815**	1368 (13.4)[e]		**1368 (13.4)**	23 (18.4)	**125**
4	1995-1996	**8628**	774 (52.4)	712 (47.6)	**1486 (14.7)**	43 (22.2)	**194**
5	1997-1999	**7870**	882 (41.3)	1250 (58.7)	**2132 (21.3)**	64 (20.9)	**306**
6	2001	**7355**	975 (38.7)	1553 (61.3)	**2528 (25.6)**	83 (19.5)	**425**
7	2002-2004	**6967**	1246 (45.2)	1511 (54.8)	**2757 (28.4)**	102 (17.5)	**584**
8	2006	**7173**	1310 (55.5)	1051 (44.5)	**2361 (24.8)**	125 (16.1)	**774**
9	2007-2009	**6761**	1354 (52.2)	1239 (47.8)	**2593 (27.7)**	146 (15.3)	**954**
11[f]	2012-2013	**6308**	1389 (53.7)	1197 (46.3)	**2586 (29.1)**	206 (14.6)	**1414**
					Deaths to August 2017	*285 (14.7)*	***1943***

[a] Deaths are displayed separately from attrition (non-response or withdrawal)

[b] % of each attrition = [withdrawal or non-response / total attrition at each wave] * 100

[c] % attrition = [total attrition at each wave / (10308 - cumulative deaths at each wave)] * 100

[d] % CHD death = (CHD death / all-cause death) * 100

[e] Only pooled attrition is available at waves 2 and 3

[f] Wave 10 was a small pilot study of measures to be included at wave 11, and has not been included here

CHD, coronary heart disease

3.2.2. National Child Development Study (1958 British Birth Cohort)

The National Child Development Study (NCDS) follows those who were born in a single week of 1958 in England, Wales, and Scotland.[175] Initially NCDS started with 17 415 participants, as the Perinatal Mortality Survey, which was designed to investigate social and obstetric factors related to stillbirth and death in early infancy. As of December 2021, 11 waves of data collection, including the Perinatal Mortality Survey, had been completed. Wave 12 data collection (age 62) was paused in 2020 due to COVID-19 pandemic. In waves 2 (age 7), 3 (age 11), and 4 (age 16), migrants born in 1958 were recruited, which increased the number of total cohort members to 18 558. The response rate has been over 58% across all waves. Table 3-2 summarises the time period of each wave, response status, and all-cause mortality at each wave. Due to the way of data collection, classifications of attrition are not equivalent to those in the Whitehall II cohort study. Of 18 558 cohort members, 9137 people participated at the most recent wave in 2013 with attrition rate of 46.3%, and there had been 1548 deaths.

At the Perinatal Mortality Survey, the questionnaire was completed by midwives collecting data from mothers and medical records. Information was collected through parents, school teachers, medical examinations, and participants between wave 2 (age 7) and 4 (age 16), and from participants in all subsequent waves.

As the NCDS started in 1958, the procedures for ethical approval have changed across time. In waves 1 (1958) to 6 (1991), internal ethical reviews were conducted because this was prior to the establishment of the multicentre research ethics committees (MREC) system, which began in 1997. From wave 7 (2000) onwards, MREC ethical approval has been sought, apart from for wave 8 (2004) which did not include any medical assessment.

The way of obtaining participants' consent has also changed since the NCDS started. In the Perinatal Mortality Survey, consent was gained in the form of a participants' agreement to be interviewed or completion of questionnaire. For the waves 2 (age 7), 3 (age 11), and 4 (age 16), informed parental consent was sought, but with no written consent. From wave 5, when the participants were aged 23, the consent was sought directly from the participants in a similar way as the previous waves, while in the wave 6 (age 33) written consent was obtained for permission that doctors or hospitals named during the interview might be contacted.

Table 3-2. Response status and cumulative all-cause death at each wave in the NCDS

Wave	Year	Age	Participants (responders)	Attrition[a]			Cumulative all-cause death
				Not issued[b] (%)	*Unproductive[c](%)*	*Total (%)[d]*	
1 (PMS)[e]	1958	Birth	**17415**	925 (-)	218 (1.2)	218 (1.2)	-
2	1965	7	**15425**	548 (-)	1764 (10.8)	1764 (10.8)	821
3	1969	11	**15337**	275 (-)	2106 (12.7)	2946 (12.7)	840
4	1974	16	**14654**	0 (0)	3031 (18.0)	3031 (18.0)	873
5	1981	23	**12537**	862 (17.0)	4199 (83.0)	5061 (28.8)	960
6	1991	33	**11469**	993 (16.4)	5047 (83.6)	6040 (34.5)	1049
7	2000	42	**11419**	1415 (23.8)	4524 (76.2)	5934 (34.2)	1200
8	2002	44	**9377**	2908 (37.0)	4951 (63.0)	7859 (45.6)	1322
9	2004	46	**9534**	4248 (55.2)	3452 (44.8)	7700 (44.7)	1324
11	2008	50	**9790**	3553 (48.6)	3755 (51.4)	7308 (42.7)	1460
12	2013	55	**9137**	4653 (59.1)	3220 (40.9)	7873 (46.3)	1548

[a] Deaths are displayed separately from attrition

[b] Not issued; wave 1-3 due to not being resident in Great Britain, wave 5 onward due to withdrawal or loss to follow-up.

[c] Unproductive; refusal, non-contact, or emigrant.

[d] % attrition = [total attrition at each wave / (18558 - cumulative deaths at each wave)] * 100, except for waves 1-3 where % attrition was calculated as; [Unproductive / (participants + unproductive - cumulative death at each wave)] * 100

[e] PMS, perinatal mortality survey

3.3. Exposure and outcomes

3.3.1. Exposure: Adverse childhood experiences

ACEs were collected at waves 1 (1985-1988) and 5 (1997-1999) in the Whitehall II cohort study, and at waves 2 (age 7), 3 (age 11), and 4 (age 16) in the NCDS. In the Whitehall II cohort study, the information was collected retrospectively through self-completion questionnaire. In NCDS data were collected prospectively from parents by health visitors when participants were aged 7, 11, and 16, as well as through participants' self-completion questionnaire at age of 16. I firstly selected relevant questions in the Whitehall II cohort study based on questions relating to abuse, neglect, and household challenges, and then sought comparable questions in the NCDS to enable cross-cohort comparison.

Table 3-3 and Table 3-4 summarise questions which were selected to measure ACEs in the Whitehall II cohort study and NCDS. The measures in both cohort studies cover the domains of "maternal/parental separation", "parental death", "hospitalisation of self", "parental mental illness", "parental substance use", "parental arguments/domestic tension", "parental unemployment", "financial problems", "orphanage/out-of-home care", "relationships with parents." As only retrospectively measured "physical abuse" was available in NCDS, I did not include this component

in NCDS analysis. In the Whitehall II cohort study, measures relate to experiences up to the age of 16, while in NCDS some questions are asked up to 16 years old, but others cover only the period up to 7 years old.

Table 3-3. Questionnaires of ACEs in Whitehall II cohort study and National Child Development Study

Whitehall II cohort study (up to age 16: retrospective)	Wave[a]	National Child Development Study (prospective)	Age
Maternal separation 1yr+		**Parental separation**	
Were you ever separated from your mother for a year or more as a child	5	Child's longest period of separation from the mother - longer than 1 year (*answered by parents*)	7
		The actual relationship-person acting mother and father *(answered by parents)*	11, 16
Parental death		**Parental death**	
Is your natural father/mother still alive? How old were you when he/she died?	1	Death of child's father *(answered by parents)*	7, 11, 16
		Death of child's mother *(answered by parents)*	
Hospitalisation 4wks+		**Overnight hospitalisation**	
You spent 4 or more weeks in hospital	5	Has the child ever been admitted to hospital for any of the following: (list of diseases) (*answered by parents*)	7
		Has the child ever been admitted to hospital overnight? (*answered by parents*)	11, 16
Divorce		-	
Your parents were divorced	5	*Not available (included in the question for "parental separation")*	
Mental illness and alcohol problems		**Mental illness**	
Your parent(s) were mentally ill or drank so often that it caused family problems	5	Family difficulties - Mental illness or neurosis *(answered by parents)*	7
		Father/mother's chronic condition *(answered by parents)*	11
		Father/mother's diagnosis of illness *(answered by parents)*	16
-		**Substance use**	
Not available (included in the question for "mental illness and alcohol problems")		Family difficulties - alcoholism	7
Arguments between parents		**Domestic tension**	
Your parents very often argued or fought	5	Domestic tension *(answered by parents)*	7
Unemployment		**Unemployment**	
Your father/mother were unemployed when they wanted to be working	5	Family difficulties - Unemployment *(answered by parents)*	7
		Source of family income in the last 12 months - unemployment *(answered by parents)*	11, 16

a Mean age ± standard deviation is 44.9 ± 6.0 at wave 1 and 55.8 ± 6.0 at wave 5

Table 3-4. Questionnaires of ACEs in Whitehall II cohort study and National Child Development Study (continued)

Whitehall II cohort study (up to age 16: retrospective)	Wave[a]	National Child Development Study (prospective)	Age
Financial problems		**Financial problems**	
Your family had continuing financial problems	5	Family difficulties - Financial difficulties *(answered by parents)*	7
		Serious financial hardship in the past 12 months (*answered by parents*)	11, 16
Physical abuse		**-**	
You were physically abused by someone close to you	5	*Not available*	-
Orphanage		**Out-of-home care**	
You were in an orphanage/children's home	5	Has the child been in the care of the local authority? (answered by parents)	7
		Has the child been in local authority/voluntary care? (answered by parents)	11, 16
Lack of attachment to mothers		**Get along with mother / get along with father**	
How much did she understand your problems and worries?	5	I get on well with my mother/father (*answered by participants*)	16
How much could you confide in her about things that were bothering you?	5		
How much love and affection did she give you?	5		
How much time and attention did she give you when you needed it?	5		
Lack of attachment to fathers			
How much did he understand your problems and worries?	5		
How much could you confide in him about things that were bothering you?	5		
How much love and affection did he give you?	5		
How much time and attention did he give you when you needed it?	5		
Mother's harsh punishment			
How harsh was she when she punished you?	5		
Father's harsh punishment			
How harsh was he when she punished you?	5		

a Mean age ± standard deviation is 55.8 ± 6.0 at wave 5

3.3.2. Outcome: Incidence of CHD

The analysis of association between ACEs and incident CHD was carried out in the Whitehall II cohort study only, as objectively determined incident CHD is available. Incident CHD was identified by a combination of data collected during the medical examination, and linkage of study participants to records from the National Health Service (Hospital Episode Statistics, HES).[176] Data collected from the medical examination were based on a 12-lead resting electrocardiogram recording, and self-reported incident CHD which was confirmed by information provided by general practitioners or manual retrieval of hospital records. Non-fatal myocardial infarction, definite angina, coronary artery bypass grafting, and percutaneous transluminal coronary angiography were included in the ascertainment. The HES-ascertainment is based on the linkage with the records from hospitalisations for non-fatal CHD as a primary or secondary diagnosis, defined by the International Classification of Disease (ICD) 9 (codes 410-414) and 10 (codes I20 – I25), or procedures K40-K49, K50, K75, and U19. Records of fatal CHD death were also added from data linkage to the Office for National Statistics death registry by using the NHS identification number. The dates of events were identified through the records used to confirm the events. Incident non-fatal and fatal CHD in this study refers to the first episode only.

3.3.3. Outcomes: Neuroendocrine and autonomic nervous systems

I used salivary cortisol as a marker of the neuroendocrine system, which is available in both the Whitehall II cohort study and NCDS, and heart rate variability as a marker of the autonomic nervous system which is only available in the Whitehall II cohort study.

Table 3-5 summarises the biomarkers in the Whitehall II cohort study and in the NCDS.

Salivary cortisol

In the Whitehall II cohort study, salivary cortisol was collected at wave 9 (2007-2009), when the mean age (standard deviation) was 65.9 (5.9) years in men and 69.6 (5.8) in women. Participants were asked to provide six saliva samples in salivettes on a weekday at waking (T_1), +30 min (T_2), +2.5 hours (T_3), + 8 hours (T_4), and +12 hours (T_5) since awakening, and at bedtime (T_6). The participants were instructed not to brush teeth, drink nor eat anything in the 15 minutes before each saliva sample collection, to record the time of sample collection in the logbook, and to send back the six salivettes and the logbook to the study team in a Freepost envelope provided. Once received by the study team, salivette devices were centrifugated at 3000 rpm for 5 minutes, resulting in a clear supernatant of low viscosity. Cortisol levels were assessed using a commercial immunoassay kit with chemiluminescence detection (CLIA, IBL-Hamburg, Hamburg Germany). The lower concentration limit of this assay was 0.44 nmol/l, with intraassay and interassay precision of <8%.

In NCDS, salivary cortisol was collected at the biomedical wave when participants were aged 44 to 45 (2002-2004). Participants were asked to provide two saliva samples in salivettes on any day, 45 mins after awakening and before eating breakfast (T_{N1}), and at 3 hours after the first sample (T_{N1}). The instructions to participants regarding the collection of samples was as in the Whitehall II study. Participants were also asked to fill in the questionnaire including the information of

date and time of sample collection and awakening time. The two salivettes and the questionnaire were sent back via post to the study team. Cortisol levels were assessed using a commercial immunoassay kit with chemiluminescence detection (CLIA, IBL-Hamburg, Hamburg Germany). The lower sensitivity of the assay is 0.44 nmol/l, with intraassay and interassay precision of <10% for a wide range of cortisol concentrations. Cortisol levels > 50 nmol/l were rerun in a second assay for confirmation.

Summary measures from the cortisol profiles were derived from these multiple measures (

Table 3-5). The description of these derivations is found in the relevant results chapter (Chapter 5).

Heart rate variability

Heart rate variability (HRV) and resting heart rate (rHR) were recorded at three waves (waves 5, 7 and 9) in the Whitehall II cohort study, when participants' mean age (standard deviation) was 55.8 (6.0), 61.1 (6.0), and 65.9 (5.9). Five-minute supine 12-lead electrocardiograms were performed at rest using SEER MC recorders (GE Medical Systems, Milwaukee, Wisconsin). Electrocardiogram signals show PQRST complexes. The P wave represents the depolarisation of the atrium following the sinoatrial node activity; the QRS wave shows the depolarisation of the ventricle produced by the atrioventricular node; and the T wave arises from the repolarisation of the ventricle. The recorders were set to capture 10-second electrocardiograms every 10 seconds, meaning that the records were continuous, with no lost electrocardiographic samples between adjacent 10-second recordings. The tachograms describing the sequences of RR intervals, the time between two successive R waves of the QRS complex, were exported from SEER MC data files. Five minutes of beat-to-beat data were re-sampled at a frequency of 500 Hz to obtain digitised sequences of R waves. Electrocardiographic abnormalities, such as ectopic beats, right bundle-branch block, respiratory arrhythmia, blocked atrial extrasystole, and high-amplitude and wide T waves, were detected using an automatic algorithm, and normal QRS complexes adequate for HRV analyses were identified.

HRV was analysed in two domains: time and frequency. The standard deviation of normal-to-normal RR intervals (SDNN; milliseconds), as an estimate of the changes in heart rate due to cycles longer than five minutes, and the square root of successive differences of normal-to-normal RR intervals (RMSSD; milliseconds), as an estimate of the short-term changes in heart rate, were used to represent the time domain. Frequency-domain measures were the low-frequency (LF: 0.04 to 0.15 Hz) and high-frequency (HF: 0.15 to 0.4Hz) spectral power (milliseconds squared), which were computed using a Blackman-Tukey algorithm. In resting conditions, higher HF and larger RMSSD indicate greater parasympathetic control, while lower HF and shorter RMSSD reflect predominance of sympathetic activation.[161] The rHR, as beats per minute (bpm), was recorded simultaneously to the HRV measurement among participants who were assessed for HRV. In the remaining participants, rHR was measured from a standard 10-second 12-lead electrocardiograms by the Burdick Eclipse 850 ECG recorder.

Table 3-5. Biomarkers which represent the neuroendocrine and autonomic nervous systems in Whitehall II cohort study and National Child Development Study (NCDS)

	Whitehall II	NCDS
Neuroendocrine system		
Measure	Salivary cortisol	Salivary cortisol
Number of measures in the day	6	2
Timing	At waking, +30 min, +2.5 hours, + 8 hours, +12 hours, at bedtime	45 min after waking, +3.0 hours
Age at sample collection[a]	65.9 (6.0)	44/45
Derived measures	Cortisol secretion during the day (area under the curve), cortisol awakening response, diurnal slope	Not applicable
Autonomic nervous system		
Measure	12-lead electrocardiogram recordings	Not available
Frequency of measures	3	
Timing	5 years interval over 10 years	
Age at sample collection[a]	55.8 (6.0), 61.1 (6.0), 65.9 (5.9)	
Derived measures	SDNN, RMSSD, HF, LH, rHR	

[a] Mean age (standard deviation) is presented in the Whitehall II cohort study

3.4. Analytic samples and exclusion criteria

Table 3-6 presents a summary of exclusion criteria, and further details are described in each of the results chapters. For analyses, participants with complete information on all ACEs and relevant outcomes and other covariates were included. Participants were excluded from the analysis of CHD and HRV if they had a record of CHD at baseline, where baseline is wave 5 when the ACEs were collected. For the cortisol analysis, participants were excluded if they were taking corticosteroid medications, and for the Whitehall II cohort study only, were taking menopausal hormone therapy or had smoked on the day of saliva sampling. This information was not available in the NCDS.

Table 3-6. Exclusion criteria and follow-up

1. ACEs and incident CHD		
Data	*Exclusion*	*Study design*
Whitehall II	• Missingness in ACEs, CHD, sex, age, ethnicity, and childhood socioeconomic position • Prior episode of CHD at baseline	Longitudinal. Baseline is wave 5 when ACEs were assessed. Timescale of follow-up is in years.
2. ACEs and salivary cortisol		
Whitehall II	• Missingness in ACEs, all measures of salivary cortisol and time at sample collection, sex, age, ethnicity, childhood and adult socioeconomic positions • Intake of cortico-steroid medications • Taking menopausal hormone therapy • Smoking on the day of saliva sampling	Cross-sectional. Six measures over a day.
NCDS	• Missingness in all ACEs[a] • Intake of cortico-steroid medications	Cross-sectional. Two measures over a day.
3. ACEs and rHR and HRV		
Whitehall II	• Missingness in ACEs, all measures of rHR and HRV, sex, age, ethnicity, childhood and adult socioeconomic positions • Prior episode of CHD at baseline	Longitudinal. Baseline is wave 5 when ACEs were measured. Timescale of follow-up is in years.

ACEs: adverse childhood experiences; CHD: coronary heart disease; rHR: resting heart rate; HRV: heart rate variability

3.5. Statistical analysis

3.5.1. Statistical models

I used Cox proportional hazard regression to investigate the relationship between ACEs and incident CHD (study 1); multivariable regression and, for repeated outcome measures, multilevel linear regression to investigate associations between

ACEs and salivary cortisol (study 2), and between ACEs and rHR and HRV (study 3). I describe each statistical model used in my PhD in this section and provide further details of the specific models fitted in the relevant results chapters.

Cox proportional hazard regression

Cox proportional hazard regression, or simply Cox regression, is a type of survival analysis, which uses information on time until the event of interest for each person. Using Cox regression, it is possible to calculate the hazard, which is the instantaneous probability of the event occurring at a given time, conditional on already having survived that long in a particular group, and the ratio of hazards (hazard ratio) between groups. In addition the 95% confidence intervals for the hazard ratio may be obtained.[177]

The equation for a general Cox regression is written as;

$h(t) = h_0(t)\exp\left(b_1x_1 + b_2x_2 + \cdots + b_px_p\right)$, where h(t) is the expected hazard at time t; $h_0(t)$ is a baseline hazard function which is multiplied by an exponential function of the set of model covariates (b1,….,bp). The baseline hazard represents the hazard when all the covariates are equal to zero, and the covariates are assumed to have proportional effects on the expected hazard h(t). Thus, the hazard ratio, which is the ratio of the expected hazards between groups, does not depend on time t.

The hazard ratio is often interpreted as a risk ratio, but the main difference is the involvement of time. Risk ratios are estimates over a specific time period, while the hazard ratios are estimates of the instantaneous probability of the event occurring.

Multilevel model

Multilevel models, also known as hierarchical models, recognise a hierarchical or clustered data structure by allowing for residual components at each level in the hierarchy. For instance, a two-level model in which students (level 1) are nested within schools (level 2) would include residuals at the student and at the school level. The residual variance is divided into a between-school (inter) component, which is the variance of the school-level residuals, and a within-school (intra) component, which is the variance of the student-level residuals. The school-level residuals (level 2) constitute unobserved characteristics which lead to correlation between student outcomes (level 1) in the same school. The residuals are also termed random effects, while coefficients of the covariates, where covariates can be measured at any level in the hierarchy, are called fixed effects.

In the same way that students can be considered nested within schools, repeated measurements on the same individual can be considered as nested within an individual.

The simplest multilevel model for repeated measures can be written as;

$$y_{ij} = \beta_0 + u_j + e_{ij}$$

where y_{ij} is the outcome for measurement *i* in individual *j*; β_0 is the overall mean (fixed part); and u_j is the individual level (level 2) residual (random effects) and e_{ij} is the measurement level (level 1) residual (random effects). The model which allows the intercept and slope to vary across individuals, with x_{1ij} representing the time at which measurement *i* in individual *j* is recorded, is written as;

$$y_{ij} = \beta_{0j} + \beta_{1j} x_{1ij} + e_{ij}$$

where $\beta_{0j} = \beta_0 + u_{0j}$, $\beta_{1j} = \beta_1 + u_{1j}$

, Hence:

$$y_{ij} = (\beta_0 + u_{0j}) + (\beta_1 + u_{1j})x_{1ij} + e_{ij}$$

$$y_{ij} = (\beta_0 + \beta_1 x_{1ij}) + (u_{0j} + u_{1j}x_{1ij}) + e_{ij}$$

Both fixed effects and the variation in random effects may be of interest depending on the research question. Further covariates can then be added as fixed effects. Whatever the interest, the use of a multilevel model is appropriate to obtain unbiased estimates of both fixed effects and random effects.

3.5.2. Average effect size by subgroups using prediction

As described in Chapter 1, one of the challenges in studying ACEs is how to deal with multiple ACEs in analyses. In my PhD, ACEs are exposures in all studies, and I used an approach which allows me to obtain estimation by the count of ACEs without using an “ACEs score”, that is a sum of the number of ACEs as described in Chapter 1. Both approaches are based on the assumption that none of the ACEs are on the same causal pathway (i.e., no ACE is on the causal pathway between another ACE and the outcome).

Figure 3-1 shows a diagram of the model using the ACEs score, assuming that all ACEs are collected as binary variables (i.e., Yes/1 or No/0) and that the ACEs score is a continuous variable. In this model, no matter what type of experience, all ACEs have an effect of either zero or one, when calculating a score. Then, a regression coefficient, β, for a continuous ACEs score (or β1, …, βx if the score is divided into x categories) are computed (in the diagram, the model for a continuous ACE score is presented).

Figure 3-1. A model using ACEs score

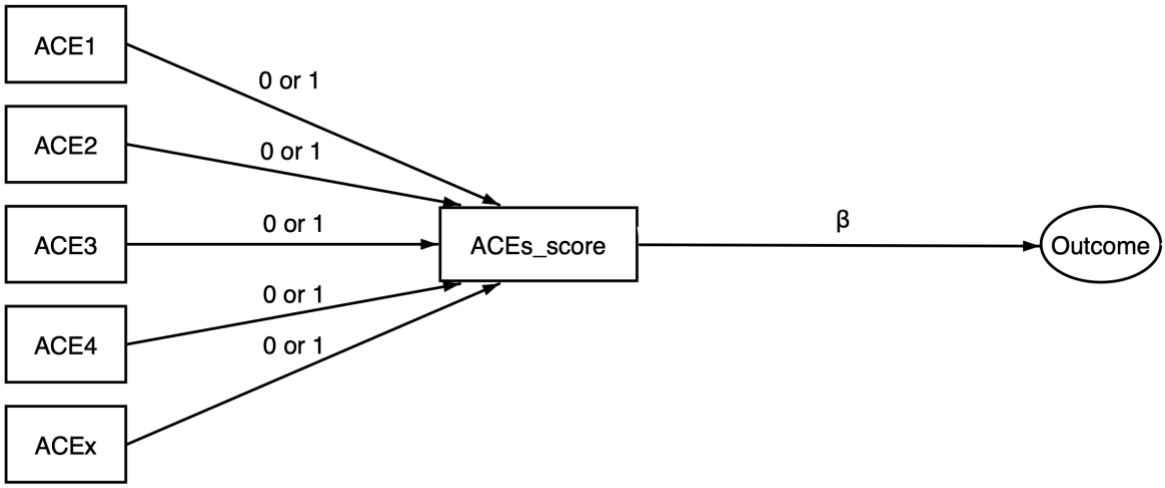
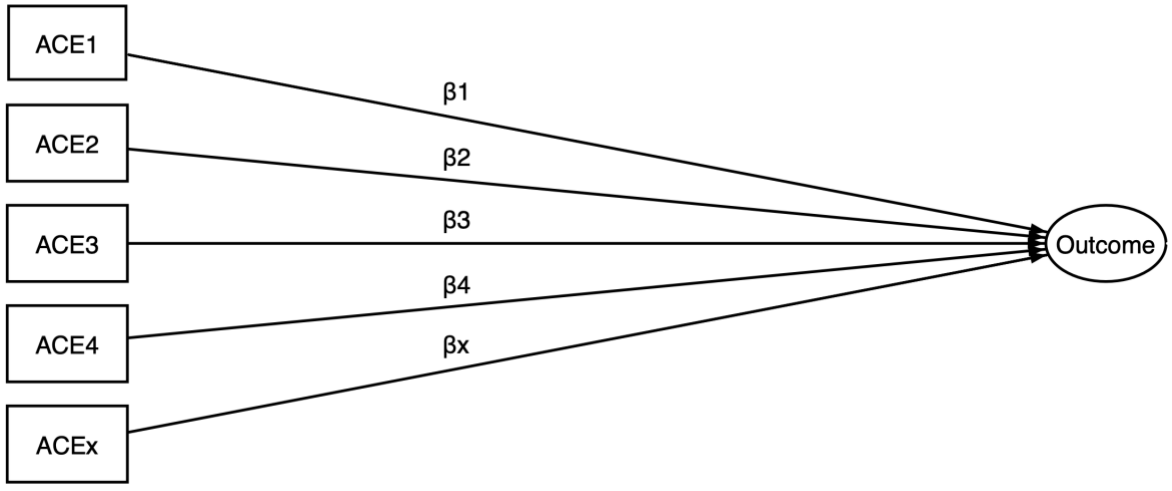


Figure 3-2 illustrates how I modelled ACEs. The approach includes variables representing all ACEs in the model simultaneously. This approach allows each ACE of x ACEs to have a different effect size (β1, β2, β3, β4...βχ in the diagram).

Figure 3-2. A model in my PhD



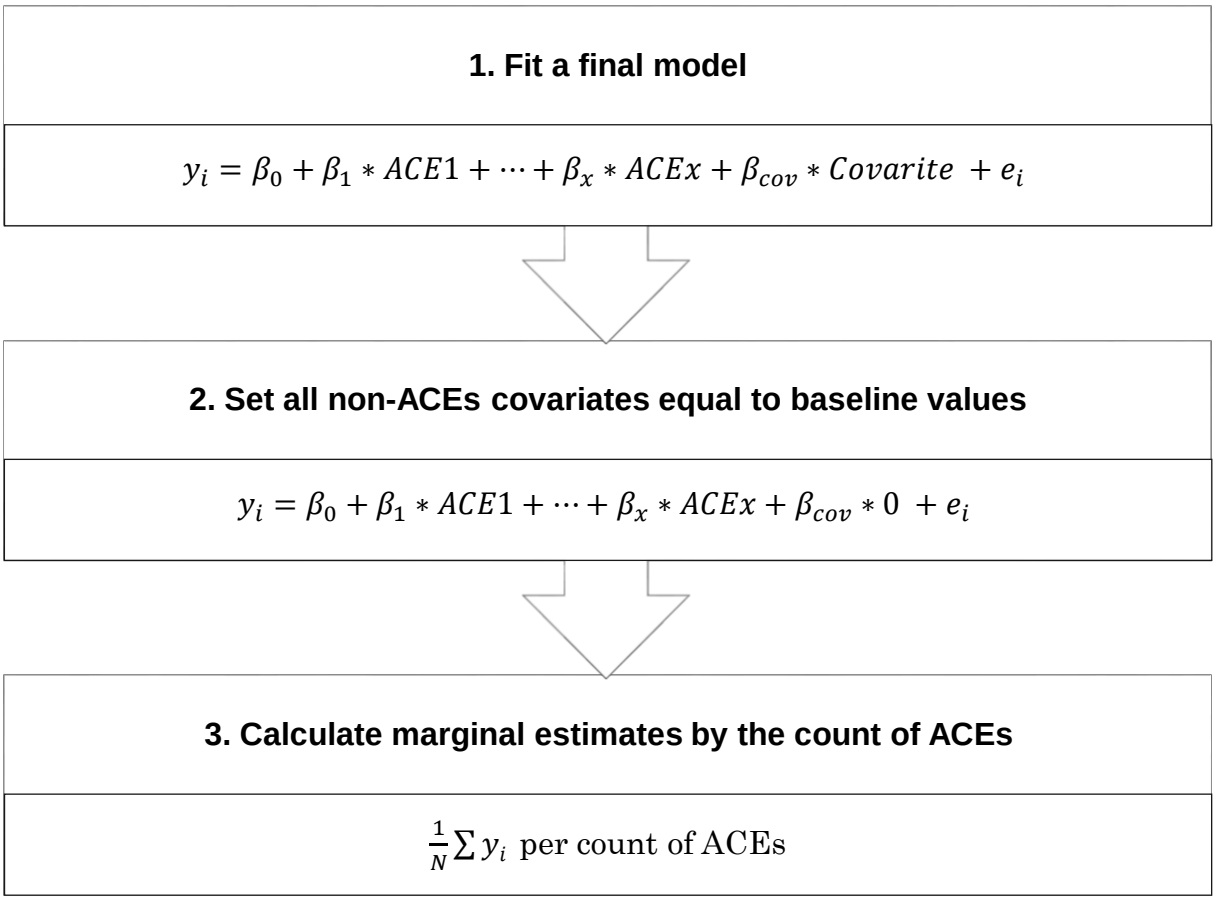
However, it is then not straightforward to assess whether there is a cumulative association between increasing number of ACEs and the outcome, since individuals with the same number of ACEs will have different combinations and therefore have

different predicted outcomes. Using the final model in which all ACEs are included, adjusted for confounders and other covariates (see 1 in Figure 3-3), I obtained marginal estimates within sub-groups with the same count of ACEs in order to see if there was a dose-response relationship.

After fitting a final model, I set all covariates, apart from the ACEs, equal to their baseline value (see panel 2 in Figure 3-3). I then calculated the marginal estimates which average the individuals' predicted values according to the count of ACEs (see panel 3 in Figure 3-3). The standard errors can also be calculated. To obtain the observed count of ACEs for each individual, the worst quartile or worst score on the Likert scale for ordinal ACEs was counted as 1 (yes) with all other responses coded as 0 (no). However, to account for severity when estimating the marginal effects, I coded the worst quartile as the difference from the highest score in the third quartile.

I then assessed whether there was a dose-response effect between the number of ACEs and the outcome. This estimate is not straightforward in the statistical software package, Stata. I therefore sought advice from a statistician who provided an approach to assess the linear trend. Further details of this approach are described in Appendix 3. This approach is, however, not applicable to multilevel models. Therefore, I was unable to calculate a dose-response effect for one analysis in Chapter 6 and all analyses in Chapter 7.

Figure 3-3. Procedure of marginal effects calculation



3.6. Conclusion

This chapter summarised the overall methodology in my PhD, while further specific details are given in each of the results chapters. As described above, the main cohort study used in my PhD is the Whitehall II cohort study. Despite some limitations, particularly that participants are all from non-manual occupations implying a possible healthy worker effect, and that ACEs are retrospectively measured, the Whitehall II cohort study has many strengths in relation to my research questions. A major strength is the availability of high-quality outcome measures: objectively measured incident CHD and multiple measures of salivary cortisol so that characteristics of the daily patterns can be derived; and measurements of HRV rather than just resting heart rate. The measures of HRV are also assessed three

times so that the effect of ACEs on change can be assessed. The large sample size means that precise estimates of associations can be obtained. I use NCDS to provide insight into the extent of potential bias due to retrospective collection of ACEs by comparing the findings for salivary cortisol from the Whitehall II cohort study with those drawn from NCDS, a population-based study with prospectively measured ACEs.

Chapter 4 Attrition in a longitudinal cohort study

The work in this chapter was based on the research question posed in my MSc dissertation. After submitting the dissertation, and as part of my PhD, I carried out a further literature review; re-planned the statistical analysis; applied for additional data; performed a new analysis applying a more appropriate statistical model; developed the discussion; and wrote the manuscript for submission to a journal. I am entirely responsible for this work. This work has been published in the Journal of Epidemiology and Community Health.[178]

4.1. Introduction

Long-term longitudinal cohort studies, such as those used in my thesis, are affected by gradual attrition due to withdrawal from the study, non-response to particular waves of data collection, and death.[179] I conducted an initial study to investigate to enable better understanding of the possible bias due to attrition in the Whitehall II cohort study. Previous analysis of the Whitehall II cohort study over waves 1 to 6 has shown differences in characteristics of participants when distinguishing between response, non-response (non-response among participants continuing in the study), or withdrawal (formal discontinued participation). In addition, previous studies have found that survey non-response is related to higher mortality rates. Therefore, understanding how non-response is associated with mortality, and CVD mortality as CVD is the focus of my research, in a longitudinal context in Whitehall II is important when considering the impact of attrition on subsequent analyses in my thesis.

Response rates in studies have generally declined over the past four decades, possibly because of increased burden on participants (e.g., increase in the number

of studies, more extensive and time-consuming questionnaires, biological sampling, the requirements of participants' consent). One challenge is to ensure that inferences drawn from a reduced sample are applicable to the members of the intended study population (internal validity).[180,181] If some study participants do not respond and they have systematically different characteristics from those who do, then estimated effects among the responders may not pertain to the original study population.[182,183] [180,181]

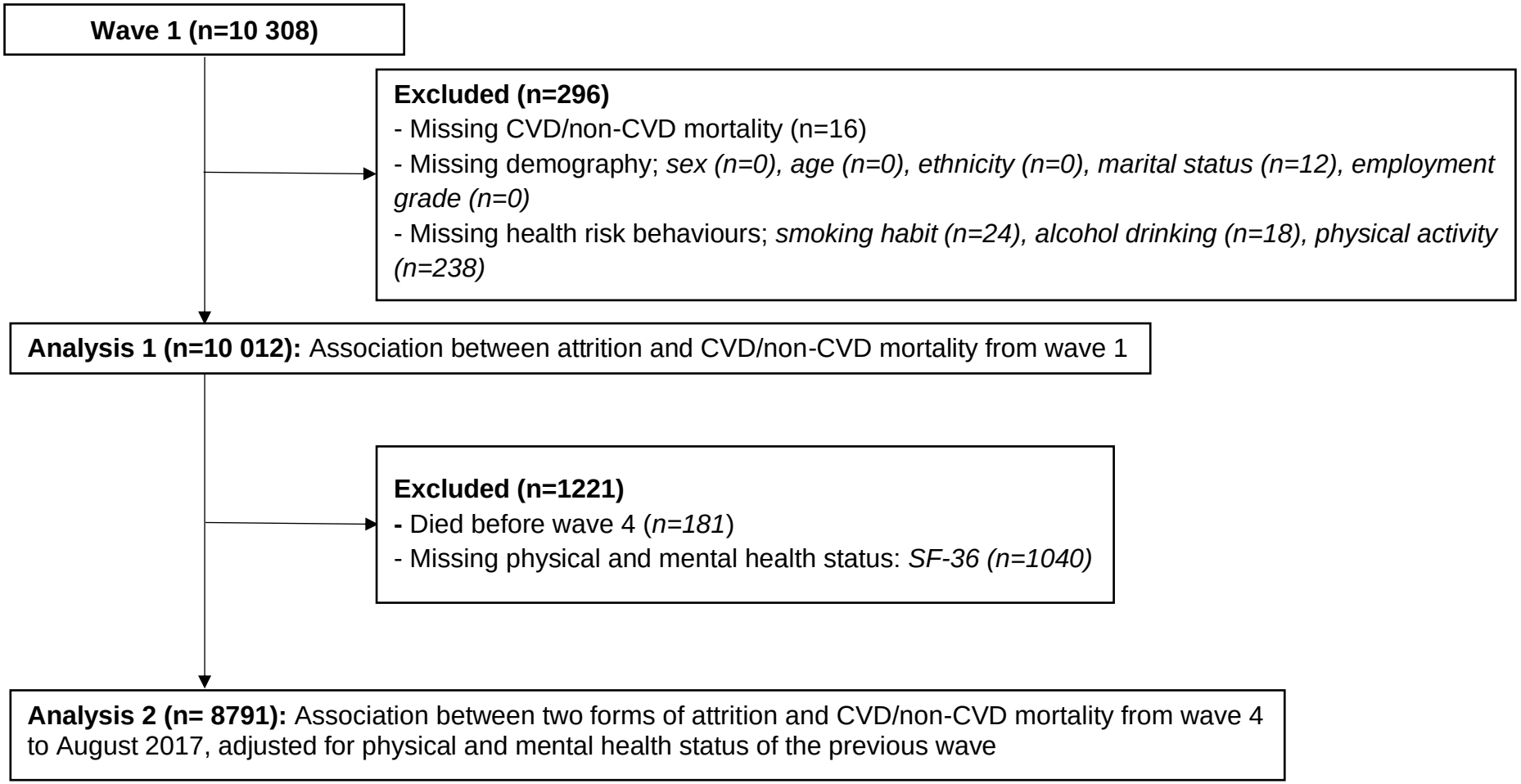
Multiple studies have investigated the characteristics of non-responders in order to understand predictors of non-response. For instance, previous studies have found that those who drop out from studies are more likely to be men,[184-186] be young or old people,[187,188] be single,[184,189] be in a lower employment grades,[190,191] have adverse smoking or alcohol drinking habits,[192,193] have greater cognitive impairment,[186,194] and have worse health.[190,195] Previous analysis of the Whitehall II cohort study over waves 1 to 6 found that compared to responders, those who withdrew from the study were more likely to be older, male, in lower employment grade, participate in fewer social activities, and have poorer general physical and mental health status, while non-responders tended to be older, male, have a tertiary level of education, be in lower employment grade, retired, not be a home-owner, and not have a long-standing illness, and among women they were more likely to be married but among men not married.[184] Thus non-responders and those who withdrew have different characteristics. Although both groups were more socially disadvantaged than those remaining in the study, those who withdrew but not non-responders were in poorer health.

Population-based studies linked with electronic health records do suggest that non-response is associated with an approximate doubling of the risk of mortality.[192,196-198] These studies considered response status at only a single time point and made no distinction between withdrawals and non-responders, who may have different characteristics and thus different mortality risks. It is therefore unclear whether the association of attrition with higher mortality applies only to non-responders at baseline, or whether the association persists and applies to all waves of data collection in longitudinal studies. If there were a difference across waves in the risk of mortality in responders compared to the risk in those lost due to attrition even after adjustment for measured factors such as age, it would suggest that differences in unmeasured risk factors between responders and those lost due to attrition change from wave to wave. That is, it is a sign that sources of bias change wave to wave.[199] As cardiovascular mortality is a major cause of death and the focus of my work, I also investigated the association between attrition and cardiovascular mortality. Accordingly, in the Whitehall II cohort study, I aimed to (i) examine the extent to which response status at each wave is associated with cardiovascular and non-cardiovascular mortality up to the following wave; (ii) investigate whether the hazard of mortality differs between two forms of attrition: withdrawal, and non-response; and (iii) assess whether there is variation across waves in the association between attrition and mortality.

4.2. Methods

I included 10 012 participants who responded at baseline and who had no missing values in sociodemographic variables and health risk behaviours and mortality from the Whitehall II cohort, the main cohort study I used in my PhD (Figure 4-1).

Figure 4-1. Flow chart of participants' recruitment



Variables

Response status

For each study participant at each wave, “response” was defined as being when the participant either completes the self-administered questionnaire (even numbered waves) or attends the medical examinations (odd numbered waves). “Withdrawal” was defined as when the participant officially informs the study research team that they wish to permanently leave the study, and “non-response” as when the participant (who has not formally withdrawn from the study) does not respond (i.e., does not complete the questionnaire or attend the medical examination). Participants who have withdrawn from the study are not contacted again at subsequent waves, whereas non-responders are re-contacted and could participate at later waves. Non-response is defined among those known to be alive. I term either withdrawal or non-response as “attrition”, and “response status” as comprising response and attrition. Prior to wave 4 it is not possible to distinguish withdrawal from non-response due to the way that information was collected. I therefore conducted two analyses. In analysis 1, I used all waves from wave 1 and considered attrition (i.e., withdrawal or non-response combined) and in analysis 2 I analysed data from wave 4 onwards, using all three categories of response status (i.e., withdrawal, non-response, response). Reasons for withdrawal and non-response were not available.

Mortality

CVD and non-CVD mortality were obtained through the National Health Services (NHS) central registry. CVD mortality includes CHD, angina, myocardial infarction, and stroke. Mortality was tracked from wave 1 to August 2017 in 10 292 participants (99.8%), with mean follow-up of 28.7 years (standard deviation: 5.1 years). CVD

mortality was based on International Classification of Disease (ICD)-9 (codes 390-459) and 10 (codes I00 - I99). Non-CVD mortality included cancer (ICD-9: 140-239; 10: C00-C97), and respiratory mortality (ICD-9: 460-519; 10: J00-J99) and any other cause not classified as CVD mortality.

Covariates

Sociodemographic characteristics

Participants' sex, age in years, ethnicity (white vs. non-white), marital status (married/cohabiting, single, divorced/widowed) and employment grade are all associated with health[8] and were taken from the first wave of the study. Employment grade was categorised as "administrative" (high grade), "professional/executive" (intermediate grade), and "clerical/support" (low grade). Information on sex, age, and employment grade at wave 1 was known for all participants. Missing values in ethnicity and marital status were replaced, where known, with responses from the wave 5 and wave 2 questionnaires, respectively.

Health risk behaviours

Health behaviours were taken from participants' questionnaire responses at wave 1. Smoking habit (never-smoker, ex-smoker, and current-smoker), alcohol drinking (<14 units per week and ≥14 and over units per week), and leisure-time physical activity (high, intermediate, low) were included. Physical activity was assessed using questions about the frequency and duration of participation per week in moderately energetic (e.g., dancing, cycling, leisurely swimming), and vigorous physical activity (e.g., running, hard swimming, playing squash). The "high" physical activity included ≥3 hours of vigorous activity, ≥6 hours of moderate and 2 to 3 hours of vigorous

activity, and 6 hours and more of moderate activity; the “intermediate” level is <6 hours of moderate and <3 hours of vigorous activity; the “low” physical activity is <3 hours moderate and <2 hours vigorous activity. Missing values were replaced with those obtained from waves 2 and 3. The cut-off points for alcohol consumption and physical activity were determined in line with the NHS guideline.[200]

General health status

The 36-item Short Form Health Survey (SF-36) Physical Component Score (PCS) and Mental Component Score (MCS) were included. The PCS is derived from; general health perceptions (5 items), physical functioning (10 items), role limitations due to physical functioning (4 items), and bodily pain (2 items). The MCS is derived from; vitality (4 items), general mental health (5 items), role limitations due to emotional problems (3 items), and social functioning (2 items). Higher scores represent better health. The PCS and MCS are not available prior to wave 3 and were therefore omitted from analysis 1. In analysis 2 PCS and MCS from the wave previous to the wave of analysis (e.g., PCS at wave 5 for the analysis of response status at wave 6) were used. Missing PCS and MCS values were replaced using the last known measurement carried forward. The PCS and MCS were categorised using wave- and sex-specific quartiles.

Statistical methods

Individual response rate across all waves of the study was calculated as the number of waves in which they did respond divided by the number of waves that they could

have responded to while still alive.[201] The mean response rate and 95% confidence interval (CI) was calculated by levels of each covariate.

Competing-risks analysis, a type of survival analysis to estimate the probability of an event in the presence of competing events,[202] was used to assess the association of attrition status (analysis 1) or response status (analysis 2) at each wave as a time-varying exposure with subsequent mortality. The time scale used was study wave. Analysis 2 addresses the primary question of interest as it separates withdrawal from non-response. The sub-distribution hazard ratios (SHRs) and 95% CIs of CVD mortality were estimated with non-CVD mortality as a competing risk. Similarly, those SHRs for non-CVD mortality were estimated with CVD mortality as a competing risk. Interaction terms between attrition/response status and sex, age, and employment grade, were included in models to assess whether these factors modified associations between attrition/response status and mortality. Also, to investigate whether SHRs showed evidence of a trend across waves point estimates of SHRs were regressed against wave. I conducted two analyses as follows (Figure 4-1).

Analysis 1: A total of 10 012 participants with complete information on all covariates were included to investigate the association of attrition status with CVD and non-CVD mortality from wave 1 up to August 2017, adjusted for sex and age, and finally additionally adjusting for marital status, ethnicity, employment grade, smoking, alcohol drinking, and physical activity.

Analysis 2: I included participants who had responses in both PCS and MCS from at least one wave between wave 3 and wave 11. Therefore 8791 participants were included in analyses of the association of response status with CVD and non-CVD

mortality, from wave 4 up to August 2017, with covariate adjustments as in analysis 1, with the addition of PCS and MCS from the previous wave as time-varying covariates.

Likelihood ratio tests were used to examine whether the estimated risks of mortality differed across the two forms of attrition by comparing models of attrition status with models of response status in analysis 2.

I conducted sensitivity analyses by repeating analysis 1 using person-years, rather than wave, as the time scale. Findings drawn from the main analysis are easy to interpret due to its use of wave as the time scale, but the estimates are less accurate in terms of time to event. I therefore perform this sensitivity analysis to compare the estimates with those of the main analysis.

4.3. Results

The response status of the 10 308 participants at each wave is given in Table 4-2. The attrition rate was between one fifth and one third of the eligible study population (those who had not died) at each wave except at waves 3 and 4 when large number of participants were recontacted. The proportion of deaths attributable to CVD rose, then fell, with increasing age.

In analysis 1, of the 10 012 participants who had no missing values in covariates men made up 67.4% of the sample. Table 4-2 shows the participants' response rates (the proportion of waves attended by a participant) according to study characteristics. Response rates were higher in men (with an average of 81.9% of

waves attended) than women (74.0%), and showed a trend across employment grade, being highest in the highest grade (86.1%) and lowest in the lowest grade (66.2%). Non-white participants showed lower response rate (65.8%) than white participants (80.9%).

Table 4-3 shows the association between attrition status and CVD and non-CVD mortality. There were 495 deaths recorded from CVD and 1367 deaths from non-CVD causes. Compared to responders, participants with attrition had 1.55 (95% CI 1.26 to 1.89) times the hazard of CVD mortality after adjustment for sex, age, ethnicity, marital status, employment grade, smoking habit, alcohol drinking, and physical activity. For non-CVD mortality, the hazard ratio was 1.56 (1.39 to 1.76). The association between attrition and mortality was not modified by sex, age, or employment grade.

Table 4-4 shows the SHRs and 95% CIs for the association between attrition and CVD and non-CVD mortality from each wave to the following wave, covering, on average, a period of three years. The estimates for CVD mortality do vary across waves from 0.84 (95% CI: 0.38 to 1.86) at the most recent wave to 3.39 (1.35 to 8.53) at the earliest. However, there was no evidence of a trend in the point estimates of the SHRs across the waves (p-value = 0.11) although the 95% confidence intervals on some estimates were wide. Similarly, for non-CVD mortality, although there was some variation in SHR by wave, from 2.18 at wave 2 to 1.27 at waves 8 and 12, there was no evidence of a linear trend (p-value = 0.61). Sensitivity analyses using person-years, rather than wave, showed the same pattern of results, but with all the SHRs being slightly reduced (Table 4-5).

Among 8791 participants in analysis 2, there were 353 deaths recorded from CVD and 1056 deaths from other causes. Figure 4-2 shows the cumulative incidence function (CIF) for CVD and non-CVD mortality from wave 4 for each response category. For CVD mortality, the curves for the CIF for non-response and withdrawal diverged, with the CIF for withdrawal being lower than that for non-response, although it was still higher than for responders. In contrast, for non-CVD mortality the CIF for non-response and withdrawal were very similar with both being higher than the CIF for responders. The association of response status with mortality is shown graphically in Figure 4-3 and presented in Table 4-6. The adjusted SHR for CVD mortality was slightly greater for non-response than for withdrawal, while the reverse was true for non-CVD mortality. Sociodemographic and health risk behaviours attenuated the association of response status with CVD mortality, but there was little change in estimates for non-CVD mortality after adjustment (Table 4-6). Comparing the model with two types of response status (response or attrition) and the model with three types of response status (response, withdrawal, or non-response) using likelihood ratio tests suggested no evidence that the differentiation of two types of attrition improved the fit of the models for either CVD (p-value = 0.28) or non-CVD mortality (p-value = 0.38). Estimates from the full models are given in Appendix 1 and Appendix 2.

Table 4-1. Response status and cumulative death (CVD, all-cause) at each wave

Wave	Period	Participants (responders)	Attrition[a]			Cumulative CVD death (%) [d]	Cumulative all-cause death
			Cumulative Withdrawal (%)[b]	*Non-response (%)[b]*	*Total (%)[c]*		
1	1985-1988	**10 308**	-	-	-	-	-
2	1989-1990	**8132**	2127 (20.7)[e]		**2127 (20.7)**	14 (28.6)	**49**
3	1991-1994	**8815**	1368 (13.4)[e]		**1368 (13.4)**	36 (28.8)	**125**
4	1995-1996	**8628**	774 (52.4)	712 (47.6)	**1486 (14.7)**	59 (30.4)	**194**
5	1997-1999	**7870**	882 (41.3)	1250 (58.7)	**2132 (21.3)**	95 (31.0)	**306**
6	2001	**7355**	975 (38.7)	1553 (61.3)	**2528 (25.6)**	132 (31.1)	**425**
7	2002-2004	**6967**	1246 (45.2)	1511 (54.8)	**2757 (28.4)**	176 (30.1)	**584**
8	2006	**7173**	1310 (55.5)	1051 (44.5)	**2361 (24.8)**	226 (29.2)	**774**
9	2007-2009	**6761**	1354 (52.2)	1239 (47.8)	**2593 (27.7)**	271 (28.4)	**954**
11[f]	2012-2013	**6308**	1389 (53.7)	1197 (46.3)	**2586 (29.1)**	405 (28.6)	**1414**
12	2015-2016	**5632**	1433 (49.7)	1448 (50.3)	**2881 (33.8)**	485 (27.0)	**1795**
					Deaths to August 2017	*519 (26.7)*	***1943***

[a] Deaths are displayed separately from attrition (non-response or withdrawal)
[b] % of each attrition = [withdrawal or non-response / total attrition at each wave] * 100
[c] % attrition = [total attrition at each wave / (10308 - cumulative deaths at each wave)] * 100
[d] % CVD death = (CVD death / all-cause death) * 100
[e] Only pooled attrition is available at waves 2 and 3
[f] Wave 10 was a small pilot study of measures to be included at wave 11, and has not been included here

Table 4-2. Characteristics of study population (n=10 012)

	n (%)	**Individual response rate (95%CI)[a]**
Sex		
Men	6749 (67.4)	81.9 (81.7-82.2)
Women	3263 (32.6)	74.0 (73.6-74.5)
Age in years		
39 and below	2750 (27.5)	79.9 (79.5-80.4)
40 – 44	2607 (26.0)	80.0 (79.6-80.5)
45 – 49	2031 (20.3)	78.8 (78.2-79.3)
50 and over	2624 (26.2)	78.5 (78.0-79.0)
Ethnicity		
White	8968 (89.6)	80.9 (80.7-81.2)
Non-white	1044 (10.4)	65.8 (64.9-66.7)
Marital status		
Married/cohabit	7435 (74.3)	80.7 (80.4-81.0)
Single	1640 (16.4)	76.3 (75.7-77.0)
Divorced/widowed	937 (9.4)	74.0 (73.1-74.9)
Employment grade		
High	2979 (29.8)	86.1 (85.7-86.4)
Intermediate	4837 (48.3)	81.1 (80.7-81.4)
Low	2196 (21.9)	66.2 (65.5-66.8)
Smoking habit		
Never-smoker	4966 (49.6)	80.7 (80.4-81.1)
Ex-smoker	3225 (32.2)	81.3 (80.9-81.7)
Current smoker	1821 (18.2)	71.8 (71.1-72.4)
Alcohol drinking		
<14 units per week	7338 (73.3)	78.4 (78.2-78.7)
≥14 units per week	2674 (26.7)	81.9 (81.5-82.4)
Physical activity		
High	2175 (21.7)	80.9 (80.4-81.4)
Intermediate	2620 (26.2)	80.9 (80.5-81.4)
Low	5217 (52.1)	77.9 (77.6-78.3)

[a] Response rate = [number of waves responded / number of waves that it was possible to attend while still alive]*100

Table 4-3. Sub-distribution hazard ratios (SHRs) of CVD and Non-CVD mortality from wave 1 to August 2017, by attrition status[a] (n=10 012)

			SHR (95% CI)			
			Adjusted for			
Outcome	**Attrition status**	No. deaths	*Sex and Age*		*All factors[b]*	
CVD mortality		*495*				
	Response	312		*ref.*		*ref.*
	Withdrawal/Non-response	183	1.86	(1.53-2.24)	1.55	(1.26-1.89)
Non-CVD mortality		*1367*				
	Response	873		*ref.*		*ref.*
	Withdrawal/Non-response	494	1.62	(1.45-1.82)	1.56	(1.39-1.76)

[a] Attrition status is time dependent and varies at each wave of the study

[b] Adjusted for sex, age, ethnicity, marital status, employment grade, smoking habit, alcohol drinking, and physical activity

Table 4-4. Association of attrition status at each wave with CVD and Non-CVD mortality up to the subsequent wave. (Analysis 1)

Wave	Response status	No. alive	CVD mortality		Non-CVD mortality	
			No. deaths	SHR (95% CI)[a]	No. deaths	SHR (95% CI)[a]
1	**Responders**	10 012	12	-	29	-
2	**Responders**	8024	12	*ref.*	35	*ref.*
	Withdrawal/Non-response	1947	9	3.39 (1.35-8.53)	18	2.18 (1.24-3.84)
3	**Responders**	8647	20	*ref.*	38	*ref.*
	Withdrawal/Non-response	1250	3	1.30 (0.38-4.40)	5	0.89 (0.35-2.26)
4	**Responders**	8462	25	*ref.*	57	*ref.*
	Withdrawal/Non-response	1369	9	2.38 (1.11-5.11)	15	1.53 (0.85-2.73)
5	**Responders**	7723	23	*ref.*	51	*ref.*
	Withdrawal/Non-response	2002	9	1.65 (0.75-3.63)	24	1.86 (1.14-3.04)
6	**Responders**	7231	28	*ref.*	69	*ref.*
	Withdrawal/Non-response	2387	15	1.59 (0.84-3.01)	41	1.77 (1.21-2.61)
7	**Responders**	6855	27	*ref.*	77	*ref.*
	Withdrawal/Non-response	2610	22	2.29 (1.29-4.07)	62	2.00 (1.43-2.80)
8	**Responders**	7054	28	*ref.*	92	*ref.*
	Withdrawal/Non-response	2223	16	1.74 (0.95-3.18)	38	1.27 (0.87-1.85)
9	**Responders**	6655	73	*ref.*	183	*ref.*
	Withdrawal/Non-response	2448	55	1.99 (1.40-2.84)	133	1.88 (1.51-2.34)
11	**Responders**	6213	43	*ref.*	178	*ref.*
	Withdrawal/Non-response	2446	36	1.84 (1.15-2.93)	112	1.47 (1.17-1.85)
12[b]	**Responders**	5551	21	*ref.*	64	*ref.*
	Withdrawal/Non-response	2739	9	0.84 (0.38-1.86)	46	1.27 (0.86-1.86)
P-value for linearity				*P=0.11*		*P=0.61*

[a] Adjusted for sex and age; [b] Mortality follow-up from wave 12 is up to August 2017

Table 4-5. Sub-distribution hazard ratios (SHRs) of CVD and Non-CVD mortality from wave 1 to August 2017, by attrition status[a] in 10 012 participants (person years as time-scale)

Outcome	Attrition status	No. deaths	SHR (95% CI) Adjusted for Sex and Age		SHR (95% CI) Adjusted for All factors[b]	
CVD mortality		*495*				
	Response	312	*ref.*		*ref.*	
	Withdrawal/Non-response	183	1.76	(1.45-2.13)	1.46	(1.20-1.79)
Non-CVD mortality		*1367*				
	Response	873	*ref.*		*ref.*	
	Withdrawal/Non-response	494	1.54	(1.38-1.73)	1.48	(1.32-1.67)

[a] Attrition status is time dependent and varies at each wave of the study
[b] Adjusted for sex, age, ethnicity, marital status, employment grade, smoking habit, alcohol drinking, and physical activity

Table 4-6. Sub-distribution hazard ratios (SHRs) and 95% confidence interval (CIs) of CVD and non-CVD mortality by response status from wave 4 to August 2017 in 8791 participants (Analysis 2)

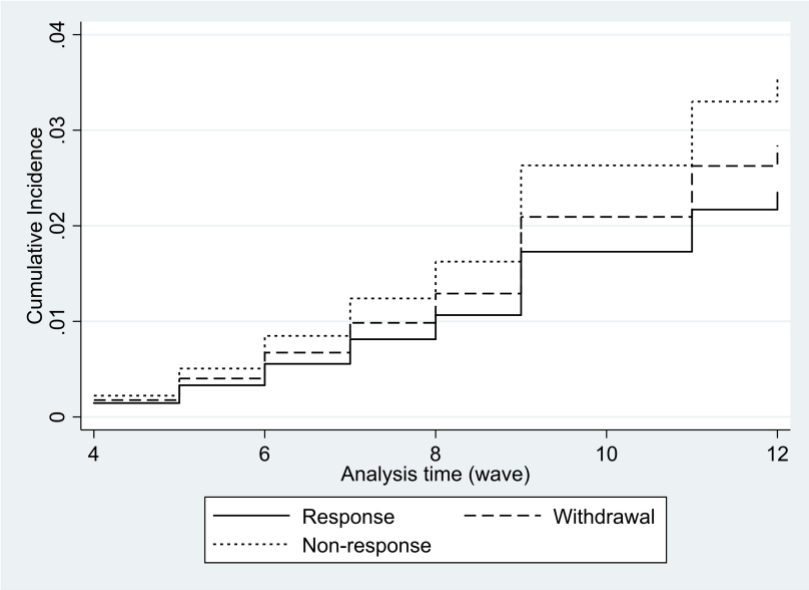
Outcome	Response status	No. Deaths	SHR (95% CI) *Sex and Age*	*p-value*[a]	SHR (95% CI) *Adjusted for +Demography and health risk behaviours*[b]	*p-value*[a]	SHR (95% CI) *+General health status*[c]	*p-value*[a]
CVD mortality		*353*						
	Response	258	*ref.*		*ref.*		*ref.*	
	Withdrawal	33	1.28 (0.89-1.84)	*0.102*	1.14 (0.79-1.65)	*0.218*	1.21 (0.84-1.75)	*0.284*
	Non-response	62	1.82 (1.37-2.41)		1.49 (1.10-2.01)		1.53 (1.13-2.06)	
Non-CVD mortality		*1056*						
	Response	748	*ref.*		*ref.*		*ref.*	
	Withdrawal	136	1.75 (1.46-2.11)	*0.617*	1.72 (1.43-2.08)	*0.593*	1.77 (1.47-2.13)	*0.377*
	Non-response	172	1.65 (1.40-1.95)		1.62 (1.36-1.92)		1.59 (1.34-1.89)	

[a] P-value of Likelihood Ratio Test between the model with attrition status (response and withdrawal/non-response) and response status (response, withdrawal, non-response)

[b] Additionally adjusted for ethnicity, marital status, employment grade, smoking habit, alcohol drinking, and physical activity

[c] Additionally adjusted for PCS and MCS from each wave

Figure 4-2. Cumulative incidence function of CVD and Non-CVD mortality by response status (left; CVD mortality, right; non-CVD mortality)



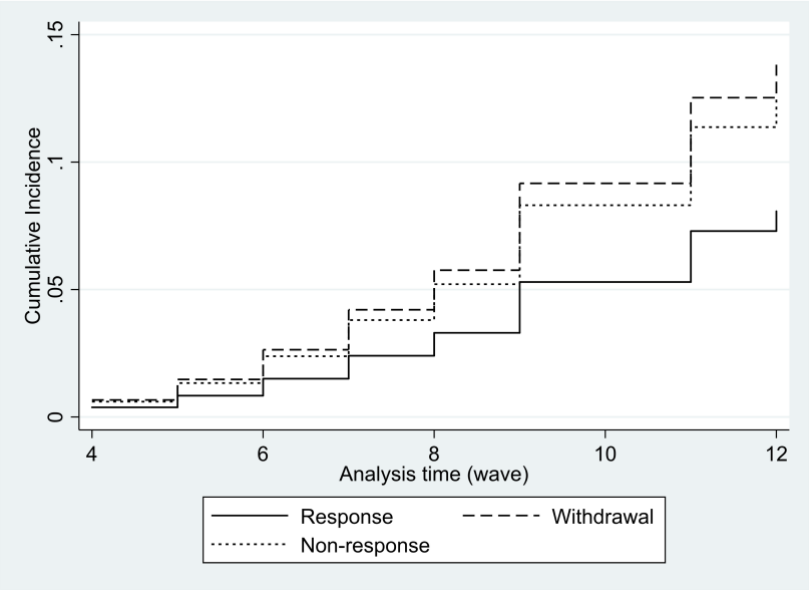
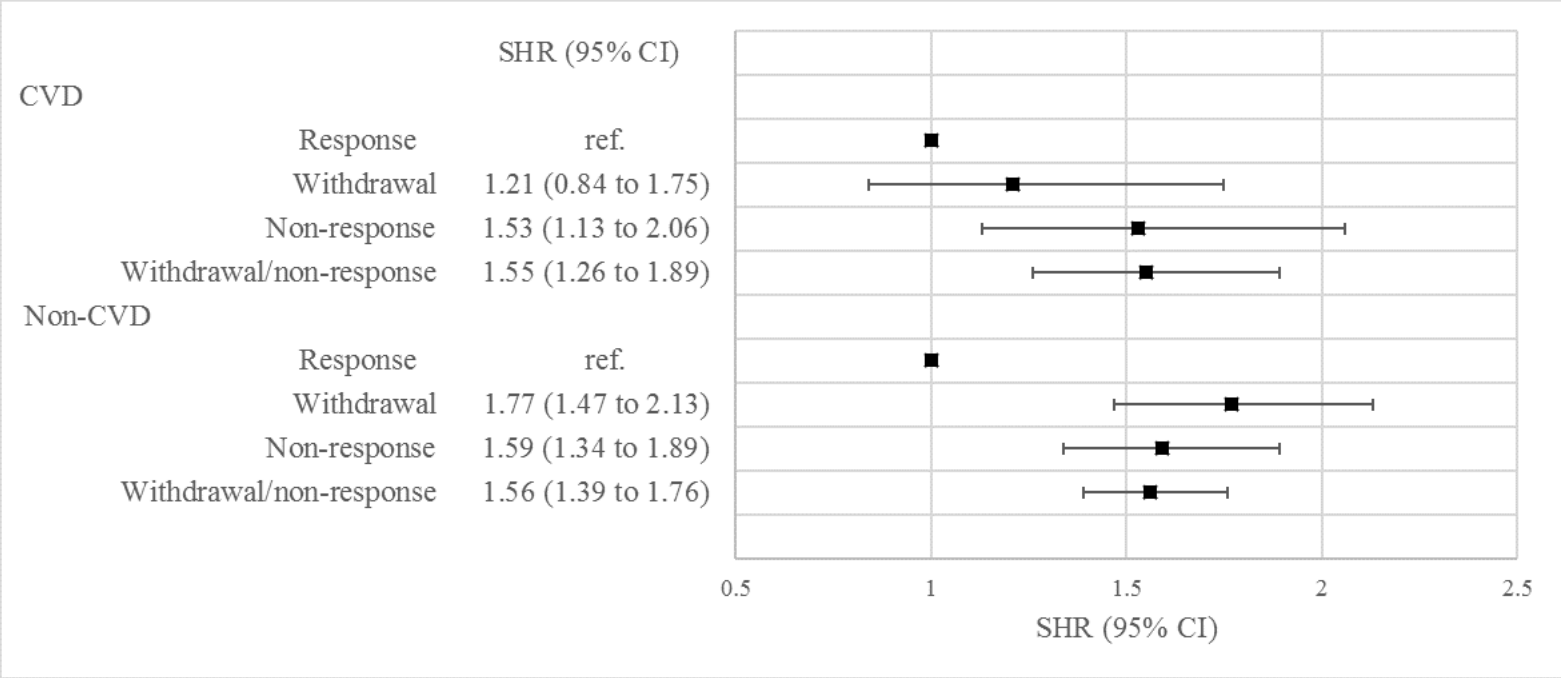


Figure 4-3. Sub-distribution Hazard Ratios (SHRs)[a] and 95% Confidence Intervals (CIs) of CVD and Non-CVD mortality by response status



[a] SHRs of *withdrawal/non-response* are based on 10 012 participants (analysis 1), adjusted for sex, age, ethnicity, marital status, employment grade, smoking habit, alcohol drinking, and physical activity. SHRs of *withdrawal* and *non-response* are based on 8791 participants (analysis 2), adjusting as in analysis 1 with the addition of PCS and MCS.

4.4. Discussion

In the Whitehall II cohort study, compared to responders, attrition after study baseline is associated with approximately 1.5 times higher hazard of mortality for both CVD and non-CVD mortality after adjustment for covariates. There is no evidence of a difference in the hazard between withdrawal and non-response and the association of attrition with mortality does not vary across waves.

The estimated association between attrition and mortality is slightly weaker than reported in previous studies. These previous studies, including one in the Whitehall II cohort study, have reported a doubling of the hazard of mortality in those with attrition compared to responders.[192,196,197] The majority of studies, including the one in the Whitehall II cohort study, used response status at baseline only, not during follow-up. It may be that at baseline, non-responders had a particularly high hazard of mortality.[192,196,197,203] The lack of difference between the hazard of withdrawal and non-response suggests that reasons for attrition in the two groups may be similar. The associations of response status with CVD mortality were attenuated with adjustment for sociodemographic factors and health risk behaviours, consistent with the previous studies[184-194,204-206] as these factors are associated both with non-response and mortality. Morbidity is also a potential predictors of attrition as some, [190,195,207] but not all, [184] of the studies in the existing literature document that those who have an illness are more likely to be lost to follow-up than those who do not. However, inclusion of physical and mental status using the SF-36 from the previous wave in my analysis, did not attenuate the association. It may be that severity of illness, whether an illness is acute or chronic, or the existence of psychological

illness, rather than general health status may have been more likely to explain the association.

The association between response status and subsequent mortality is clearly not causal; however, as my analysis shows, response status predicts mortality in later waves in the Whitehall II cohort study. This implies that the internal and external validity of analyses within the cohort may be affected.[182,208,209] For example, self-selection into a wave can lead to collider bias (a bias occurring when two variables independently affect a third variable, and that third variable is conditioned upon).[182] Complete case analysis would not be problematic if it can be assumed that missingness occurs completely at random,[209] which is a strong assumption as it implies that there are no systematic differences between those with and without missing data. This assumption clearly does not hold in the Whitehall II cohort study. Attrition, therefore, does have the potential to cause bias in studies using data from the Whitehall II cohort. Although the response rate (i.e., what proportion of people attended the study) could be an indicator of selection bias, this is not always the case.[182,208] It is therefore recommended that researchers report characteristics of those excluded from the study[210] to allow readers to evaluate the validity of findings, and consider potential methods to allow for attrition.

I hypothesised that differences in hazards between participants and those lost due to attrition would change with time. My study, however, did not support this hypothesis, which suggests that relative changes of unmeasured risk factors in responders compared to withdrawers/non-responders were either absent, or not sufficiently large to influence associations.

In conclusion, the results in this study suggest that those who are lost due to attrition, no matter when attrition occurs, have an excess mortality within three to five years. Although statistical methods which deal with missing data, such as multiple imputation, are now widely used, they are challenging to apply to models with repeated outcomes (as in Chapters 6 and 7). I therefore used complete case analysis in my subsequent analyses, but the findings in this study are taken into consideration, to assess the potential extent of any bias which may have been generated.

Chapter 5 Adverse childhood experiences and incident coronary heart disease

This work has been published in the American Journal of Preventive Cardiology.[210] I am entirely responsible for this work except the analysis to examine the dose-response association between count of ACEs and incident CHD, which required further programming, performed by co-author Owen Nicholas.

5.1. Introduction

As described in Chapter 1, CHD is the leading cause of death and disability worldwide.[211] CHD represents a long-term disease process, from the development of atherosclerosis, subclinical disease, to its clinical manifestations. This natural history of CHD appears to start early in life.[4,5]

Adverse childhood experiences (ACEs) appear to be associated with unfavourable brain development and function, resulting in potential negative behavioural and physiological changes, and unfavourable stress reactivity over life.[40,56,132] A meta-analysis estimated that those who had ACEs were three times more likely to be smokers (Odds ratio, 95% confidence intervals: 2.82, 2.38 to 3.34), six times as likely to drink alcohol problematically (5.84, 3.99 to 8.56), as well as four times more likely to have depression (4.40, 3.54 to 5.46).[80] Those who experienced ACEs have also be found to be more likely to have hypertension, obesity and hyperlipidaemia later in life.[96] Failures of adaptation to stress and insufficient recovery from the stress, in addition to engagement in health risk behaviours, may play a substantial role in the development and progression of CHD.[7,40,212]

Findings for associations of ACEs with CHD are, however, mixed; some studies documented positive associations, most of which also reported dose-response associations,[78,82,83,99,113,213,214] but not all.[98,101,215,216] These mixed results could be in part explained by the challenges in research on ACEs, as described in section 1.3.3. In particular, study design and ascertainment of incident CHD are key challenges given that the long period of time between ACEs with clinical manifestation of CHD.

To date, there are few longitudinal studies with follow-up from the time when ACEs are assessed, linked with central registry records of CHD. Linkage to health records provides more accurate ascertainment of CHD than self-report and is not subject to recall bias. Given that ACEs have been modelled in different ways in previous studies, it remains unclear whether specific ACEs are particularly important, whether some ACEs have larger associations with CHD than others or whether there is a dose-response association between the number of ACEs and CHD. Previous studies have focused on providing an estimate of the association between ACEs and CHD. However, given that any such association may be causal, and that some ACEs are modifiable, it is important for public health to quantify the extent that the elimination of ACEs could have on CHD prevention.[217]

Accordingly, the objectives of this study are (i) to examine the association of each type of ACE with incident CHD in adulthood, and the dose-response association between ACEs and CHD, and (ii) to quantify the reduction in risk of incident CHD in the absence of ACEs, using a counterfactual approach.

5.2. Methods

Study population

I used the data from the Whitehall II cohort study for these analyses. I included 5149 participants who had no missing values for ACEs, confounders, and incident CHD. The flow chart of participants' recruitment into the analytical sample is shown in Figure 5-1.

Exposures and outcome

The assessment of ACEs and the variables derived from them, and ascertainment of incident CHD are described in section 3.3, Chapter 3.

Confounders

I identified potential confounders from existing studies.[83,97,218] A diagram was used to identify the variables, which were observed in the Whitehall II cohort study, that should be adjusted for assuming the diagram is correct for the total effect of ACEs on CHD (Figure 5-2). Sex, age in years, and ethnicity (white, non-white) were derived from phase 1. Missing values in ethnicity were replaced with responses from phase 5. Fathers' occupation was used as a marker of childhood socioeconomic position, derived from phase 1, and missing values were replaced with responses at phase 6. Father's occupation was categorised as professional, managerial/technical, skilled-non-manual, skilled-manual, partly skilled, and unskilled according to the Registrar General's Social Class Scheme.

Statistical analysis

I described the prevalence of ACEs, incident CHD, and confounders among those excluded from the study, and those in the study sample (n=5149). I computed hazard ratios (HRs) and 95% Confidence Intervals (CIs) of incident CHD in associations with ACEs by applying Cox proportional hazard regression (see section 3.5.1, Chapter 3). The time scale was time in days, starting from the phase 5 data collection which was taken to be the baseline. In preliminary analyses, I included an interaction term for sex with each ACE to examine whether sex modified the association of ACEs with incident CHD. As there was no evidence of any such interaction, estimates including both men and women are presented with adjustment for sex. In order to assess whether multicollinearity between the ACEs could be a problem, I computed variance inflation factors for each ACE. All variance inflation factors were between 1.02 and 1.57 which were well below the threshold for identification of multicollinearity. I therefore included all 14 ACEs in a model, making the assumption that no ACE is on the causal pathway of another type of ACE. The initial model adjusted for sex and age, with additional adjustment for ethnicity and childhood socioeconomic position. Based on the estimates in this final model, I predicted the average marginal HRs and 95% CIs according to the count of ACEs with all confounders held constant. The marginal effects are quantities which represent changes in outcome when a specific exposure changes. These estimates were plotted with their 95% CIs to visualise the relationship. It is not simple in Stata, however, to estimate the linear trend across ACEs count. I therefore sought advice from a statistician who provided an approach to calculate the constant of proportionality to estimate how the marginal log-hazard increases in proportion to the number of ACEs. This calculation is based on the marginal estimates and the

average shift in covariates between the observed and no ACEs (further details are provided in Appendix 3). Finally, the reduction in hazard of CHD resulting from the counterfactual scenario of absence of all ACEs compared with the observed was calculated. In carrying out this estimation of the marginal log-hazard all covariates other than the ACEs are set to zero because these covariates remain constant across both scenarios and therefore do not contribute to the estimates (see Appendix 3).

I carried out two sensitivity analyses. First, I estimated HRs and 95% CIs for each ACE in a model in which one adversity was included at a time with adjustment for the same confounders as in the main analysis. These estimates were compared with those from the main analyses to see whether there was an impact on individual estimates when adjusting for all other ACEs. Second, in order to compare the dose response estimate in my main analysis with a more commonly used approach, I fitted the model with an ACEs score, which is the sum of the number of ACEs (ranging from 0 to 10), with the same adjustments.

I used Stata MP version 16.0 for all analyses, apart from when estimating a dose-response for the count of ACEs, for which Microsoft Excel was used, in my main analysis.

5.3. Results

The selection of the analytic sample is presented in Figure 5-1. Of the 10 308 participants at phase 1, 7870 participants took part in phase 5. By excluding those who had missing values in ACEs assessed at phases 1 and 5, confounders, along

with those with a prior episodes of incident CHD, the number in the analytical sample was 5149 (men: 72.6%).

In Table 5-1 I present the characteristics of the study sample according to each ACE. Compared with those excluded, the study sample was more likely to be male, be younger, be of White ethnicity, and be in a non-manual childhood socioeconomic position. The prevalence of ACEs was lower among those included in the study than those excluded.

Among the study sample, 62.9% had at least one ACE. The highest prevalence was observed for financial problems (26.1%), followed by arguments between parents (19.5%). In Table 5-2 I show the distribution of covariates according to the count of ACEs. Women were less likely to have no ACEs compared with men (29.6% versus 39.9%) and more likely than men to have a count of ACEs of 3+ (27.8% versus 18.5%). Among the non-white group there was a lower proportion with no ACEs (29.7%) compared with the white group (37.5%), but a higher proportion with 3+ ACEs (25.5%) than the white (20.8%). There was a higher proportion of those who had a father in a manual occupation in the 3+ ACEs group (27.6%) compared with those with a father in a non-manual occupation (16.7%), while a lower proportion in no ACEs category (29.4%) than the non-manual occupational background (42.1%). A mean duration of follow-up of 12.9 years (standard deviation 4.5) gave rise to 509 first episodes of CHD.

In Table 5-3 I report the HRs (95% CIs) of CHD according to each ACE. Among the 14 ACEs, experience of maternal separation and experience of parental

unemployment were associated with higher rates of CHD. For maternal separation the HR was 1.56 (95% CI: 1.25 to 1.96) and for parental unemployment it was 1.49 times (1.18 to 1.89). For all other ACEs confidence intervals included the null value of one, with six additional ACEs demonstrating a HR of greater than one, but the other six a HR less than one. ACEs such as abuse and, especially, being in an orphanage had low prevalence and therefore confidence intervals were wide.

The same two associations remained in the fully adjusted model although the estimates changed slightly. The estimate for maternal separation decreased to 1.33 (1.03 to 1.73). The HR for parental unemployment increased slightly to 1.53 (1.16 to 2.02). The effect of father's harsh punishment was strengthened in the adjusted model such that for an increase in category the HR is 1.16 (1.04 to 1.29). The estimates for other ACEs do change after adjustment, but all other 95% confidence intervals still include one and there is no consistent direction of effect.

Using the fully adjusted model, predicted HRs by the count of ACEs are presented in Figure 5-3 and show a small increase as count of ACEs increases. There are larger HRs for particularly high counts of ACEs, but confidence intervals are wide due to the small numbers in these groups. The hazard of incident CHD is estimated to increase by 5.0% for each additional ACE, but 95% CIs just included one (constant for proportionality in HR 1.05, 95% CI 0.99 to 1.11). There was an estimated 6.0% (0.94, 0.87 to 1.01) reduction in hazard of CHD in the absence of all ACEs, as the counterfactual scenario, against the observed distribution of ACEs. However the 95% CIs for the estimate included one.

Results of sensitivity analyses are presented in Table 5-4. Models which included only one adversity at a time showed only slight differences in effect sizes compared with the main analysis in which all other ACEs were included. Consistent with the main analysis, only maternal separation (HR 1.31, 95% CI:1.02 to 1.68) and parental unemployment (1.44, 1.11 to 1.85) showed positive associations with incident CHD where confidence intervals did not include one, consistent with the main analysis. Nine out of the 14 ACEs had HRs greater than one indicating the majority were in the expected direction. The estimate for the ACEs score (1.04, 0.98 to 1.10) was similar to the estimate for ACE count from the final model in the main analysis (1.05, 0.99 to 1.11).

Table 5-1. Characteristics of study population according to inclusion in the present analytical sample

	Excluded sample (n=5159)[a]	Study sample (n=5149)
Exposure[b]		
No adverse childhood experiences, *n (%)*	-	1908 (37.1)
Maternal separation 1yr+, *n (%)*	427 (22.6)	520 (10.1)
Parental death, *n (%)*	852 (18.1)	370 (7.2)
Hospitalisation 4wks+, *n (%)*	285 (16.6)	627 (12.2)
Divorce, *n (%)*	198 (11.6)	99 (1.9)
Mental illness and alcohol problems, *n (%)*	116 (6.8)	304 (5.9)
Arguments between parents, *n (%)*	378 (22.1)	1003 (19.5)
Unemployment, *n (%)*	229 (13.5)	504 (9.8)
Financial problems, *n (%)*	655 (37.4)	1342 (26.1)
Physical abuse, *n (%)*	58 (3.4)	119 (2.3)
Orphanage, *n (%)*	69 (4.1)	28 (0.5)
Lack of attachment to mothers, *median (IQR)*	8 (6 to 10)	8 (6 to 10)
Lack of attachment to fathers, *median (IQR)*	10 (8 to 12)	10 (8 to 12)
Mother's harsh punishment, *median (IQR)*	2 (1 to 2)	2 (1 to 2)
Father's harsh punishment, *median (IQR)*	2 (1 to 3)	2 (1 to 3)
Outcome		
First episode of coronary heart disease from phase 5	271	509
Covariates		
Sex, *n (%)*		
Men	3158 (61.2)	3737 (72.6)
Women	2001 (38.8)	1412 (27.4)
Age in years at baseline, *median (IQR)*	45.2 (40.1 to 51.1)	43.6 (39.3 to 49.5)
Ethnicity, *n (%)*		
White	4342 (85.7)	4839 (94.0)
Non-white	725 (14.3)	310 (6.0)
Childhood socioeconomic position, *n (%)*		
Non-manual	2073 (55.3)	3109 (60.4)
Manual	1676 (44.7)	2040 (39.6)

[a] Proportion was calculated with the number of responders to each item as denominator, which differed across items. Due to the differences in the number of denominators across items, a proportion of "No adverse childhood experiences" is not available

[b] Adverse childhood experiences are not mutually exclusive

Table 5-2. Distribution of covariates according to the count of adverse childhood experiences

	ACEs[a]			
	0	**1**	**2**	**3+**
n	*1908*	*1262*	*895*	*1084*
Sex, *n (%)*				
Men (n=3737)	1490 (39.9)	925 (24.8)	630 (16.9)	692 (18.5)
Women (n=1412)	418 (29.6)	337 (23.9)	265 (18.8)	392 (27.8)
Age in years at baseline, *mean ± SD*	43.9 ± 5.9	44.5 ± 6.0	44.7 ± 6.1	44.9 ± 5.9
Ethnicity, *n (%)*				
White (n=4839)	1816 (37.5)	1185 (24.5)	833 (17.2)	1005 (20.8)
Non-white (n=310)	92 (29.7)	77 (24.8)	62 (20.0)	79 (25.5)
Childhood socioeconomic position[b] *n (%)*				
Non-manual (n=3109)	1308 (42.1)	763 (24.5)	518 (16.7)	520 (16.7)
Manual (n=2040)	600 (29.4)	499 (24.5)	377 (18.5)	564 (27.6)

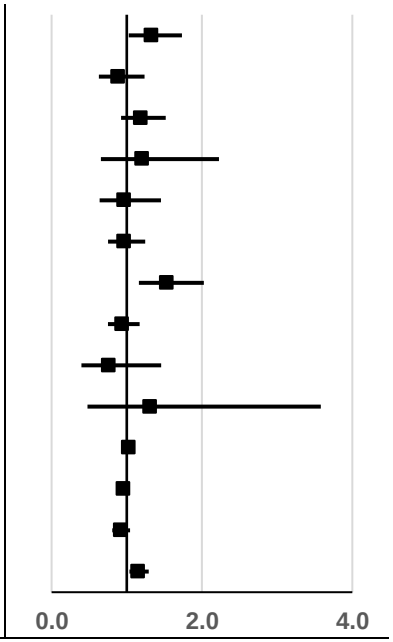
[a] In this table, the count of ACEs was categorised into four groups for convenience to describe distribution of covariates

[b] Non-manual: professional, managerial/technical, skilled-non-manual; Manual: skilled-manual, partly skilled, and unskilled

ACEs, adverse childhood experiences

Table 5-3. Hazard ratios (HRs) and 95% confidence intervals (CIs) for the association of adverse childhood experiences (ACEs) with incident coronary heart disease (CHD) in a model including all ACEs simultaneously (n=5149)

	No. CHD	RR (95% CI)[d]	HR (95% CI) *Adjusted for* Sex and age		HR (95% CI) *Adjusted for* All confounders[e]	
Total	509					
Multiple ACEs[a]						
Maternal separation 1yr+	76	1.56 (1.25, 1.96)	1.39	(1.07, 1.81)	1.33	(1.03, 1.73)
Parental death	40	1.10 (0.81, 1.49)	0.94	(0.67, 1.31)	0.88	(0.63, 1.24)
Hospitalisation 4wks+	75	1.25 (0.99, 1.57)	1.17	(0.91, 1.50)	1.18	(0.92, 1.52)
Divorce	11	1.13 (0.64, 1.98)	1.30	(0.71, 2.39)	1.21	(0.66, 2.23)
Mental illness and alcohol problems	28	0.93 (0.65, 1.33)	0.98	(0.65, 1.48)	0.96	(0.64, 1.45)
Arguments between parents	93	0.92 (0.75, 1.14)	0.96	(0.74, 1.23)	0.97	(0.75, 1.25)
Unemployment	71	1.49 (1.18, 1.89)	1.60	(1.22, 2.11)	1.53	(1.16, 2.02)
Financial problems	138	1.06 (0.88, 1.27)	0.95	(0.76, 1.18)	0.94	(0.75, 1.17)
Physical abuse	10	0.85 (0.47, 1.54)	0.78	(0.41, 1.50)	0.76	(0.40, 1.46)
Orphanage	4	1.45 (0.58, 3.60)	1.27	(0.47, 3.48)	1.31	(0.48, 3.58)
Lack of attachment to mothers	96[b]	0.94 (0.76, 1.16)	1.03	(0.99, 1.07)	1.03	(0.99, 1.07)
Lack of attachment to fathers	86[b]	0.90 (0.72, 1.12)	0.95	(0.92, 0.99)	0.96	(0.92, 1.00)
Mothers' harsh punishment	13[c]	0.81 (0.48, 1.37)	0.92	(0.80, 1.04)	0.92	(0.81, 1.04)
Fathers' harsh punishment	37[c]	1.22 (0.89, 1.67)	1.19	(1.06, 1.33)	1.16	(1.04, 1.29)



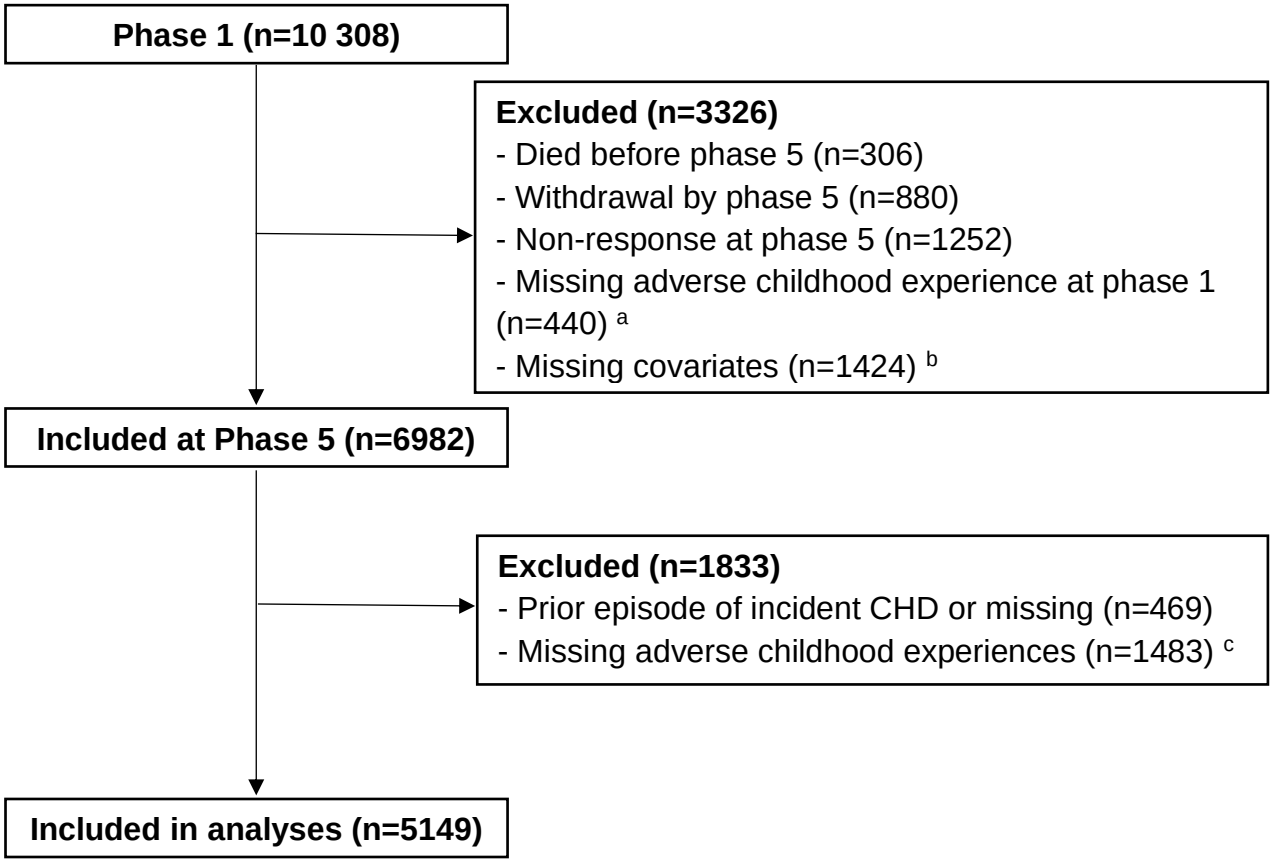
[a] A model in which all ACEs were adjusted for simultaneously. "Lack of attachment to mothers/fathers" and "Mothers/fathers' harsh punishment" are ordinal, and the other variables are binary in which reference groups are people who have no corresponding ACEs
[b] Number of incident coronary heart disease (CHD) among people in the worst quartile
[c] Number of incident coronary heart disease (CHD) among people who answered "great deal" in 4-likert scale
[d] Risk ratios (RRs) for the associations of each type of ACEs with incident coronary heart disease (CHD)
[e] A model adjusted for sex, age, ethnicity, and childhood socioeconomic position

Table 5-4. Hazard ratios (HRs) and 95% confidence intervals (CIs) in a separate model with one adversity at a time, and in a model with a cumulative ACEs score, in the association with incident coronary heart disease (CHD)

	No. CHD	HR[a]	95% CI[a]
A model with one adversity at a time			
Maternal separation 1yr+	76	1.31	(1.02, 1.68)
Parental death	40	0.96	(0.69, 1.33)
Hospitalisation 4wks+	75	1.22	(0.95, 1.56)
Divorce	11	1.17	(0.64, 2.13)
Mental illness and drunk	28	1.01	(0.69, 1.47)
Arguments between parents	93	0.99	(0.79, 1.24)
Unemployment	71	1.44	(1.11, 1.85)
Financial problems	138	1.02	(0.84, 1.25)
Physical abuse	10	0.88	(0.47, 1.64)
Orphanage	4	1.40	(0.52, 3.76)
Lack of attachment with mothers	96[b]	1.01	(0.98, 1.04)
Lack of attachment with fathers	86[b]	0.99	(0.96, 1.02)
Mother's harsh punishment	13[c]	0.99	(0.88, 1.11)
Father's harsh punishment	37[c]	1.10	(0.99, 1.21)
A model with a cumulative ACEs score			
ACEs score	509	1.04	(0.98, 1.10)

[a] Adjusted for sex, age, ethnicity, and childhood socioeconomic position
[b] Number of incident CHD among those who answered "Not at all," but the model was fit with original variables in 4-likert scale
[c] Number of incident CHD among those in the worst quartile, but the model was fit with original variables ranging from 1 to 4

Figure 5-1. Flow chart of participants' recruitment



[a] Parental death, n=440
[b] Sex, n=0; age, n=0; ethnicity, n=92; childhood SES, n=1410
[c] Maternal separation 1yr+, n=694; hospitalisation 4wks+, n=836; divorce, n=842; mental illness and alcohol problems, n=848; argument, n=840; unemployment, n=851; financial problems, n=807; physical abuse, n=851; orphanage, n=863; lack of attachment with mothers, n=809; lack of attachment with fathers, n=1096; mother's harsh punishment, n=771; father's harsh punishment, n=1042

Figure 5-2. Diagram for the association between adverse childhood experiences and incident coronary heart disease (CHD)

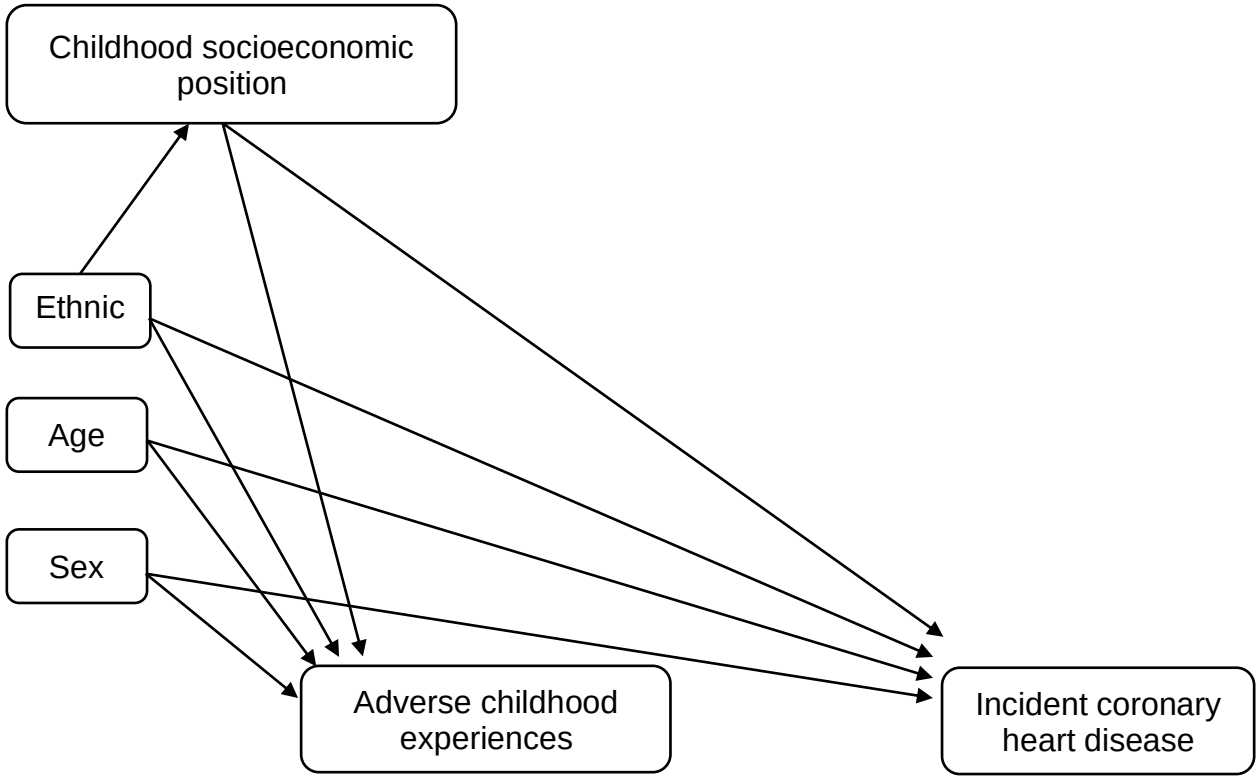
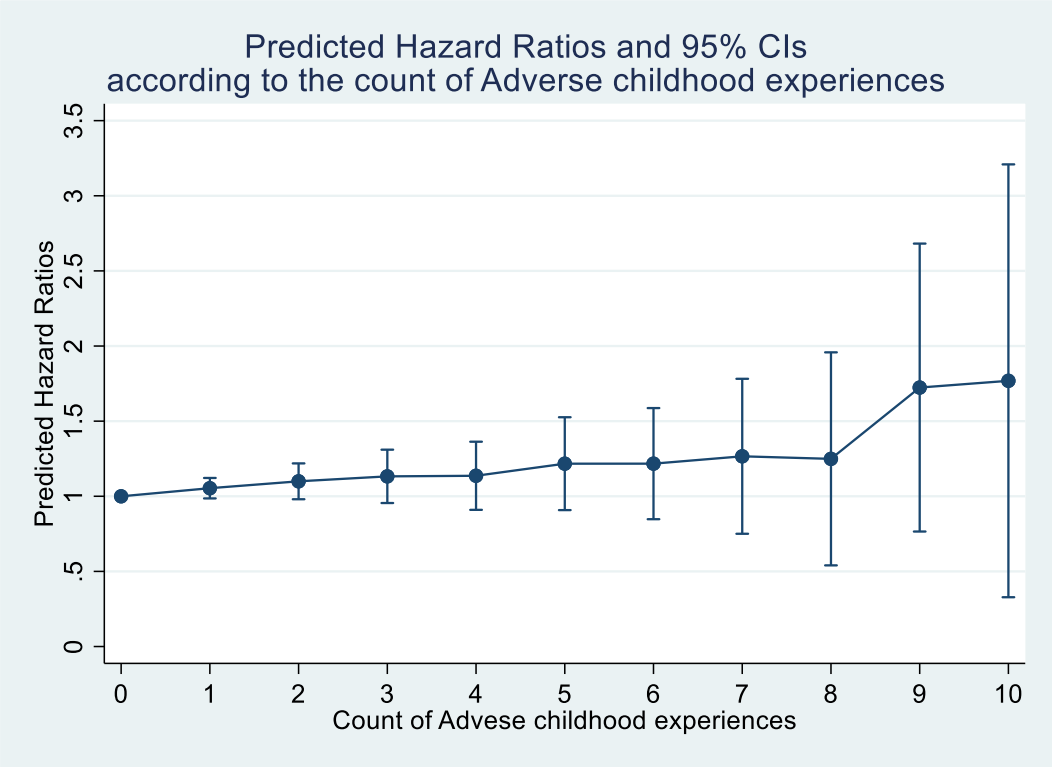


Figure 5-3. Predicted hazard ratios and 95% confidence intervals (CIs) of incident coronary heart disease (CHD) by the counts of adverse childhood experiences (ACEs)[a]



[a.] Adverse childhood experiences in the presented study range from zero to 10

5.4. Discussion

Associations with CHD in later life were not consistent across all ACEs in this analysis of the Whitehall II cohort study. There were only two ACEs which were independently associated with incident CHD. There was weak evidence of a small dose-response between the count of ACEs and incident CHD. I estimated a 6.0% reduction in CHD in the absence of ACEs, but 95% CIs included one.

The percentage of people who experienced at least one ACE in my study was 62.9%, in close agreement with a systematic review of this field.[219] Parental unemployment and maternal separation were the only ACEs that were independently associated with increased risk of CHD. It may be that specific ACEs have different

mechanisms through which they influence CHD. Unemployment in adulthood has been shown to be related to increased risk of CHD possibly due to cumulative chronic stress,[220] but my study shows unemployment may also have intergenerational effects. One of the potential pathways from parental unemployment to adult CHD may be mediated by children's educational attainment, and subsequent adult socioeconomic position,[221] an established risk factor of CHD.[8] On the other hand, the association with maternal separation may be due to alterations in biological mechanisms during a sensitive period of early life which have been shown in previous human[118] and in animal studies.[222] Some of the ACEs have low prevalence and thus have wide confidence intervals and so, for example, an association with being in an orphanage cannot be ruled out. There is a need to study ACEs in a larger population where they will be more prevalent.

To address challenges of ACE score as described in section 1.3.3, Chapter 1, I fitted a model including all ACEs simultaneously similar to some previous research.[218,223] This approach retained the information on the severity of ordinal ACEs rather than categorising as a binary variable. The predicted hazard ratio according to the count of ACEs,[180] was however, very similar to that obtained using a more traditional ACE score which simply counted the number of ACEs. Given that ACEs are likely clustered and co-occurring, estimated risks for each ACE may provide limited information on their own, [224,225] while estimates for the overall effect of ACEs may be more reliable due to their smaller standard errors. My counterfactual estimate for the overall effect of all ACEs, 6.0% (HR 0.94; 95% CI: 0.87 to 1.01), is close to a finding from a systematic review based on cross-sectional studies that reported an approximately 10% reduction for all ACEs.[219] Despite this relatively small figure,

particularly when comparing with corresponding reported figures for health risk behaviours, of around 20 to 35%,[219] 6.0% is noteworthy given that CHD is the most common cause of DALYs, 1713.6 years per 100 000 population in 2019, followed by Alzheimer disease and other dementias, in UK.[17]

The mixed results from existing studies can be partly explained by the differences in the measurement of ACEs and CHD and definition of ACEs, as described in section 1.3.3, Chapter 1, and which are further discussed in Chapter 7. My study models ACEs in a different way to most previous studies, although I carried out a sensitivity analysis using the ACEs score which is more commonly used. It may be that if there is an effect of ACEs on CHD, then the effect is small and existing studies are not large enough to estimate the association with adequate precision. Other possible factors contributing to the inconsistencies in findings are the covariates adjusted for in analytical models and differences in study design.[114,116] Some studies included adjustment for mental health and health behaviours, which are likely to be mediators (i.e., on the causal pathway between ACEs and CHD, rather than confounders). Given that there is no established pathway between each ACE and the development of CHD, adjustment for a priori mediators can violate the accuracy of estimation, or decrease its precision.[226] I thus adjusted only for variables which are considered to confound the association between ACEs and CHD. However, I am aware of other potential confounders (e.g., parental longstanding illness at the time of participant's birth) which are not available in the cohort study and there may also be unidentified confounders. My findings are consistent with longitudinal studies using electronic health records to obtain CHD and retrospectively measured ACEs reporting little evidence for the association.[98,101]

Retrospectively measured ACEs, such as used in the Whitehall II cohort study, may be influenced by the health status (e.g., depression) at the time of assessment. On the other hand, prospective measurement of ACEs could also be biased (e.g., under-report of sexual abuse) due to being scared to report/social desirability bias. There are possible discrepancies between prospective and retrospective measurement of ACEs where ACEs are more subjective such as emotional abuse, while objective or factual ACEs such as parental death are more likely to be consistent.[102,109] It would be interesting to examine associations of prospectively measured ACEs with CHD, and to compare findings with longitudinal studies in which retrospectively measured ACEs were used.

The 14 ACEs used in my study did not include distal relationships (e.g., school peers), societal or environmental events due to lack of information on these in the Whitehall II cohort study. It has been shown that these ACEs are also important because psychological and physiological influence on lifelong health has been reported.[227,228] Investigation of these adversities is of interest to develop policies for those vulnerable population.

There is debate as to whether the duration over which an ACE is experienced, and age at the occurrence of the ACE are particularly important. However, I was unable to investigate this because of a lack of information. I cannot rule out the possibility of overadjustment when including all ACEs in the same model, because some ACEs may potentially lie on the causal pathway between another ACE and CHD. For example, family financial problems could lead to parental mental illness and

exposure to parental mental illness may lead to CHD. Almost half of the original participants were excluded from the study because they had died before phase 5 or did not respond at phase 5 when most of ACEs were assessed. I also excluded those with missing values in confounders and those having had CHD before phase 5 in order to study incident CHD. A prior episode of CHD, or subsequent deaths among those with missing ACEs information can bias the estimates. Both outcome, CHD, and exposure, ACEs, thus influence selection into a study and because carrying out complete case analysis is equivalent to adjustment for selection this can result in collider bias.[182]

Almost two thirds of population have a legacy of ACEs throughout life and thus there is a considerable burden of ACEs and therefore any effect on health is of public health importance. My research, however, demonstrates that the majority of ACEs may not be associated with the development of CHD later in life. The suggestion that specific experiences may have an impact on health needs to be investigated in further research in larger studies which have adequate power to assess rarer ACEs. Investigations on duration, timing, and severity of exposure to ACEs may also shed light on whether there are particular types of ACEs which are more important.

Chapter 6 Adverse childhood experiences and adult salivary cortisol

6.1. Introduction

One of the possible underlying biological pathways in the association between ACEs and CHD is via the hypothalamic–pituitary–adrenal (HPA) axis, a central component of the stress response system.[40] The final effectors in the HPA axis are glucocorticoids, primarily cortisol, which plays important roles in metabolism, immune function, and inflammatory responses.[40] Short-term fluctuations of the cortisol are essential to respond to the environment to maintain allostasis. However, prolonged deviations, due to sustained or repeated exposures to stressors, may gradually dysregulate system functioning.[40]

As described in Chapter 1, findings on the association between ACEs and cortisol secretion are heterogeneous, and there has been little research on the potential sex difference in the association. Accordingly, my objectives are (i) to investigate whether ACEs are related to diurnal cortisol secretion in adult life and whether there is a sex difference in the association; and (ii) to examine whether there is a dose-response association between the number of ACEs and cortisol secretion after taking the effect sizes of each adversity into account.

6.2. Methods

Study sample

I used data from the Whitehall II cohort study and the National Child Development Study (NCDS). I excluded participants who had missing values in exposure, outcome, and covariates. Additionally, I excluded those who were taking steroids

(Whitehall II cohort study and NCDS) or using menopausal hormone therapy[229] (Whitehall II cohort study only because such information is not available in NCDS) at the time when salivary cortisol was measured because these treatments may change cortisol levels.[230] Consequently, my analytic sample was 3419 in the Whitehall II cohort study (Figure 6-1) and 2117 in the NCDS (Figure 6-2).

Exposures and outcome

The assessment of ACEs and the variables derived from them, and assessment of salivary cortisol in the Whitehall II cohort study and in the NCDS are described in section 3.3, Chapter 3.

Confounders and predictors

I identified potential confounders and predictors using a theoretical model indicated in a diagram and based on knowledge about relationships between variables from existing studies. The diagram was then used to identify the set of variables that should be adjusted for on the assumption that the diagram is correct (Figure 6-3).

In the Whitehall II cohort study, I included sex, age in years, ethnicity (white vs non-white), childhood socioeconomic position, and adult socioeconomic position, smoking (yes vs no), and waking time on the day of sample collection. Age in years was measured in the wave when saliva was sampled. Fathers' occupational grade was used as a marker of childhood socioeconomic position and employment grade at the wave of saliva sampling, as a marker of adult socioeconomic position. Father's occupation was classified according to the Registrar General's Social Class Scheme, with categorisations of 'professional', 'managerial/technical', 'skilled-non-manual',

‘skilled-manual’, ‘partly skilled’, and ‘unskilled’. Employment grade was categorised as ‘administrative’ (high grade), ‘professional/executive’ (intermediate grade), and ‘clerical/support’ (low grade). Information on smoking and waking time on the day of saliva collection was obtained from the logbook.

In the NCDS, I included sex, childhood socioeconomic position at birth, and waking time on the day of sample collection. I did not adjust for age in NCDS as the cohort members were born in a single week of 1958, and for ethnicity because only 2% of the cohort members identify themselves as non-White. Father’s occupation was classified using the same Scheme as for the Whitehall II cohort study. Information on waking time on the day of saliva collection was obtained from the form enclosed with the samples, but information on smoking at the time of saliva sampling was not available.

Statistical analysis

The Whitehall II cohort study

Area under the curve (AUC) was used as an index for the amount of cortisol secretion during a day, and cortisol awakening response (CAR) and diurnal slope as the indices of the diurnal cortisol pattern.

I calculated the AUC for each individual, as an index of the amount of cortisol secretion during a day, based on the six observed values using the Trapezoidal rule.[231] The equation used was given by;

$$\text{AUC} = \frac{1}{2}\sum_{k=1}^{n-1}(T_{k+1} - T_k) \times (C_{k+1} + C_k)$$

where T_k denotes the time at each sample collection, and C_k the cortisol value at each time point, where *k*=1,….,*n*-1., and n is the number of samples collected, which equals six in this study.

I computed the CAR by subtracting cortisol measured at awakening time (T_{W1}) from cortisol measured at 30 minutes after awakening (T_{W2}). The analytical sample was restricted to those who had the cortisol measure at T_{W1} taken within 10 minutes after awakening, and measure at T_{W2} taken within 60 minutes after awakening,[232] irrespective of time of the day at awakening,[229] to minimise measurement error in CAR.

I first described cortisol levels across a day, and covariates by the count of ACEs. As the distribution of salivary cortisol values was right skewed, I used median and inter-quartile range for descriptive analysis, apart from for the cortisol awakening response, which was normally distributed.

I applied linear regression to examine the associations of ACEs with AUC (ln(nmol/l*hr)x100) and CAR (nmol/l). The natural logarithm of the AUC measures was taken, to reduce skewness, and then multiplied by 100 so that the resulting regression coefficients are interpreted as percent change in AUC per unit change in an exposure.[233] In preliminary analyses, I included interaction terms for sex by individual adversities and likelihood ratio tests to assess whether sex modified the association of ACEs with each index of cortisol. With no evidence for interactions, I

present estimates from models including men and women together as the primary analyses. I present sex-stratified estimates, in the light of sex differences in HPA axis stress response,[154,234] as a supplementary analysis.

Model 1 included all 14 ACEs simultaneously, sex, and age, and model 2 additionally adjusted for ethnicity, childhood and adult socioeconomic position, and smoking and waking time on the day of sample collection. Based on the final model, I estimated the average outcome values according to the count of ACEs, and tested a dose-response effect in the sex-pooled models, following the same procedure as outlined in Chapter 5 (see section 5.2).

For the diurnal slope, I used a multilevel regression model in which measurement occasions were considered level 1, nested within individuals as level 2. This model takes into account the correlation of the repeated cortisol measurements within individuals, as well as the variance between individuals, by estimating a random slope and intercept for each individual. The outcome was log cortisol (x 100) at T_{W1}, T_{W3}, T_{W4}, T_{W5}, and T_{W6}, but did not include cortisol at T_{W2} because the awakening response appears to have different biological system from diurnal slope.[235,236] Model 1 included only the linear and quadratic term for time since awakening as fixed effects. I estimated the random effect only for linear time since awakening, because the random quadratic term was very small, and its inclusion did not improve the model fit. Model 2 additionally included all 14 ACEs; Model 3 added awakening time, and the interaction terms between the ACEs and time in order to assess whether ACEs were associated with the diurnal slope; and Model 4, a final model, was additionally adjusted for age in years, ethnicity, childhood and adult socioeconomic

position, smoking on the day of sample collection, and their interaction terms with time since awakening. From the final model, I estimated predicted diurnal slopes for each individual, and plotted the average by the count of ACEs, when all the covariates were set to their baseline values (coded as zero in the model). It was not possible to use the method described in section 5.2 to estimate the correct constant of proportionality because this method cannot be applied to multilevel models. Instead, I regressed the predicted point estimates against the count of ACEs to assess whether there is a trend in the association. This approach, however, underestimates the error and provides 95% confidence intervals which are too narrow and therefore should not be interpreted.

I performed sensitivity analyses for all three outcomes where I used the cumulative score of ACEs (i.e., single exposure summing the number of ACEs), instead of the 14 separate ACEs (i.e., multiple exposures) used in the main analysis.

The National Child Development Study

Unlike the Whitehall II cohort study, only two measures over the course of a day of saliva samples are available in the NCDS; 45 mins after awakening (T_{N1}) and 3 hours after the first sample (T_{N2}: i.e., 3 hrs 45 mins after awakening). I used the T_{N1} sample as an index of early morning cortisol, and the T_{N2} sample as an independent marker of daytime cortisol level.

I used a multivariate linear regression to examine association of ACEs with cortisol level at T_{N1} and T_{N2}. The outcomes were log cortisol levels (x 100) at T_{N1} and T_{N2}. The Breusch-Pagan test was used to examine whether the residuals of cortisol

levels at T_{N1} and T_{N2} in the equations are independent of each other. If they are not independent, then it is not appropriate to consider the two outcomes separately and they should be modelled using a multivariate model.

I included interaction terms for sex with each adversity to examine whether sex modified the association of ACEs with cortisol levels at T_{N1} and T_{N2} as a preliminary analysis. With no such evidence, I present estimates from models including men and women together. I firstly included all ACEs simultaneously, adjusted for awakening time and time since awakening, and then additionally adjusted for sex and childhood socioeconomic position. Based on the estimates from the final model, I estimated predicted values of cortisol levels at T_{N1} and T_{N2} according to the count of ACEs, and tested a dose-response effect, following the same procedure as Chapter 5 (see section 5.2).

6.3. Results

The Whitehall II cohort study

Table 6-1 summarises the reports of ACEs in the analytical sample of n=3419. Among the participants included in the analysis, 67.8% reported at least one adversity. The highest prevalence was observed for “financial problems” (24.8%), followed by “arguments between parents” (19.0%). Orphanage was observed among only 0.4% of the study sample. In Table 6-2 I present the distribution of covariates according to the count of ACEs. A higher proportion in no ACE category was observed among men (34.0%) than women (26.7%), while a proportion in 7+ ACEs category was higher among women (1.5%) than men (0.9%). Compared to non-white participants, white participants had a higher proportion of no ACE group (32.3% vs 29.5%), but a lower

proportion in 7+ ACEs group (0.9% vs 2.2%). People who grew up in non-manual socioeconomic position were more likely to report no or smaller number of ACEs, while people from manual socioeconomic background tend to report larger number of ACEs. Similarly, the lower the employment grade was, the lower proportion of no ACE group was, but the higher proportion was observed in 7+ ACEs category. People who smoked on the day of saliva sample collection had a higher proportion in no ACE group (32.7%) than people who did not (24.1%), but there was no consistent trend in the other categories of ACEs.

In Table 6-3 estimates and 95% confidence intervals (CIs) for the associations of ACEs with the AUC and CAR are presented. In the sex and age adjusted model, there was no clear pattern of association between ACEs and AUC. The largest effect size was observed for parental death and this was strengthened after additionally adjusted for ethnicity, childhood and adult socioeconomic position. Those experiencing this adversity showed a 7% increase in AUC (95%CI 0.25 to 13.83) compared to those who did not. Parental death was also associated with increased CAR. Those who had experience of parental death showed 2.724 nmol/l (0.32 to 5.128) increased CAR than those who did not in the sex and age adjusted model. This association remained in the fully adjusted model, where the estimate increased slightly to 2.801 nmol/l (0.398 to 5.204). Confidence intervals of all other types of ACEs included zero and there was no consistent direction of association observed.

Figure 6-4 and Figure 6-5 present predicted AUC and CAR with 95% CIs, respectively, from the fully adjusted models by the count of ACEs. For AUC, the predicted values show no pattern across the count of ACEs. When calculating the

constant of proportionality, there was no evidence of any association with on average each additional adversity being related to a 0.008% decrease (-0.980 to 0.964) in AUC. For the CAR, there was only a slight upward trend with increasing number of ACEs (Figure 6-5) where the increase in CAR peaks at five adversities with no further increase observed for six and seven plus adversities, although 95% CIs were wide. There was an estimated 0.206 nmol/l (-0.144 to 0.556) increase in CAR per ACE count, but the 95% confidence interval included zero.

Sex-stratified analysis showed that for AUC, there were no clear trends by ACE count in either men or women (Figure 6-4, Appendix 4). Among men, parental death showed the strongest association with CAR, similar to the sex-combined analysis, while 9 out of the 14 ACEs demonstrated an association in the same positive direction (i.e., ACE associated with greater CAR). Therefore, a slight progressive increment in predicted CAR per ACE count was observed among men up to a count of five, although confidence intervals for higher counts were wide (Figure 6-5, Appendix 4). No such trend for CAR was observed in women, although the sample size was considerably smaller than for men which meant that the estimates for individual ACEs had much wider confidence intervals (Appendix 4). Prevalence of hospitalisation and orphanage was low among the analytical sample of women in AUC, resulting in particularly wide confidence intervals.

Table 6-4 reports estimates and 95% CIs for the association of ACEs with diurnal slope. The decreasing diurnal slope was non-linear showing a steeper decline earlier in the day (Model 1). None of the adversities was associated with either the awakening value (i.e., the intercept) or with the (negative) slope. There was no

consistent direction of association between the ACEs and slope, with seven producing negative estimates and seven positive estimates in the final model. Based on the final model, I predicted diurnal slopes and their relative changes by the count of ACEs (Figure 6-6). As the number of adversities increased, the cortisol values on waking decreased by 0.642%, and the diurnal slope became flatter (i.e., a positive coefficient indicated a less steep decline) by 0.079% per hour and therefore the bedtime cortisol level was elevated.

In sex-stratified analyses, no ACEs were associated with either intercept or slope in either men or women (Appendix 5). Among men, there was a more consistent pattern with slope with the majority of ACEs having positive coefficients indicating a less steep decrease in cortisol. Among men, therefore, a similar trend was observed to that seen in the main analysis (Figure 6-6). No clear indication of patterns according to number of adversities was observed among women (Figure 6-6, Appendix 5).

Appendix 6 reports results of sensitivity analyses of the association between the cumulative score of ACEs, and AUC and CAR. Likelihood ratio tests suggested that the associations of the cumulative score with AUC and CAR were linear. On average AUC decreased by 0.640% (-1.675 to 0.396), and CAR increased by 0.113 nmol/l (-0.263 to 0.489), per additional count of ACEs, but 95% CIs included zero. These findings are consistent with the null findings from the main analysis, although estimates per ACE count are slightly different. Appendix 7 shows the results of the models for count of ACEs and diurnal slope. Cortisol on wakening decreased by 0.745% (-1.982 to 0.492) with 95% CIs including zero, which was consistent with the

effect estimated in the main analysis. The association with diurnal slope was very small at 0.031% (-0.097 to 0.159) per hour per ACE count (Appendix 8) being half the size of the coefficient from the main analysis.

The National Child Development Study

Table 6-1 presents the prevalence of ACEs. Among the analytic sample, 45.7% of participants reported at least one ACE. People who had not got along with father had the highest proportion, followed by overnight hospitalisation (13.0 %) and financial problems (11.9%). In Table 6-5 I present characteristics of study sample according to the count of ACEs. Similar distribution by the count of ACEs was observed between men and women. A higher proportion was observed in 3+ ACEs group among people who grew up in the family where fathers' occupation was manual (9.8%) than people from non-manual background (4.5%).

In Table 6-6 I present estimates and 95% CIs for the association between ACEs and two measures of salivary cortisol. None of the individual ACEs was associated with early morning cortisol (T_{N1}), and cortisol level 3.45 hours after awakening (T_{N2}). The Breusch-Pagan test showed that the residuals of these two cortisol levels in the equations are not independent of each other and therefore the multivariate model is appropriate.

Figure 6-7 shows estimated cortisol levels with 95% CIs at T_{N1} and T_{N2}. As the number of ACEs increases, cortisol level at T_{N1} shows an upward trend, while the level at T_{N2} exhibits a downward trend, although 95% CIs are wide. Per additional count of ACEs, I estimated 1.035% increase (-0.919, 2.989) for the cortisol at T_{N1}

and 1.412% decrease (-3.704, 0.880) for the cortisol T_{N2}, but confidence intervals included zero.

6.4. Discussion

I provide evidence of an effect of increasing number of retrospectively measured ACEs on the diurnal pattern of cortisol in the Whitehall II cohort study. An increasing number of adversities had no association with AUC, or CAR. As the number of ACEs increased, the cortisol values on waking decreased and the diurnal slope became flatter, but the estimated effects were small and I was unable to calculate the appropriate confidence intervals. Among 14 individual ACEs, parental death showed associations with AUC and CAR. Sensitivity analyses using a simple sum indicated little evidence of any effect. There was no association between prospectively measured ACEs and two measures of cortisol levels in daytime in the NCDS.

The percentage of participants who reported at least one ACE was 67.8% in the Whitehall II cohort study, which is in close agreement with a systematic review of studies in Europe and North America.[219] The percentage in the NCDS was, however, notably lower (45.7%), which might be due to selection bias (i.e., bias due to selection into this study from the study population), or to the fact that ACEs were measured prospectively in the NCDS.

To date, there are a limited number of studies examining the amount of cortisol secretion during the day in relation to ACEs.[152] My findings, drawn from six saliva samples across the day, indicate that a greater number of adversities is not related

to AUC, consistent with a previous study, with cortisol measured from four samples over the day.[152] One systematic review reported no association between adversities and CAR.[144] Most of the studies included in the review used data from people with underlying health conditions, such as psychosis, and their sample sizes were small.[144] My study, which has the relatively large sample size, provided little evidence to support an association between increasing number of ACEs and CAR, although an upward trend was observed, particularly in men. The CAR is thought to be associated with anticipated stress in the upcoming day.[237] For example, it has been shown that CAR on workdays are larger than on weekends with unchanged awakening cortisol levels.[238] Given that saliva samples were collected on weekdays, my results may suggest that people who experienced multiple adversities in childhood do not show physiologically heightened stress activation in the beginning of the day than those who did not.

I did not find any difference in cortisol levels on waking or on diurnal slope among people who experienced multiple adversities in childhood. In contrast, previous studies have documented an association, but these studies were conducted with specific at risk groups such as people with fibromyalgia,[147] depression,[148] and international adoptees.[149] Of the studies based on samples from the general population, findings have been limited by small sample size (61 healthy adults),[146] a smaller number of cortisol samples in a day (three samples),[150] or the examination of only one form of adversity (maternal separation).[118] Thus, my study does not corroborate these findings, but in a large study sample with multiple cortisol measures characterising diurnal patterns more precisely than previous studies. If there is an association between number of ACEs and diurnal slope it is small. The

diurnal pattern of cortisol output of a smaller slope is similar to that previously shown to be associated with adverse health outcomes.[140,142].

I performed additional analyses using the NCDS in which ACEs were measured prospectively. There was a difference in the distribution of people reporting experiencing ACEs between the Whitehall II cohort study and the NCDS. A possible explanation is due to the difference in whether ACEs were measured prospectively or retrospectively, while there are other potential reasons, such as selection into the study or cohort effect. The initial study population in the NCDS is 18 558, but only 11% of these were included in this study because of missingness in variables of interest (Figure 6-2). Although the NCDS is more nationally representative than the Whitehall II cohort study, this selection into the analytic sample might have altered the distribution of ACEs. The participants in the NCDS are, in general, younger than those in the Whitehall II cohort study. Therefore it is possible that there could be a cohort effect where types of adversities experienced by children could have changed with time.[239]

Comparisons of the findings from the Whitehall II cohort study with those from the NCDS were challenging. The Whitehall II cohort study had six saliva samples over the day, while only two measures were available in the NCDS. Apart from one measure to represent a peak value of awakening response (collected 30 min after wakening in the Whitehall II cohort study and 45 min after awakening in the NCDS), the other measures including derived variables were difficult to compare between these. Furthermore, types of ACEs collected in the NCDS are not the same as those in the Whitehall II cohort study. It is because data collection took place before it was

recognised that ACEs needed to be measured, so there were no standard questions. Despite direct comparison not being possible, no evidence of association was observed in either study for any measure considered.

I reported sex-stratified estimates in the Whitehall II cohort study as supplementary analyses because there is a potential biological reason for sex differences in the stress response among humans.[154,155,234] This difference in stress response therefore may translate into differences in associations between ACEs and cortisol, although the direction of the effect remains unclear. The Whitehall II cohort study has more men (67%) than women (33%) and thus any test for sex interaction lacks power and estimates in women were less precise than for men. However, I did observe possible different patterns in cortisol secretion in the association with adversities between men and women where the association appeared slightly larger in men than women. This possible sex-difference requires further investigation in larger studies with more equal numbers of men and women. Among various possible reasons for a sex difference, the direction of dysfunction (heightened or suppressed) may partly depend on the timing of biological assessment,[40] type of stressors,[234] as well as sex. It is, however, unlikely that participants recognised ACEs as acute stressors in middle age in the current study because of the time from childhood to adulthood. Another possibility is an interaction with underlying health conditions.[234] It has been increasingly recognised that dysfunction of the HPA axis is associated with adverse health outcomes, although the causality of association is uncertain.[138-140] It is therefore possible that there are differences in the direction of associations before and after the onset of certain diseases. I excluded people taking steroid treatments including hormone replacement therapy, but I am unable to rule out possibility that

any other diseases or conditions (e.g., menopause) may have led to the differences between sexes. Men and women are known to have different risk of developing certain diseases. For example, men are more likely to be at higher risk of CHD,[240] while women have higher prevalence of depression.[241] Two factors, sex hormones and genetic factors related to sex chromosomes, appear to play an important role, but sex differences in response to chronic stress remain poorly elucidated.[154,234,242] It is also uncertain whether how an individual's emotional and behavioural management of stress alters physiological stress reactivity, and these can differ by sex.[243] Hence, accumulating evidence for how men and women respond to chronic stress will enhance our understanding of aetiology for sex dimorphism in diseases.

While I found little evidence to suggest ACEs were related to cortisol in my study, cortisol secretion changes with age across the life course. The association of indicators of ageing with cortisol secretion is intriguing. A meta-analysis based on cross-sectional studies has documented that better physical capability, such as faster walking speed, at mid- and older ages was observed in people who had a steeper diurnal slope.[244] Similarly, cognitive capability, particularly fluid cognitive ability has been shown to be positively related to a steeper diurnal slope and lower bed-time cortisol,[245] but no association was observed with CAR.[244,245] Another marker of ageing is telomere length,[246] of which an inverse association with ACEs was reported in a meta-analysis.[247] A cross-sectional study of adults aged 18 to 65 years found that larger CAR was associated with shorter leukocyte telomere length,[248] implying a link between elevated cortisol response after awakening and accelerated ageing. My observations, showing a slight upward trend of CAR as the number of ACEs increases, might suggest that people who experienced multiple

adversities in childhood are more likely to be at risk of premature ageing. I adjusted for participants' age to take into account its effect on the association between ACEs and cortisol level given that cortisol level is known to change with age.[249] It would, however, be of interest to investigate whether adversities in childhood were associated with the trajectory of cortisol level with age, and whether the association is stronger at younger ages, which are closer to the exposure to ACEs.

My study used saliva samples collected only during the daytime from a single day. It is therefore prone to measurement error, and it is not possible to assess the total circadian rhythm of cortisol secretion. Additionally, only the self-reported time of sample collection was available. Although study participants in the NCDS are from the general population, the study population in the Whitehall II cohort study were all civil servants at the time of recruitment, so were probably healthier than the general population, which may potentially reduce the generalisability of my findings. Currently, most studies of ACEs have applied a cumulative score, which is a simple sum of adversities, possibly due to its convenience of use. However, this approach has been criticised for not taking account of different effect sizes for each adversity on outcome.[114,116] To address this issue, I fitted models which included all types of adversities as separate variables, and then averaged effect sizes according to the count of adversities, similar to some previous research.[210,218,223] I compared these results with those from models in which the cumulative score was used as a sensitivity analysis. Similar patterns were seen in the findings of AUC, CAR, and diurnal slope. One of the assumptions for a cumulative score is that each adversity contributes to the outcome equally. This assumption is, however, unlikely and each adversity has different effect size and direction of the association, as demonstrated

by my results as well as in existing literature. A downside of my methodological approach to test a dose-response effect is a difficulty in applying the approach to multilevel models. I was therefore unable to test the dose-response effect for the analysis of diurnal slope.

Although my main interest in this study was to examine the effect of multiple adversities, a particular type of adversities may be most harmful, or it might depend on the severity or timing of the adversity. I observed the association of “parental death” with increased total amount of cortisol secretion during the day and with elevated CAR. Although a study of youth aged 10 to 29 years has reported that parental death dysregulated the HPA axis during five years of follow-up,[250] the long-term association remains unclear,[251,252] requiring further research.

In conclusion, multiple ACEs appear to contribute little to dysregulation of the HPA axis in later life. My findings highlight a possible sex difference in the association, which may throw light on physiological explanations for sexual differences in stress-related diseases, and which warrants further study. As well as the HPA axis, the autonomic nervous system plays an important role in stress reactivity. I examine the association of ACEs with the autonomic nervous system in Chapter 7.

Table 6-1. Prevalence of adverse childhood experiences

Whitehall II cohort study (n = 3419)	N (%)	NCDS (n = 2117)	N (%)
Adverse childhood experiences			
Maternal separation 1yr+	330 (9.7)	Parental separation, divorce	184 (8.7)
Parental death	241 (7.1)	Parental death	34 (1.6)
Hospitalisation 4wks+	408 (11.9)	Overnight hospitalisation	275 (13.0)
Divorce	58 (1.7)		
Mental illness and alcohol problems	196 (5.7)	Mental illness	96 (4.5)
		Substance use	12 (0.6)
Arguments between parents	651 (19.0)	Domestic tension	69 (3.3)
Unemployment	334 (9.8)	Unemployment	103 (4.9)
Financial problems	848 (24.8)	Financial problems	252 (11.9)
Physical abuse	69 (2.0)		
Orphanage	15 (0.4)	Out-of-home care	27 (1.3)
Lack of attachment to mothers	8 (6 to 10)[a]	Get along with mother[b]	234 (11.1)
Lack of attachment to fathers	10 (8 to 12) [a]	Get along with father[b]	384 (18.1)
Mother's harsh punishment	2 (1 to 2) [a]		
Father's harsh punishment	2 (1 to 3) [a]		
No adverse childhood experiences, n (%)	1101 (32.2)	No adverse childhood experiences, n (%)	1150 (54.3)

a Median (inter quartile range)

b Cumulative percentage of "uncertain", "untrue", and "very untrue" is presented.

Table 6-2. Characteristics of study sample according to the counts of adverse childhood experiences) (n = 3419)

			Count of adverse childhood experiences							
		Total	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7+**
	N	**3419**	**1101**	**816**	**665**	**393**	**229**	**129**	**52**	**34**
Sex, *n (%)*										
Men	2601	100.0	883 (33.95)	628 (24.14)	502 (19.3)	284 (10.92)	153 (5.88)	94 (3.61)	35 (1.35)	22 (0.85)
Women	818	100.0	218 (26.65)	188 (22.98)	163 (19.93)	109 (13.33)	76 (9.29)	35 (4.28)	17 (2.08)	12 (1.47)
Age in years at phase 9, *mean ± SD*	3419	65.9 ± 6.0	65.24 ± 5.94	66.22 ± 6.06	66.03 ± 6.06	66.68 ± 5.65	66.34 ± 5.84	66.87 ± 6.58	64.83 ± 5.37	64.77 ± 6.09
Ethnicity, n (%)										
White	3236	100.0	1047 (32.35)	782 (24.17)	623 (19.25)	367 (11.34)	216 (6.67)	125 (3.86)	46 (1.42)	30 (0.93)
Non-White	183	100.0	54 (29.51)	34 (18.58)	42 (22.95)	26 (14.21)	13 (7.1)	4 (2.19)	6 (3.28)	4 (2.19)
Childhood socioeconomic position, *n (%)*										
Non-manual	2082	100.0	783 (37.61)	479 (23.01)	415 (19.93)	208 (9.99)	115 (5.52)	44 (2.11)	25 (1.2)	13 (0.62)
Manual	1337	100.0	318 (23.78)	337 (25.21)	250 (18.7)	185 (13.84)	114 (8.53)	85 (6.36)	27 (2.02)	21 (1.57)
Adult socioeconomic position, n (%)										
Administrative (high)	1683	100.0	590 (35.06)	403 (23.95)	329 (19.55)	183 (10.87)	98 (5.82)	53 (3.15)	13 (0.77)	14 (0.83)
Professional/executive	1421	100.0	439 (30.89)	325 (22.87)	283 (19.92)	166 (11.68)	103 (7.25)	60 (4.22)	29 (2.04)	16 (1.13)
Clerical/support	315	100.0	72 (22.86)	88 (27.94)	53 (16.83)	44 (13.97)	28 (8.89)	16 (5.08)	10 (3.17)	4 (1.27)
Smoking on the day of sample collection, n (%)										
No	3232	100.0	1056 (32.67)	765 (23.67)	621 (19.21)	370 (11.45)	217 (6.71)	123 (3.81)	47 (1.45)	33 (1.02)
Yes	187	100.0	45 (24.06)	51 (27.27)	44 (23.53)	23 (12.3)	12 (6.42)	6 (3.21)	5 (2.67)	1 (0.53)
Salivary cortisol, nmol/l (IQR)										
Wakening (T1)	3399	13.87 (9.15)	14.07 (9.40	14.55 (8.93)	13.81 (9.16)	13.43 (7.73)	13.53 (9.51)	12.70 (7.91)	12.57 (9.41)	13.05 (15.40)
+ 30 min (T2)	3399	19.85 (14.11)	19.86 (13.97)	20.51 (14.43)	19.69 (14.23)	19.13 (12.28)	20.65 (14.71)	18.33 (15.11)	20.00 (13.97)	21.99 (12.95)
+ 2.5 hrs (T3)	3404	8.5 (6.23)	8.43 (6.23)	8.78 (6.13)	8.54 (6.43)	8.55 (6.13)	8.18 (6.07)	8.18 (6.70)	8.73 (6.57)	8.83 (6.71)
+ 8.0 hrs (T4)	3383	5.27 (4.12)	5.31 (4.21)	5.36 (3.95)	5.12 (4.32)	5.37 (4.07)	5.28 (4.20)	4.97 (3.46)	4.93 (4.13)	5.64 (5.80)
+ 12.0 hrs (T5)	3380	2.71 (2.53)	2.76 (2.63)	2.63 (2.50)	2.72 (2.45)	2.64 (2.33)	2.92 (2.51)	2.62 (2.62)	2.58 (2.69)	2.71 (1.52)
Bedtime (T6)	3393	1.93 (2.00)	1.97 (1.98)	1.95 (2.07)	1.86 (2.06)	1.86 (1.85)	2.07 (2.07)	1.85 (1.54)	1.93 (2.46)	2.25 (2.76)
Cortisol awakening response	2950	5.95 (13.73)	5.90 (13.88)	6.12 (14.60)	5.74 (13.24)	5.39 (12.47)	7.76 (14.92)	5.68 (13.87)	7.70 (14.31)	7.48 (12.20)
Area under the curve (T1, T6[a]; nmol/l*hr), mean ± SD	3232	109.52 (57.12)	110.6 (58.62)	112 (54.75)	109.73 (64.14)	106.4 (48.04)	109.65 (56.72)	102.26 (49.89)	109.38 (45.82)	109.87 (72.27)

a Trapezoidal rule was used to calculate the area under the curve

Table 6-3. Coefficients and 95% confidence intervals (CIs) for the association of adverse childhood experiences with area under the curve (AUC) and cortisol awakening response (CAR) of the salivary cortisol

	AUC (n = 3232) a % (95% CI)		**CAR** (n = 2950) a b (95% CI)	
	Sex and age	All covariates	Sex and age	All covariates
Adverse childhood experiences				
Maternal separation 1yr+	-0.501 (-6.448, 5.446)	0.301 (-5.585, 6.188)	1.219 (-0.93, 3.367)	1.28 (-0.881, 3.44)
Parental death	6.51 (-0.389, 13.41)	7.04 (0.250, 13.830)	2.724 (0.32, 5.128)	2.801 (0.398, 5.204)
Hospitalisation 4wks+	2.338 (-2.816, 7.492)	2.189 (-2.885, 7.262)	-0.417 (-2.224, 1.389)	-0.534 (-2.343, 1.275)
Divorce	-2.889 (-15.547, 9.769)	-0.314 (-12.806, 12.178)	-0.889 (-5.534, 3.756)	-0.342 (-5, 4.315)
Mental illness and alcohol problems	0.686 (-6.983, 8.355)	0.512 (-7.031, 8.054)	0.329 (-2.409, 3.067)	0.368 (-2.37, 3.105)
Arguments between parents	-1.435 (-6.129, 3.26)	-1.522 (-6.138, 3.095)	-0.464 (-2.134, 1.206)	-0.441 (-2.11, 1.228)
Unemployment	4.195 (-1.657, 10.047)	4.077 (-1.713, 9.867)	2.012 (-0.072, 4.096)	1.851 (-0.245, 3.947)
Financial problems	-2.229 (-6.431, 1.972)	-1.99 (-6.146, 2.166)	0.541 (-0.962, 2.045)	0.487 (-1.026, 2)
Physical abuse	-1.52 (-13.74, 10.7)	-2.312 (-14.324, 9.70)	-1.153 (-5.715, 3.409)	-1.183 (-5.74, 3.375)
Orphanage	0.695 (-25.658, 27.049)	0.323 (-25.625, 26.271)	-2.743 (-12.075, 6.588)	-3.194 (-12.535, 6.146)
Lack of attachment to mothers	-0.274 (-1.017, 0.47)	-0.373 (-1.104, 0.359)	-0.166 (-0.432, 0.1)	-0.174 (-0.44, 0.092)
Lack of attachment to fathers	-0.107 (-0.837, 0.624)	-0.108 (-0.829, 0.613)	0.088 (-0.174, 0.35)	0.069 (-0.194, 0.331)
Mother's harsh punishment	-1.899 (-4.335, 0.536)	-1.94 (-4.335, 0.455)	-0.42 (-1.294, 0.454)	-0.465 (-1.339, 0.408)
Father's harsh punishment	0.785 (-1.359, 2.929)	0.866 (-1.245, 2.978)	0.18 (-0.591, 0.95)	0.207 (-0.565, 0.978)
Covariates				
Sex	-5.271 (-9.175, -1.366)	-4.638 (-8.737, -0.539)	1.488 (0.088, 2.889)	1.39 (-0.099, 2.879)
Age in years	0.416 (0.136, 0.695)	0.422 (0.146, 0.698)	-0.066 (-0.166, 0.034)	-0.068 (-0.168, 0.033)
Ethnicity		-13.678 (-21.157, -6.199)		-2.39 (-5.187, 0.408)
Childhood socioeconomic position		0.483 (-0.852, 1.818)		0.302 (-0.184, 0.789)
Adult socioeconomic position		-0.039 (-2.812, 2.733)		0.377 (-0.63, 1.385)
Smoking on the day of saliva sampling		5.143 (-2.078, 12.364)		0.591 (-1.953, 3.136)
Awakening time		-7.702 (-9.180, -6.223)		-0.792 (-1.342, -0.241)
Intercept	4.488 (4.284, 4.692)	5.016 (4.789, 5.243)	11.621 (4.314, 18.928)	16.455 (8.181, 24.73)

a Log-transformed values (ln(nmol/l)*hr) are presented for intercept, otherwise % in AUC, while CAR is presented in an original scale (nmol/l).
b Sex, age in years, ethnicity, childhood socioeconomic position, adult socioeconomic position, and awakening time and smoking on the day of saliva sampling.

Table 6-4. Estimates[a] and 95% Confidence Intervals (CIs) of diurnal slope of log cortisol in the association with adverse childhood experiences (n = 3400)

	b (95% CIs)			
	Model 1	**Model 2**	**Model 3**	**Model 4**
Fixed part: reference trajectory				
Log salivary cortisol at awakening (ln(nmol/l))	2.529 (2.507, 2.552)	2.523 (2.43, 2.616)	2.722 (2.566, 2.877)	2.92 (2.645, 3.194)
Time since awakening (linear, hr)	-14.032 (-14.672, -13.393)	-13.12 (-14.255, -11.984)	-9.798 (-11.576, -8.019)	-17.806 (-20.745, -14.866)
Time since awakening (quadratic, hr^2)	0.16 (0.122, 0.198)	0.16 (0.122, 0.197)	0.145 (0.107, 0.183)	0.147 (0.109, 0.185)
Fixed part: intercept				
Adverse childhood experiences; binary (ref. no experience)				
Maternal separation 1yr+		-3.297 (-10.318, 3.724)	-3.254 (-10.261, 3.754)	0.037 (-7.078, 7.153)
Parental death		2.138 (-5.811, 10.088)	2.035 (-5.9, 9.969)	3.193 (-4.698, 11.083)
Hospitalisation 4wks+		4.859 (-1.22, 10.939)	4.999 (-1.069, 11.067)	4.661 (-1.388, 10.71)
Divorce		-2.575 (-17.918, 12.768)	-2.45 (-17.764, 12.863)	1.058 (-14.238, 16.353)
Mental illness and alcohol problems		-0.77 (-9.891, 8.352)	-0.828 (-9.932, 8.276)	-1.517 (-10.567, 7.534)
Arguments between parents		-3.962 (-9.537, 1.613)	-3.863 (-9.427, 1.702)	-3.364 (-8.904, 2.176)
Unemployment		4.737 (-2.273, 11.746)	4.753 (-2.243, 11.749)	4.511 (-2.487, 11.508)
Financial problems		-3.898 (-8.919, 1.124)	-3.752 (-8.764, 1.261)	-3.382 (-8.407, 1.642)
Physical abuse		-10.798 (-25.219, 3.623)	-10.928 (-25.321, 3.466)	-8.768 (-23.09, 5.554)
Orphanage		7.387 (-22.964, 37.739)	8.002 (-22.296, 38.3)	5.799 (-24.338, 35.937)
Adverse childhood experiences; ordinal				
Lack of attachment to mothers		-0.055 (-0.943, 0.832)	-0.072 (-0.958, 0.814)	-0.112 (-0.994, 0.771)
Lack of attachment to fathers		0.407 (-0.466, 1.28)	0.431 (-0.44, 1.303)	0.247 (-0.624, 1.117)
Mother's harsh punishment		-0.948 (-3.846, 1.95)	-0.964 (-3.856, 1.928)	-0.572 (-3.455, 2.31)
Father's harsh punishment		-0.004 (-2.547, 2.54)	-0.046 (-2.585, 2.493)	-0.163 (-2.707, 2.382)
Covariates				
Awakening time			-2.914 (-4.716, -1.111)	-2.788 (-4.585, -0.99)
Sex (ref. men)				-5.278 (-10.225, -0.331)
Age in years				-0.211 (-0.544, 0.122)
Ethnicity: non-White (ref. White)				-22.373 (-31.388, -13.359)
Childhood socioeconomic position (ref. professional - highest grade)				0.675 (-0.936, 2.286)
Smoking (ref. non-smoking)				-8.594 (-17.218, 0.03)
Adulthood socioeconomic position (ref. administrative - highest grade)				-2.567 (-5.904, 0.771)

Fixed part: slope				
Adverse childhood experiences; binary (ref. no experience)				
Maternal separation 1yr+		0.753 (0.023, 1.483)	0.754 (0.027, 1.482)	0.106 (-0.629, 0.841)
Parental death		0.397 (-0.436, 1.23)	0.383 (-0.448, 1.213)	0.267 (-0.555, 1.089)
Hospitalisation 4wks+		-0.074 (-0.709, 0.561)	-0.061 (-0.693, 0.571)	-0.168 (-0.795, 0.459)
Divorce		-0.114 (-1.712, 1.484)	-0.105 (-1.697, 1.487)	-0.083 (-1.666, 1.5)
Mental illness and alcohol problems		0.252 (-0.696, 1.2)	0.252 (-0.693, 1.197)	0.444 (-0.491, 1.378)
Arguments between parents		0.433 (-0.146, 1.013)	0.434 (-0.143, 1.012)	0.376 (-0.197, 0.948)
Unemployment		0.124 (-0.605, 0.854)	0.105 (-0.623, 0.832)	0.043 (-0.682, 0.767)
Financial problems		0.054 (-0.47, 0.577)	0.088 (-0.433, 0.61)	-0.07 (-0.59, 0.451)
Physical abuse		0.774 (-0.712, 2.261)	0.725 (-0.755, 2.206)	0.596 (-0.871, 2.062)
Orphanage		-0.654 (-3.8, 2.493)	-0.518 (-3.654, 2.618)	-0.418 (-3.523, 2.687)
Adverse childhood experiences; ordinal				
Lack of attachment to mothers		-0.029 (-0.122, 0.063)	-0.032 (-0.124, 0.06)	-0.018 (-0.109, 0.074)
Lack of attachment to fathers		-0.094 (-0.185, -0.004)	-0.089 (-0.18, 0.001)	-0.072 (-0.162, 0.018)
Mother's harsh punishment		-0.217 (-0.519, 0.085)	-0.219 (-0.519, 0.082)	-0.253 (-0.552, 0.045)
Father's harsh punishment		0.216 (-0.049, 0.48)	0.205 (-0.058, 0.469)	0.241 (-0.022, 0.504)
Covariates				
Awakening time			-0.451 (-0.638, -0.264)	-0.47 (-0.655, -0.284)
Sex (ref. men)				0.09 (-0.421, 0.601)
Age in years				0.106 (0.072, 0.14)
Ethnicity: non-White (ref. White)				1.592 (0.656, 2.529)
Childhood socioeconomic position (ref. professional - highest grade)				0.023 (-0.144, 0.189)
Smoking (ref. non-smoking)				1.938 (1.04, 2.835)
Adulthood socioeconomic position (ref. administrative - highest grade)				0.436 (0.092, 0.78)
Random part				
Variance: individual level intercept	0.087 (0.071, 0.106)	0.086 (0.07, 0.105)	0.084 (0.069, 0.103)	0.079 (0.064, 0.098)
Variance: individual level slope (linear time since awakening, hr)	0.001 (0.001, 0.001)	0.001 (0.001, 0.001)	0.001 (0.001, 0.001)	0.001 (0.001, 0.001)
Covariance: individual level intercept and slope change	0 (-0.001, 0.001)	0 (-0.001, 0.002)	0 (-0.001, 0.001)	0 (-0.001, 0.002)
Variance: measurement occasions in a day level intercept	0.454 (0.442, 0.467)	0.454 (0.442, 0.467)	0.454 (0.442, 0.467)	0.455 (0.442, 0.467)

[a] In fixed part, log-transformed values (ln(nmol/l)*hr) are presented for intercept, otherwise %.

Table 6-5. Characteristics of study sample according to the count of adverse childhood experiences in the National Child Development Study (n = 2117)

		Count of adverse childhood experiences			
	Total	**0**	**1**	**2**	**3 +**
	2117	**1150**	**537**	**260**	**170**
Sex, *n (%)*					
Men	1036 (100)	574 (55.4)	268 (25.9)	115 (11.1)	79 (7.6)
Women	1081 (100)	579 (53.3)	269 (24.9)	145 (13.4)	91 (8.4)
Childhood socioeconomic position, *n (%)*					
Non-manual	692 (100)	423 (61.1)	166 (24.0)	72 (10.4)	31 (4.5)
Manual	1425 (100)	727 (51.0)	371 (26.0)	188 (13.2)	139 (9.8)
Salivary cortisol (nmol/l), median (IQR)					
+ 45 min (T1N)	19.7 (14.0 to 26.7)	19.3 (13.9 to 26.4)	20.1 (13.5 to 26.2)	20.7 (15.5 to 27.9)	19.9 (14.0 to 28.6)
+ 3.45 hrs (TN2)	6.7 (4.6 to 9.5)	6.8 (4.6 to 9.6)	6.7 (4.6 to 9.4)	6.7 (4.7 to 9.4)	6.2 (4.1 to 9.3)
Waking time (hr), mean ± SD	7.4 ± 1.2	7.4 ± 1.1	7.5 ± 1.4	7.5 ± 1.2	7.4 ± 1.4

Table 6-6. Estimates[a] and 95% Confidence Intervals (CIs) of log cortisol at age 44/45 in the National Child Development Study (n = 2117)

	Model 1			Model 2			Model 3		
Cortisol measured at 45 min after awakening									
Constant	3.553	3.331	3.775	3.545	3.32	3.77	3.498	3.264	3.733
Awakening time (hr)	-8.509	-10.3	-6.718	-8.501	-10.3	-6.705	-8.469	-10.26	-6.675
Time since awakening (hr)	3.035	-19.67	25.74	3.003	-19.76	25.76	-0.028	-22.84	22.78
Adverse childhood experiences; binary (ref. no experience)									
Parental separation, divorce				1.866	-5.389	9.121	1.855	-5.39	9.101
Parental death				11.12	-7.087	29.32	11.95	-6.245	30.15
Hospitalisation due to longstanding illness				-0.683	-7.312	5.947	-0.587	-7.209	6.035
Mental illness				0.613	-10.57	11.79	0.788	-10.38	11.96
Substance abuse				1.662	-30.08	33.41	1.965	-29.74	33.67
Domestic tension				0.112	-13.33	13.56	-0.195	-13.62	13.23
Unemployment				2.353	-8.769	13.47	2.234	-8.891	13.36
Financial problems				-0.263	-7.656	7.129	0.182	-7.284	7.648
Out-of-home care				0.511	-19.73	20.75	1.764	-18.49	22.02
Adverse childhood experiences; ordinal									
Get along with mother				-0.569	-3.852	2.715	-0.423	-3.704	2.858
Get along with father				0.756	-2.067	3.58	0.53	-2.299	3.36
Confounders									
Sex							5.561	1.098	10.02
Childhood socioeconomic position							-0.642	-2.492	1.209
Cortisol measured at 3.45 hours after awakening									
Constant	3.026	2.611	3.44	3.035	2.619	3.451	3.166	2.739	3.593
Awakening time (hr)	-8.741	-10.85	-6.634	-8.745	-10.85	-6.635	-8.762	-10.87	-6.655
Time since awakening (hr)	-12.17	-21.98	-2.366	-12.29	-22.08	-2.491	-12.29	-22.07	-2.501
Adverse childhood experiences; binary (ref. no experience)									
Parental separation, divorce				3.662	-4.849	12.17	3.858	-4.639	12.36

Parental death		-19.86	-41.24	1.51	-21.24	-42.59	0.116
Hospitalisation due to longstanding illness		2.815	-4.963	10.59	2.808	-4.958	10.57
Mental illness		0.46	-12.66	13.58	0.491	-12.61	13.59
Substance use		6.897	-30.35	44.14	5.769	-31.41	42.95
Domestic tension		-5.002	-20.79	10.78	-4.627	-20.38	11.13
Unemployment		-10.79	-23.85	2.266	-10.08	-23.14	2.983
Financial problems		-5.553	-14.24	3.13	-5.169	-13.93	3.594
Out-of-home care		3.351	-20.41	27.11	2.638	-21.13	26.4
Adverse childhood experiences; ordinal							
Get along with mother		-0.682	-4.535	3.171	-0.874	-4.722	2.974
Get along with father		0.865	-2.449	4.178	1.255	-2.063	4.574
Confounders							
Sex					-7.463	-12.68	-2.247
Childhood socioeconomic position					-0.772	-2.946	1.401
Correlation of residuals							
Coefficient	0.133			0.135			0.138
Breusch-Pagan test of independence	p <.001			p <.001			p <.001

[a] Estimates are shown in %, apart from constant presented in logarithm scale

Figure 6-1. Flow chart of follow-up in the Whitehall II cohort study

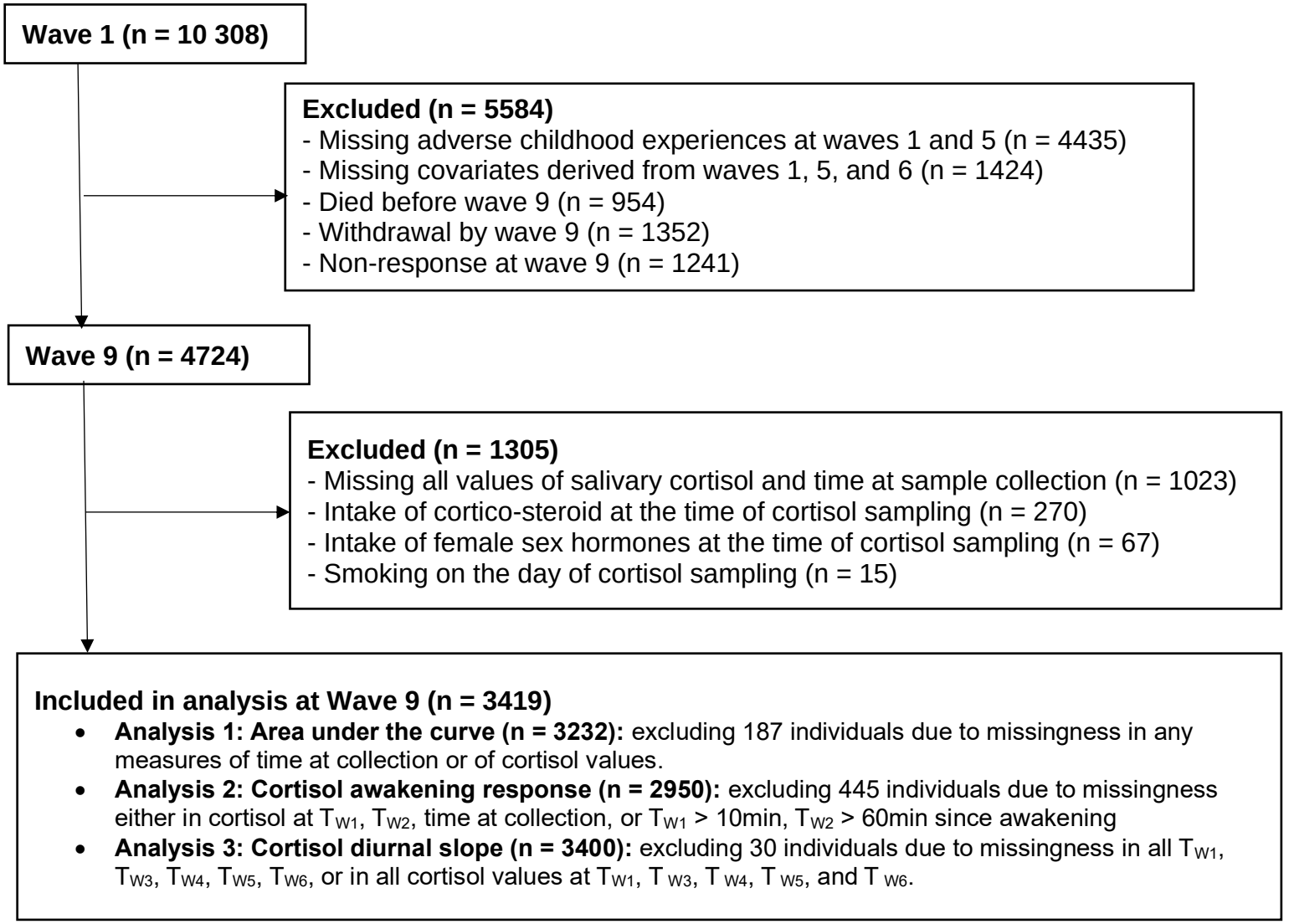


Figure 6-2. Flow chart of participants in the National Child Development Study

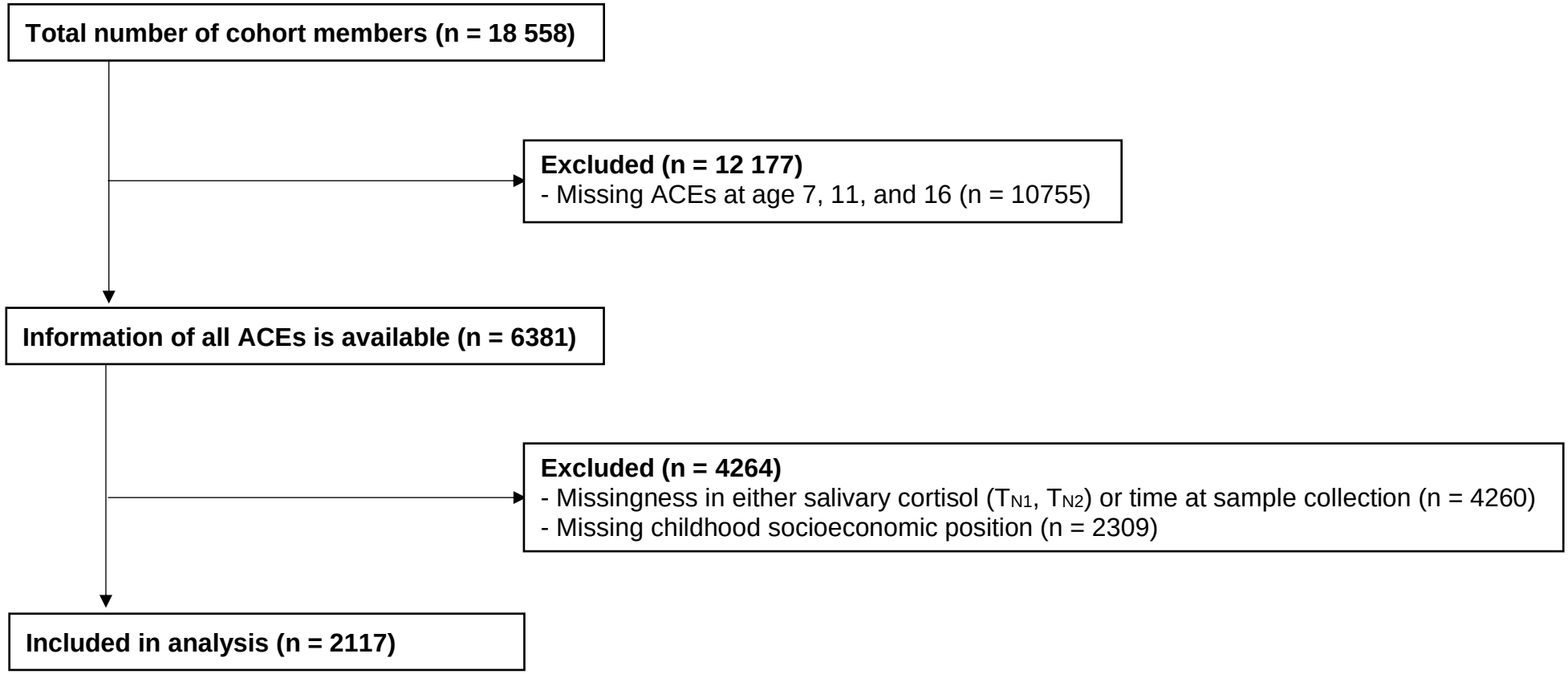


Figure 6-3. Diagram for the association between adverse childhood experiences and incident coronary heart disease (CHD)

: mediators

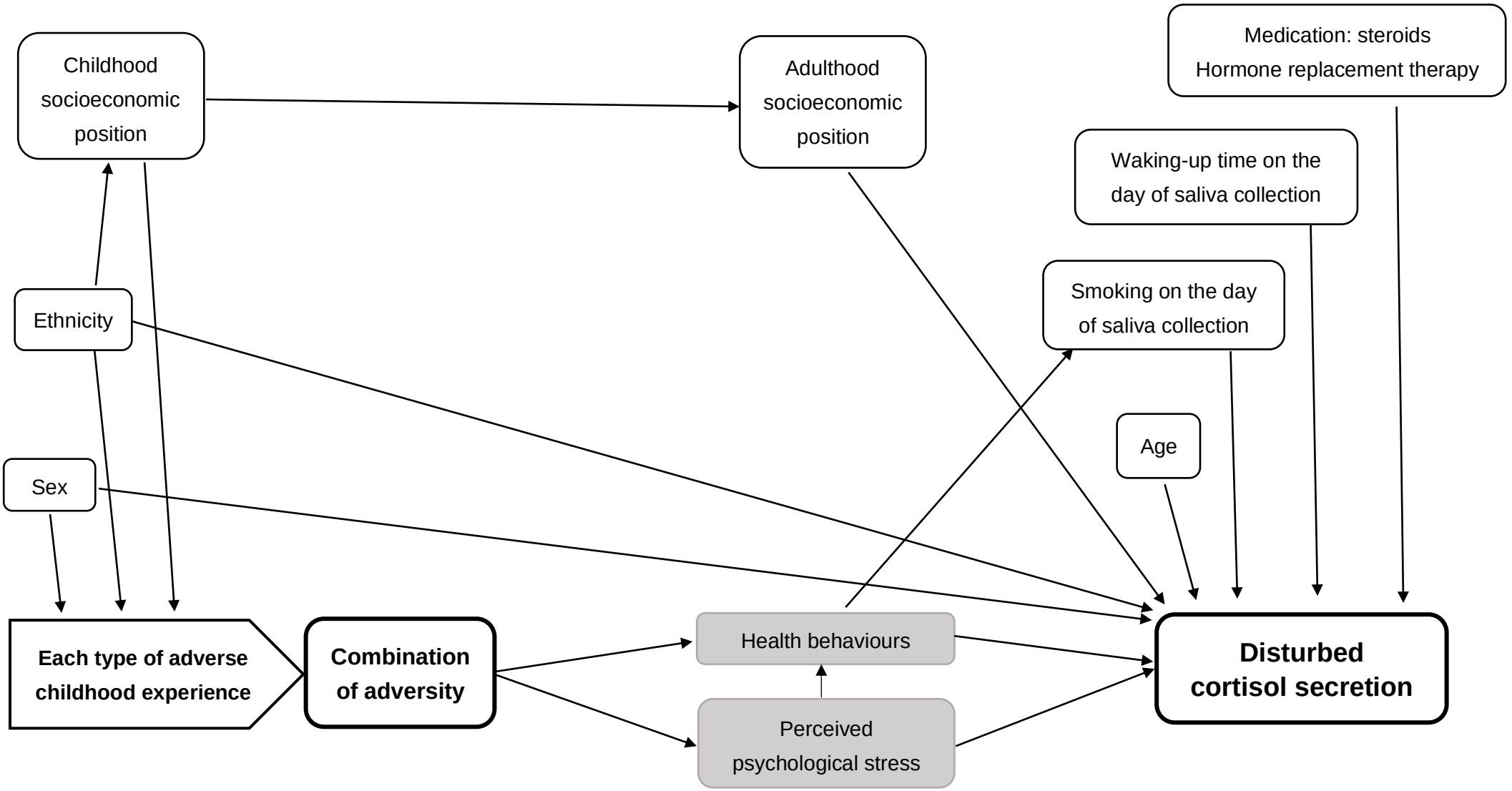
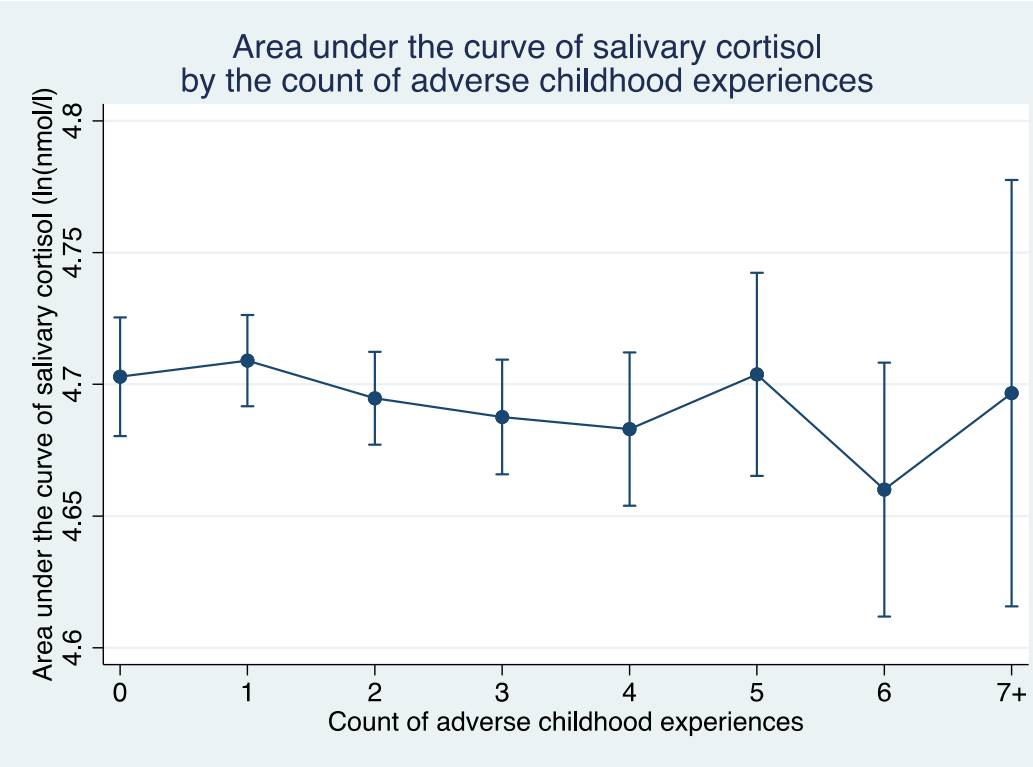
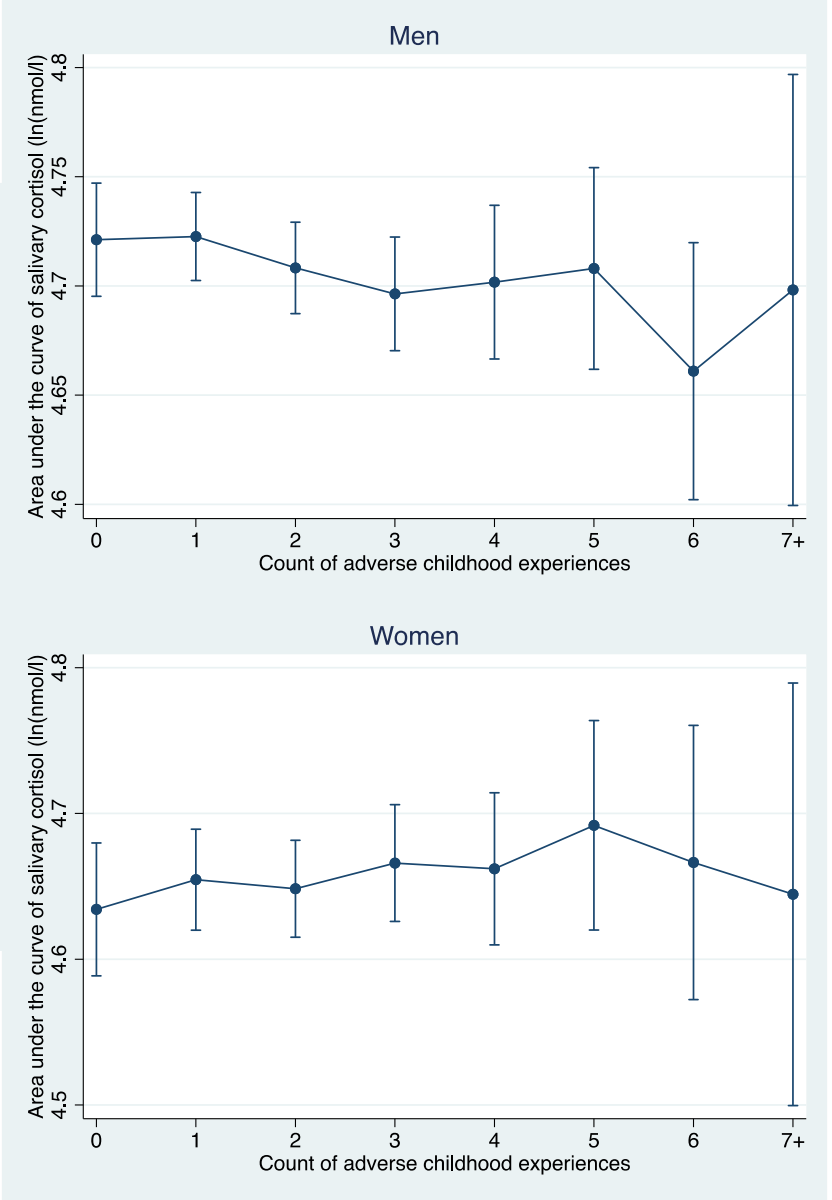


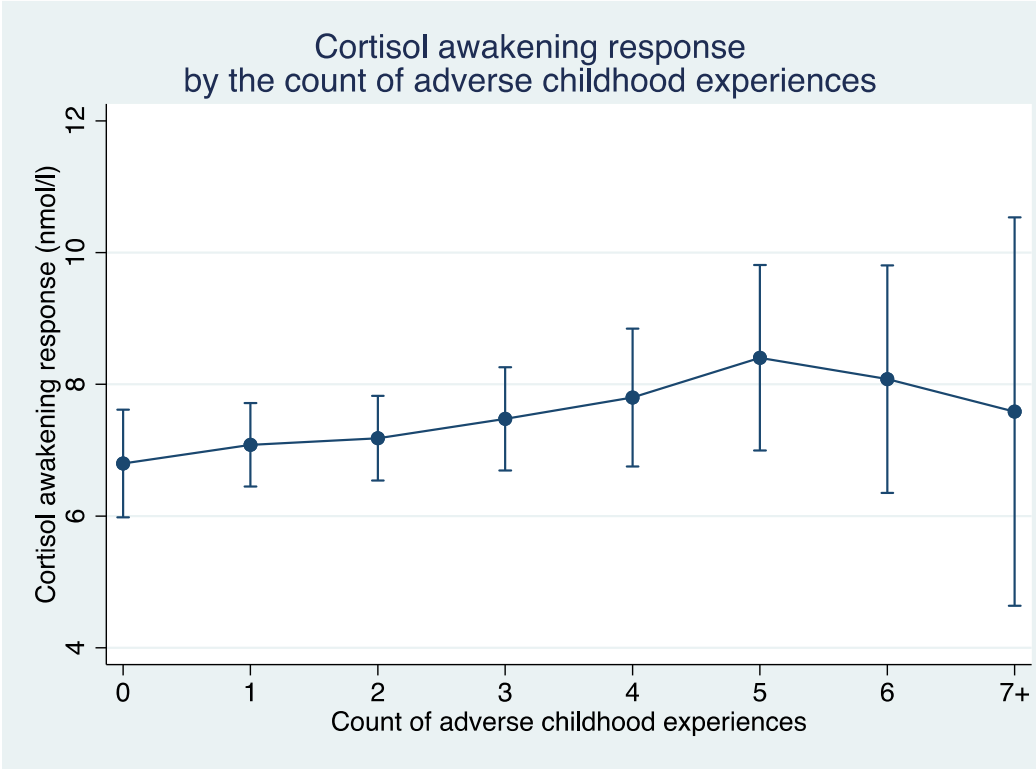
Figure 6-4. Estimated area under the curve with 95% CIs by the count of adverse childhood experiences (n=3232)

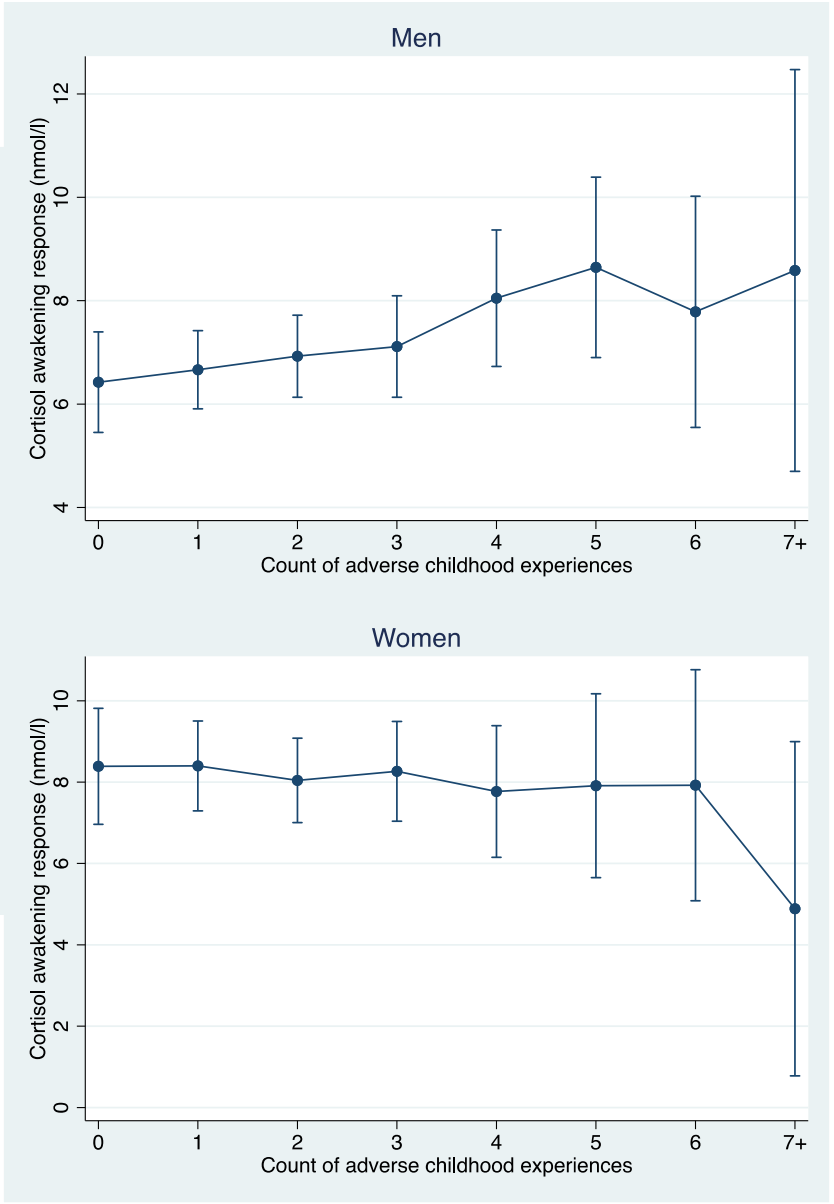




a. Model including all 14 ACEs, and adjusted for sex, age, ethnicity, childhood and adult socioeconomic position, and smoking and awakening time on the day of sample collection

Figure 6-5. Estimated cortisol awakening response (CAR) and 95% CIs by the count of adverse childhood experiences (n = 2950)





a. Model including all 14 ACEs, and adjusted for sex, age, ethnicity, childhood and adult socioeconomic position, and smoking and awakening time on the day of sample collection

Figure 6-6. Predicted diurnal cortisol slope and relative changes by the count of adverse childhood experiences (n = 3400)

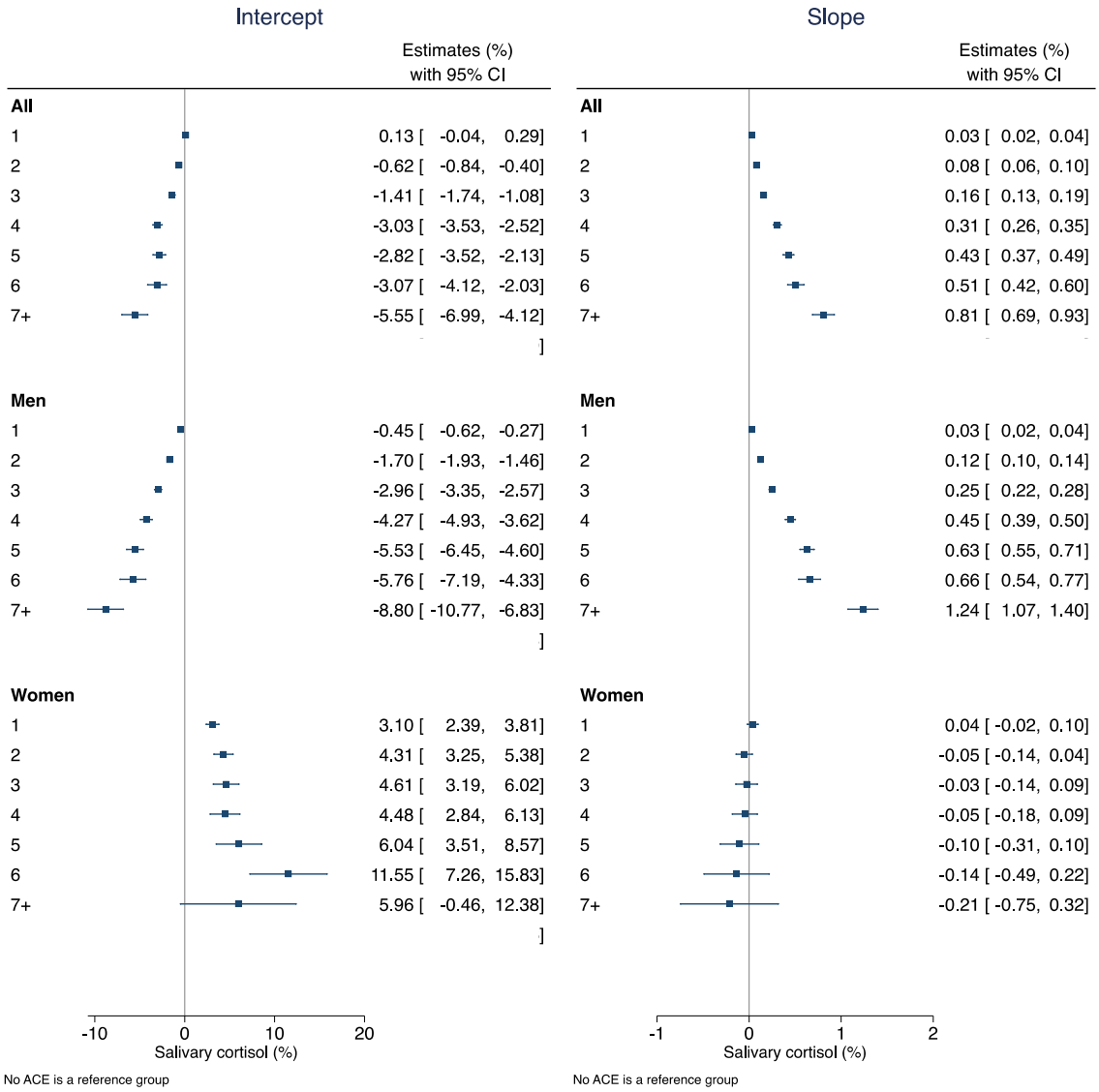
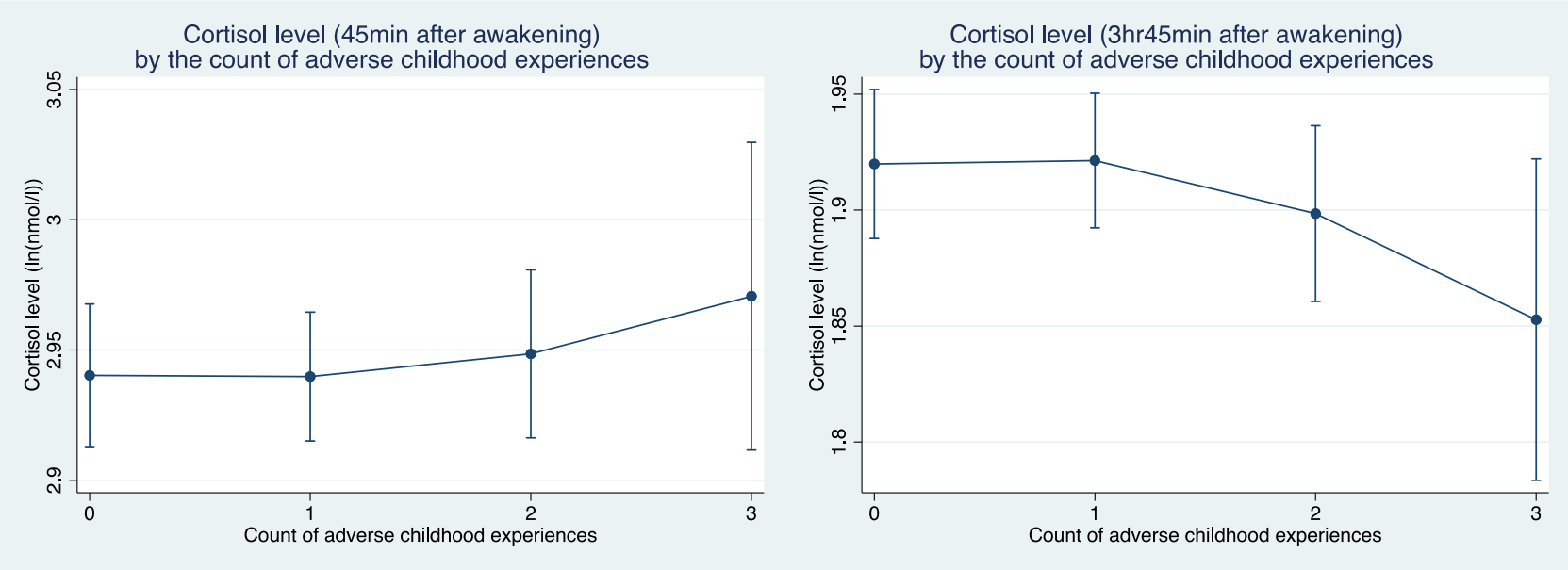


Figure 6-7. Estimated cortisol levels and 95% CIs by the count of adverse childhood experiences (left: 45 min after wakening, right: 3 hrs 45 min after awakening)



Chapter 7 Association of adverse childhood experiences with heart rate variability

7.1. Introduction

I demonstrated, in Chapter 5, a weak relationship between number of ACEs and increased hazard of CHD. I hypothesised two potential biological pathways, linking ACEs with physical diseases, particularly CHD. In chapter 6 I show that one of these hypothesised pathways – via the endocrine system – is unlikely to be a major pathway in the relationship as I found no evidence of associations between ACEs and cortisol in the Whitehall II cohort study and if associations do exist, they appear to be small. In this chapter I investigate the association between ACEs and the autonomic nervous system, the second hypothesised biological pathway.

A pathway linking ACEs with CHD via the HPA axis has been increasingly researched, as described in Chapter 6. Few studies, however, have examined the possible pathway via the autonomic nervous system.[172,173] A cross-sectional study of 10 260 people with mean age of 44.3 years (standard deviation, SD: 13.2) documented no association of child maltreatment (emotional neglect, emotional abuse, physical abuse, and sexual abuse) with SDNN and RMSSD measured at a single time point, after adjusting for confounders.[172] Similarly no association with childhood trauma was observed in another study which used heart rate, respiratory sinus arrhythmia, and pre-ejection period as markers of HRV.[173] Sex differences were, either not found[172] or not addressed.[173] These studies used a particular type of severe ACEs and HRV was measured at a single time point when the mean age of participants was 44.3 (13.2)[172] and 41.7 (13.1).[173] It also remains unclear whether HRV changes with age differently in people who experience adversity in childhood

compared with those who did not. HRV decreases with increasing age and therefore associations may be observed between ACEs and rate of change in HRV across later life. As the autonomic nervous system is known to function differently in men and women,[170,171] and ACEs have a potentially different effect on women compared with men it is important to consider possible sex differences in associations. Moreover, given that the HRV is related to sinus node function, careful consideration is required as to whether to include people who have had cardiac events at baseline and over the follow-up period.

Accordingly, I hypothesised that people who had adversity in childhood exhibit disrupted autonomic activity later in life. The objectives of the current study are (i) to examine whether people who reported ACEs have lower rHR and HRV in mid to later life from that of those who did not, and (ii) to assess whether the association of adversity with the rHR and HRV is constant over age by sex.

7.2. Methods

Study population

The study population are the participants in the Whitehall II cohort study. I included participants who had at least one recording of rHR and HRV, no missing values in covariates and ACEs, and no history of cardiac events by the time when rHR and HRV were measured. My analytical sample includes 3827 (Figure 7-1).

Exposure and outcome

The assessment of ACEs and variables derived from them, and assessment of HRV and rHR are described in section 3.3, Chapter 3.

Covariates

Potential confounders and predictors were identified based on existing literatures and by a theoretical diagram. Assuming that this diagram is correct (Figure 7-2). I included sex, age at baseline, ethnicity (white vs. non-white), and childhood and adult socioeconomic position, as demographic covariates. Sex, age, ethnicity, and father's occupational grade, a marker of childhood socioeconomic position, were derived from baseline (1985 to 1988). Employment grade, a marker of adult socioeconomic position, was derived from the waves when electrocardiographic samples were recorded, as a time-varying variable. Father's occupation was measured in Registrar General's Social Class Scheme, with categorisations of 'professional', 'managerial/technical', 'skilled-non-manual', 'skilled-manual', 'partly skilled', and 'unskilled'. Employment grade in adulthood was categorised as 'administrative' (high grade), 'professional/executive' (intermediate grade), and 'clerical/support' (low grade). I also included medication intake at the time of electrocardiographic samples collection as potential predictors. Types of medication were beta-blocker, angiotensin-converting enzyme inhibitors, calcium channel blocker, and diuretics.

Statistical analysis

I described the prevalence of ACEs in the analytical sample, and the distribution of covariates and indices of rHR and HRV by the number of ACEs.

I performed sex-stratified analyses in the light of sex differences in the autonomic nervous system. Log-transformations were used for SDNN, RMSSD, LF, and HF as their distributions were right skewed, while rHR was not transformed given its normal distribution.

I applied a multilevel linear regression in which measurement occasions was level 1, nested within individuals as level 2, as described in section 3.5, Chapter 3. Models were fitted separately for each index of HRV and rHR. The natural logarithm of the HRV was used to fit the model, and then multiplied by 100 in order that the results were interpreted as percent changes in HRV per unit change in an exposure.[233]

I estimated random effects for intercept and for the linear term of age, which was centred at age 60. The inclusion of a random quadratic term, and interaction terms of ACEs and other covariates with age did not improve the model fit and were thus not included in the models. For the fixed part of the model, model 1 included the linear and quadratic terms for centred age in years, and age at baseline (i.e., age at the first measurement); model 2 additionally included all 14 ACEs; and model 3 included ethnicity, childhood socioeconomic position, adult socioeconomic position (time-varying), and medication intake (time-varying). Based on model 3, I predicted individual rHR and HRV for each individual, and plotted the average by the count of ACEs, when all the covariates were set to their baseline values (coded as zero in the model). As for diurnal slope (Chapter 6), this approach, however, underestimates the error and provides 95% CIs which are too narrow and therefore should be interpreted with caution. It was not possible to use the method described in 5.2 to estimate the correct constant of proportionality.

7.3. Results

Among the 3932 participants in the analytical sample, 67.9% (men: 65.9%, women: 73.9%) reported at least one adverse experience in childhood (Table 7-1). Financial problems had the highest prevalence both in men (23.6%) and women (30.0%), followed by arguments between parents (men: 16.9%, women: 25.0%). Median age in years (interquartile range) at baseline was 54.3 (10.3) for men and 54.7 (10.2) for women. Around two thirds of the analytical sample, dominated by white ethnicity, were from non-manual childhood socioeconomic position. In men, approximately half of them was in administrative posts (high grade), while half of women was in professional/executive posts (intermediate grade).

Average rHR and HRV by the covariates at baseline, 5 years, and 10 years follow-up time are shown in Table 7-2 (rHR), Table 7-3 (SDNN), Table 7-4 (RMSSD), Table 7-5 (LF), and Table 7-6 (HF). All indicators of HRV exhibited a downward trend from the baseline to 10 years follow-up time. Consistent with this, decreases in means for all measures were also seen from the younger to older age category. Women showed higher mean rHR, RMSSD, and HF, but lower LF than men. Non-white people showed slightly higher RMSSD and HF, but lower LF, than white people. Those of whom father's occupational grade were non-manual had higher LF than those from manual occupational background, as did participants in the highest employment grade compared to those in the lowest employment grade.

Figure 7-3 presents average trajectories of rHR and HRV from models including linear and quadratic terms of age and adjusted for baseline age. In both men and

women, rHR showed a U-shaped relationship with a gentle decline until age 61.4 in males and age 60.5 in females, followed by an increase with age, although the range of change is small. A downward trend was observed in all indicators of HRV with age, consistent with previous reports, with no evidence of any quadratic effect.[253,254]

Estimates for three models for each outcome by sex are reported in Table 7-7, Table 7-8, Table 7-9, Table 7-10, Table 7-11, Table 7-12, Table 7-13, Table 7-14, Table 7-15, and Table 7-16. The associations between each ACE and the outcomes in models 2 and 3 are constant across longitudinal age, as preliminary analysis found no evidence of ACE by age interaction.

In model 3, as age increases, the average linear decline of rHR is 0.16 bpm per year in both men (95% confidence intervals (CIs): -0.205 to -0.115) (Table 7-7) and women (-0.239 to -0.086) (Table 7-8). Among men there is evidence of a deviation from linear decline (age squared: coefficient, 95% CIs; 0.004, 0.001 to 0.007). There is little evidence of a consistent effect on rHR across individual ACEs, with some showing a positive and others a negative coefficient. Men who had been in an orphanage had a 5.521bpm (-10.769 to -0.273) lower mean rHR compared to others, although the confidence intervals were wide. There was no such association observed among women. The largest effects for women were for parental unemployment and physical abuse. Those experiencing parental unemployment had a mean rHR 2bpm (-4.007 to -0.001) lower than others, while those experiencing abuse had a mean 2.5bpm higher (-0.493 to 5.429) but the confidence intervals included zero. Those taking beta-blockers exhibited lower rHR than those not.

All indicators of HRV showed decline with age, and no consistent association with individual ACEs was observed. In men, there was no evidence that any ACEs were associated with SDNN (Table 7-9), RMSDD (Table 7-11), LF (Table 7-13) or HF (Table 7-15). Women who had been in an orphanage did have higher SDNN, RMSDD, and HF than others (Table 7-10, Table 7-12, Table 7-16) in both model 2 and in the fully adjusted model, model 3. Women who had experienced parental unemployment also had slightly higher SDNN (0.082 in model 3) although the 95% CIs (-0.004 to 0.168) did include zero and RMSDD (0.126; 0.006 to 0.246). Being in an orphanage also produced the largest estimate for LF (0.63 in model 3), although the 95% CIs included one (Table 7-14) and for HF (1.401, 0.306 to 2.495) (Table 7-16). Intake of beta-blockers showed a positive association with RMSSD and HF in both sexes. In both time and frequency domains, there was no clear difference between men and women by the count of ACEs (Figure 7-4 and Figure 7-5)

7.4. Discussion

This study provides little evidence for the association between increasing number of adversities and disrupted autonomic activity, consistent with existing studies.[172,173] I hypothesised disrupted autonomic activity as a potential pathway of the association between ACEs and CHD by contributing to the development of CHD, through for example elevated blood pressure, or through triggering the incidence of CHD via for example, heightened coagulation. However, my study did not support the hypothesis.

It is well-recognised how the autonomic nervous system responds to acute stress. However, it remains unclear whether chronic stress across the life course leads to

disruption of autonomic activity, and if so, how the activity, which is a consequence of interaction between sympathetic and parasympathetic systems, changes over age. In recent years, two systematic reviews have reported low vagal activity, characterised as low RMSSD and HF, in relation to chronic stress[255] and posttraumatic stress disorder (PTSD).[256] The main difference from my study, which showed no association with HRV, is that it was unknown whether stress was present at the time of HRV assessment. I did not distinguish whether participants reporting ACEs were currently experiencing stress, although it is likely that people who experienced adversities in childhood have difficulties in coping with stress in adulthood,[257] or have PTSD caused by ACEs later in life.[258,259] On the other hand, the focus of the two existing systematic reviews was the presence of stress at the time of assessment. It might be possible that a key determinant of disrupted autonomic activity is the presence of current stress status, which is known to be strongly associated with ACEs.[219] This may mean that current stress could act as a modifier either, in a positive or negative way, of ACEs.

This is the first study to show profiles of age-related changes in rHR and HRV by ACEs in men and women across middle to older age. Both men and women exhibited little evidence in the association between adversity and rHR in my study. In contrast, existing studies have documented that disadvantaged childhood socioeconomic position is associated with elevated rHR over the lifecourse,[260] and subsequently the elevated rHR could predict increased risk of cardiovascular and all-cause mortality.[261] Given that ACEs are events rather than circumstances, findings of my study and of the previous study are not directly comparable, but it may imply that disadvantaged circumstances are a more powerful driver of lifelong rHR than

adverse events in childhood. It also raises the question of whether there is clustering of ACEs in people from disadvantaged backgrounds and whether this may play a role in explaining any social gradient.

Trajectories of SDNN, a summary measurement of variability, appeared to be different between men and women (

Figure 7-4) while the existing study reported no association of adversity with SDNN, and no sex difference.[172] This might be partly due to differences in types of adversities included in the studies, and reliability of SDNN measurements. The task force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology warns that it is not appropriate to compare SDNN measures recorded over different durations.[161] It is therefore difficult to compare the results of my study using a more reliable measure of ECG recorded for five minutes, with the previous study, which did not report the length of ECG recording.[172] The RMSSD, HF, and LF are highly correlated measures, among which RMSSD and HF reflect vagal activity.

In resting conditions, LF is thought to demonstrate baroreflex activity, negative-feedback of blood pressure control, which is mainly vagally mediated.[262] Disturbed vagal modulation is reported to be associated with traditional risk factors of CVD and mortality,[263] and medically unexplained physical symptoms.[264]

This study has limitations and strengths. Due to the availability of good quality HRV measures, I used the Whitehall II cohort study for this analysis. However, as mentioned previously, this study has only retrospectively measured ACEs which may be prone to reporting bias.[102] As described in Chapter 3 the participants of the Whitehall II cohort study were civil servants (men: 67%, women: 33%) at the time of recruitment, so they were more likely to be healthier than general population potentially reducing the generalisability of the findings. The sex imbalance in the study sample also led to less precise estimation among women than men, making it challenging to draw conclusions about the women and about sex differences.

As rHR and HRV were recoded for five minutes in resting conditions, the measurements mainly represents parasympathetic nervous control, rather than dynamic inter-relation between sympathetic and parasympathetic nervous systems.[265] That is, my findings are limited to variation of parasympathetic activity, as well as possible errors due to short-term measurement. As in my other analyses, I dealt with ACEs by taking account of different effect sizes of each type of adversity in relation to the outcome, while there is limitation in underestimating the errors, leading to narrow confidence intervals. Furthermore, my study used rHR and HRV measured at three time points to estimate the trajectory of measures across age, while the existing studies used measurements collected at a single time point.[172,173] This means that I am able to investigate the association of ACEs on change with age as well as level. Given that analysis using a single measurement is susceptible to regression dilution bias due to measurement errors,[266] my estimation may be less biased than existing studies.

In conclusion, there is little evidence that ACEs are associated with rHR and HRV in either men or women. It is therefore unlikely that experience of adversities in childhood triggers incidence of CHD through the impact on HRV. To examine whether a dynamic inter-relation between sympathetic and parasympathetic nervous systems is dysregulated by ACEs, it would be interesting to investigate the circadian rhythm of autonomic activity in association with adversity in future research.

Figure 7-1. Flow chart of participants' recruitment

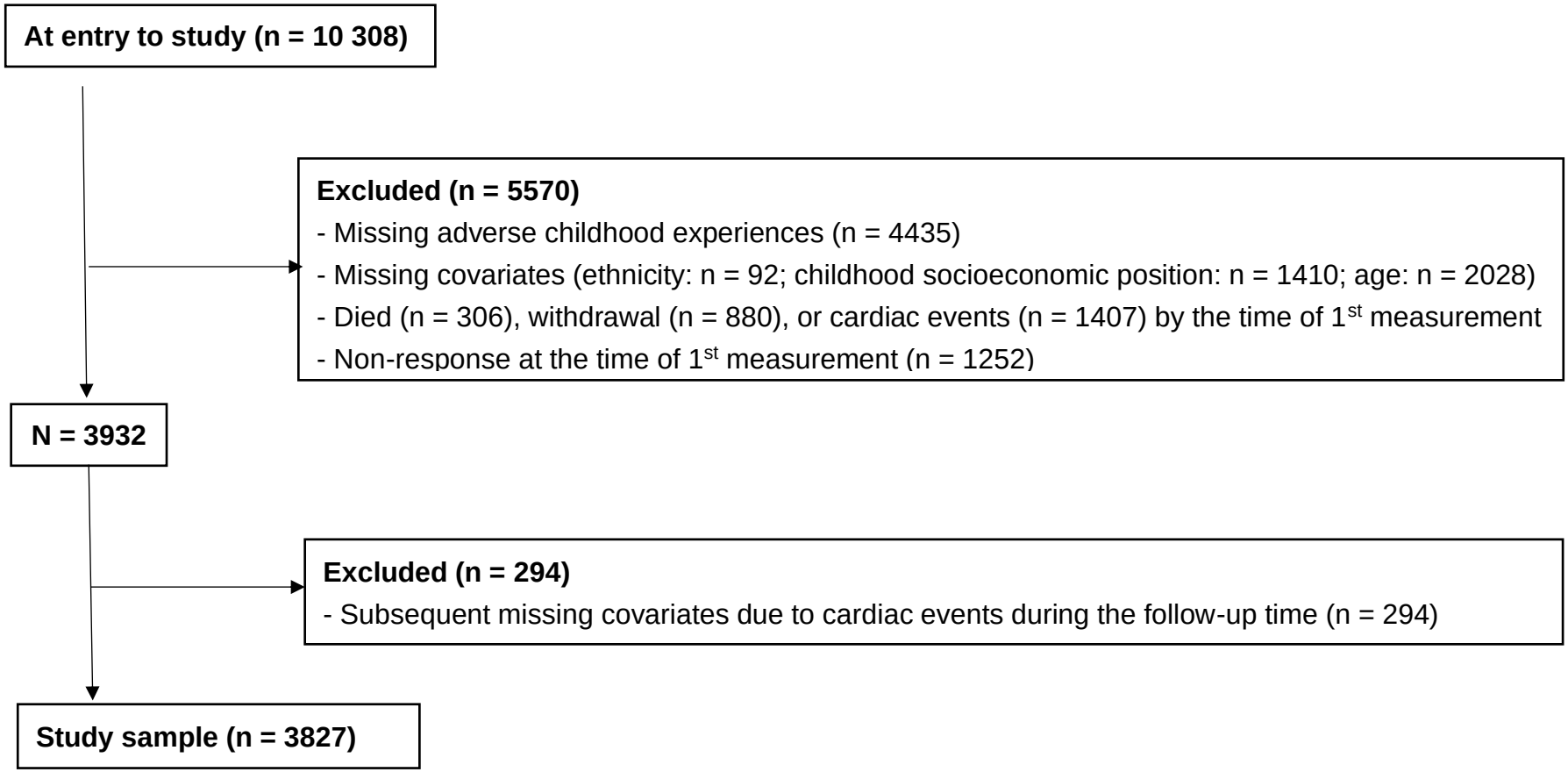


Figure 7-2. Directed acyclic graph for the association between adverse childhood experiences and resting heart rate and heart rate variability

: mediators

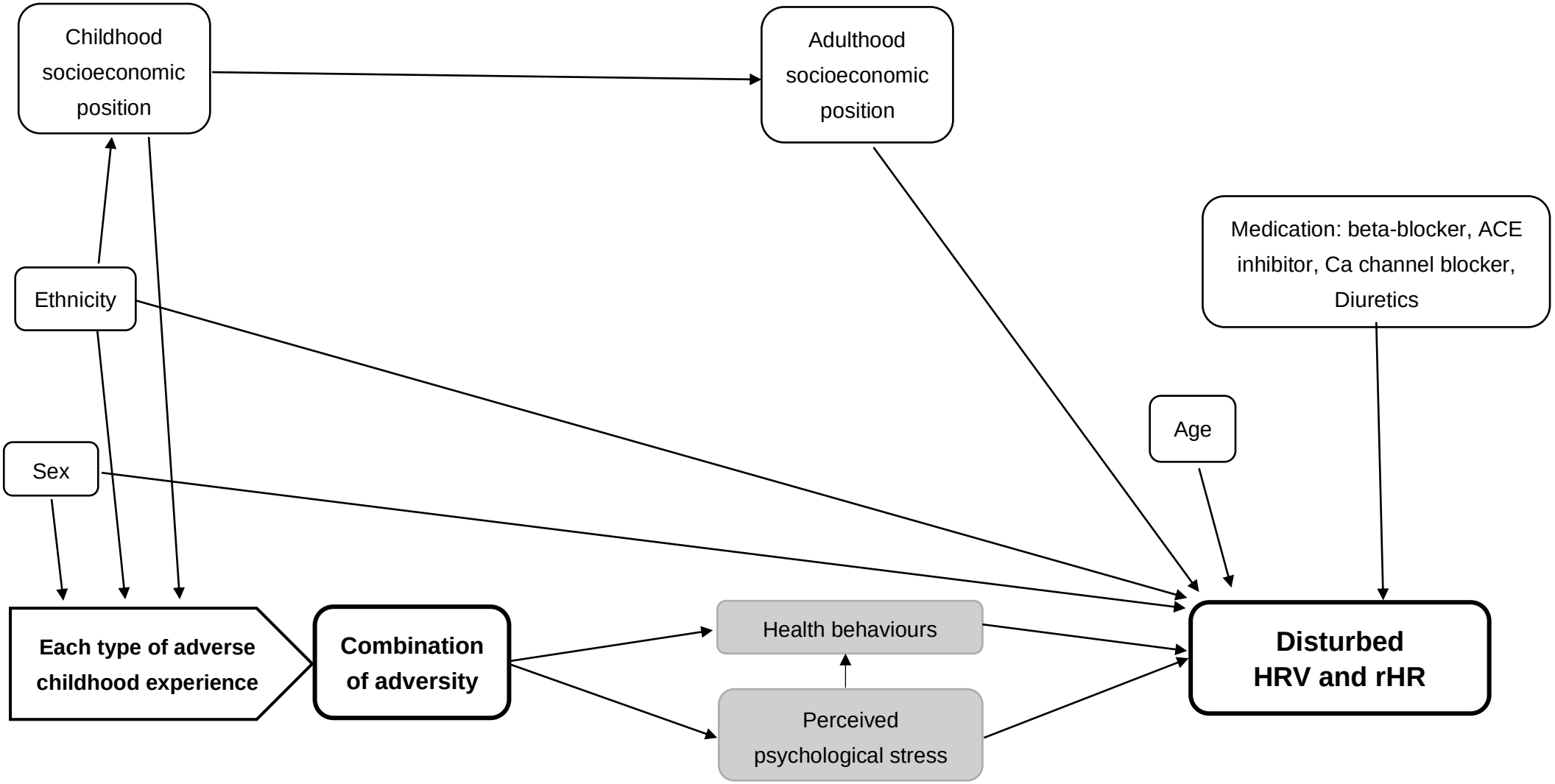
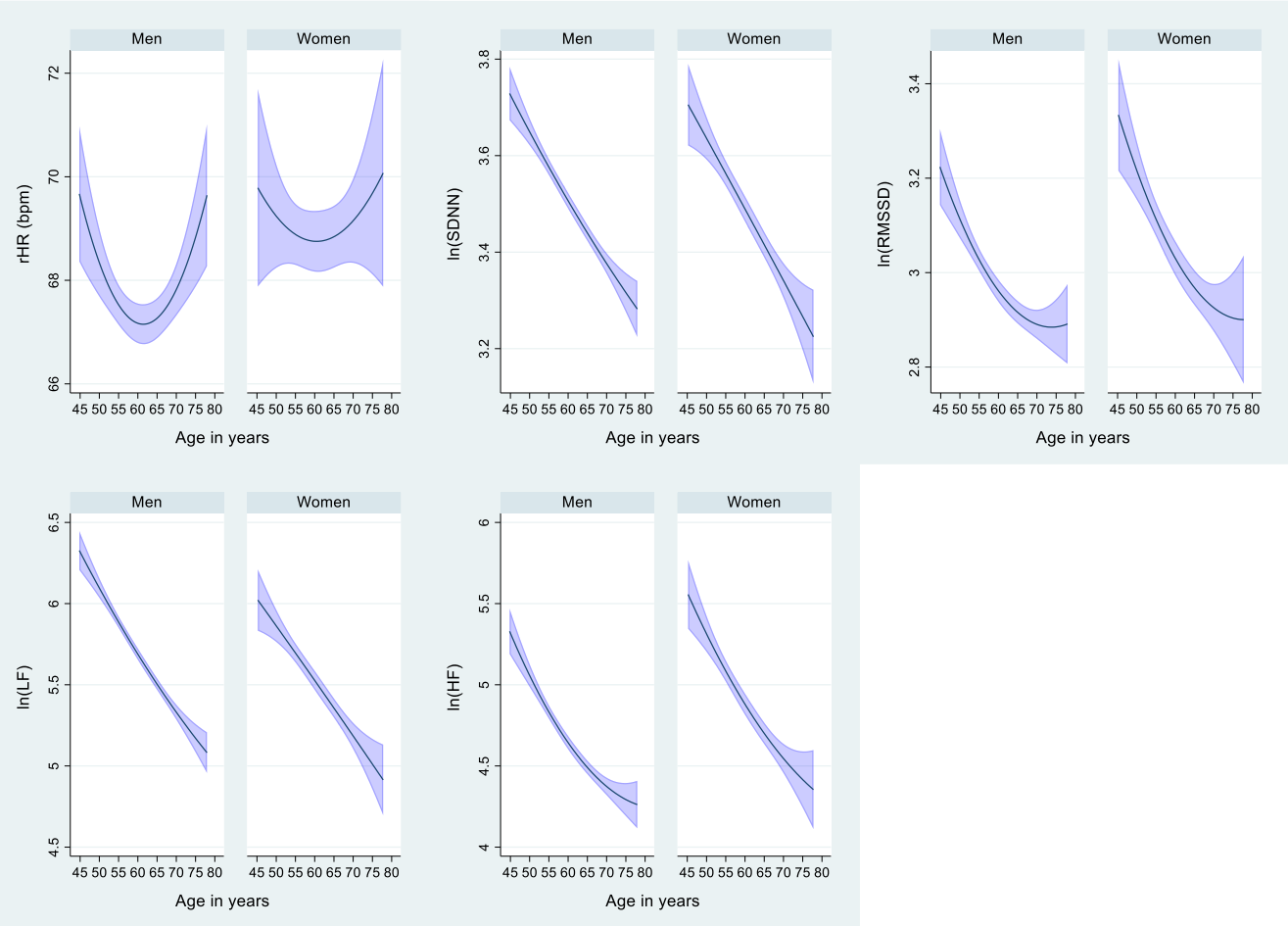


Figure 7-3. Average trajectories of resting heart rate and heart rate variability among men and women



Adjusted for linear term and quadratic term of age.

Figure 7-4. Relative changes in resting heart rate and heart rate variability (time domain) according to the count of adverse childhood experiences

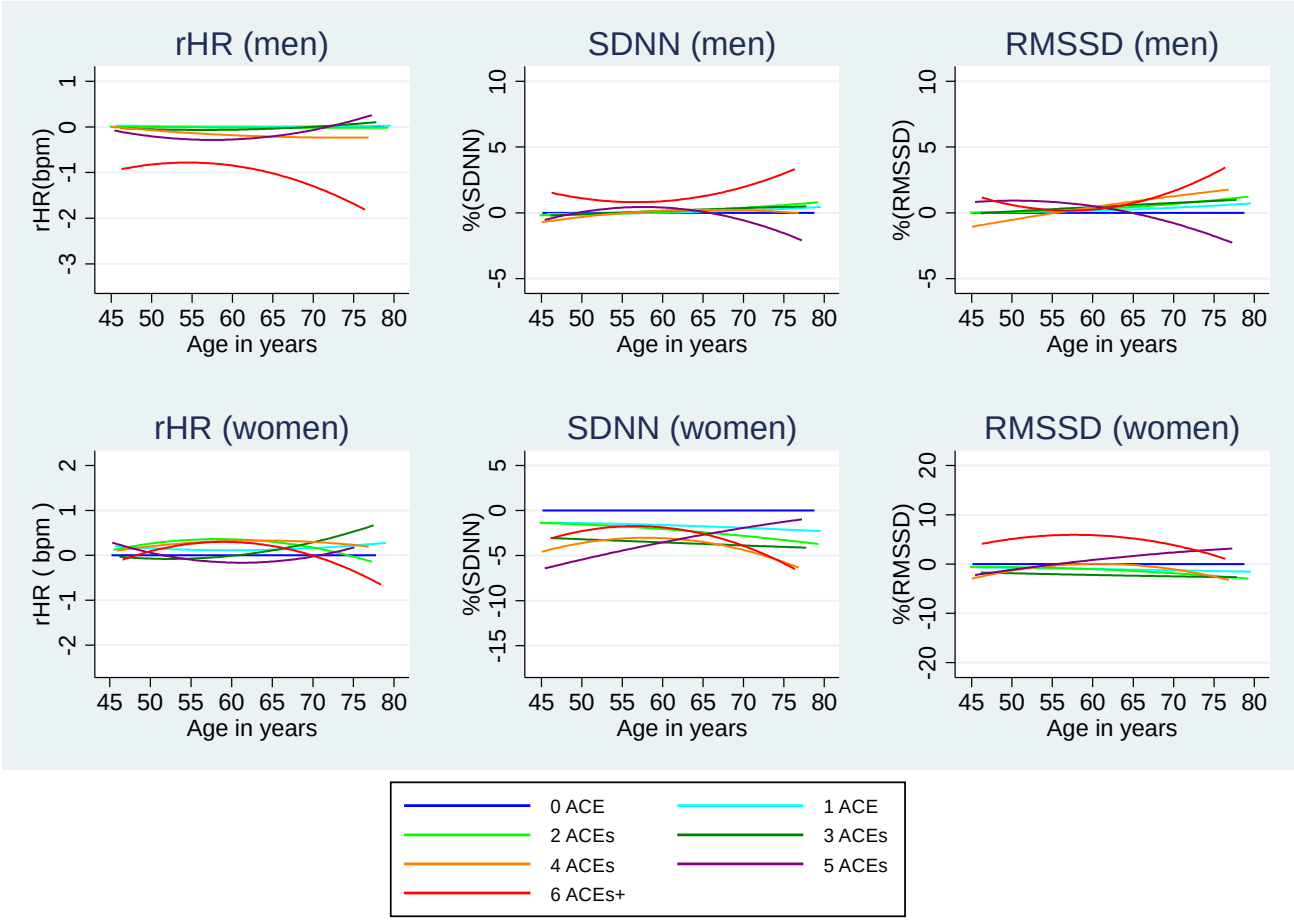


Figure 7-5. Relative changes in heart rate variability (frequency domain) according to the count of adverse childhood experiences

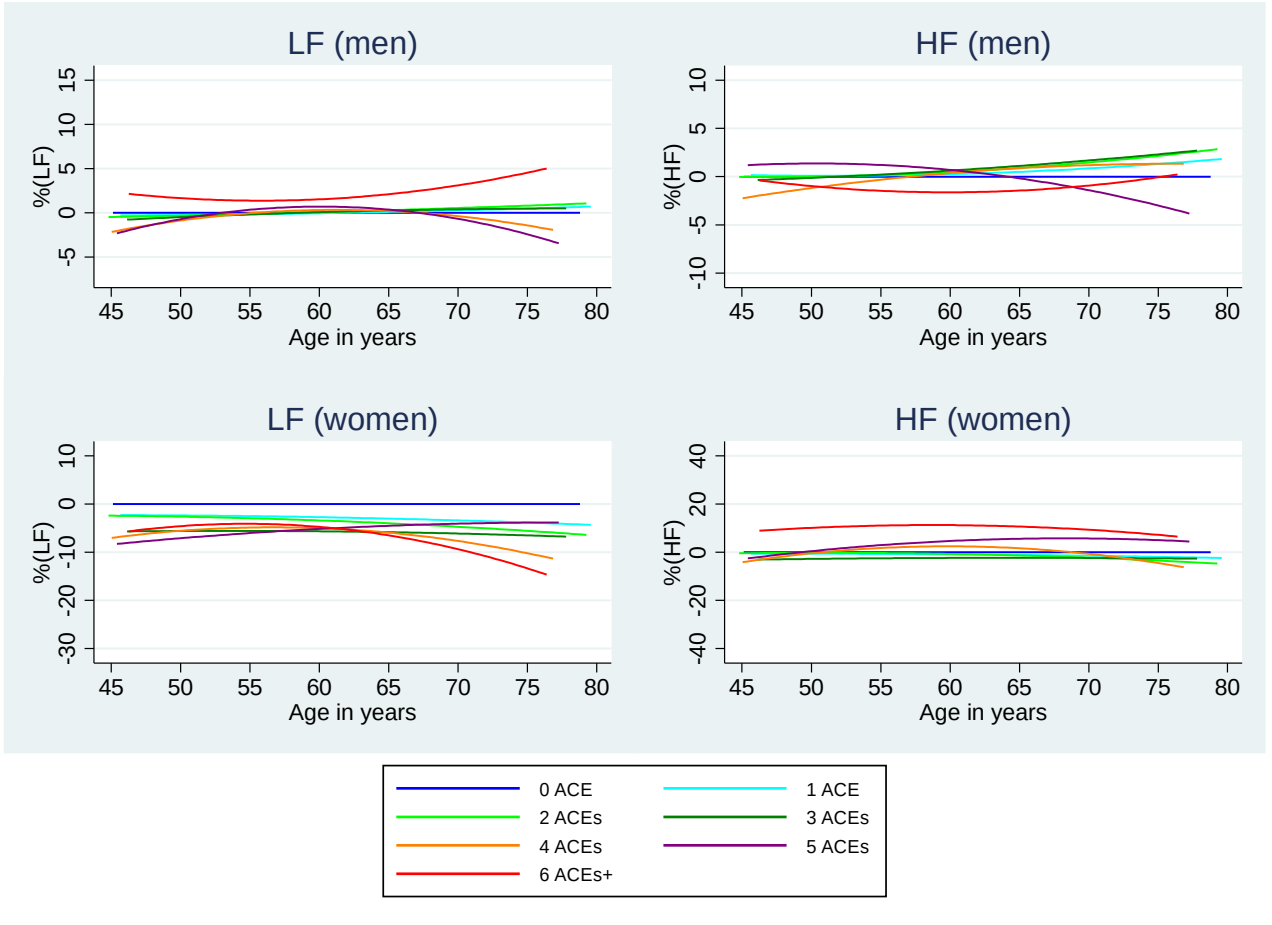


Table 7-1. Characteristics of analytical sample at baseline

	n	Men	Women
Adverse childhood experiences, n (%)			
Maternal separation 1yr+, n (%)	3932	261 (8.9)	109 (10.9)
Parental death, n (%)	3932	195 (6.7)	71 (7.1)
Hospitalisation 4wks+, n (%)	3932	334 (11.4)	121 (12.1)
Divorce, n (%)	3932	50 (1.7)	22 (2.2)
Mental illness and alcohol problems, n (%)	3932	142 (4.9)	66 (6.6)
Arguments between parents, n (%)	3932	496 (16.9)	251 (25.0)
Unemployment, n (%)	3932	272 (9.3)	100 (10.0)
Financial problems, n (%)	3932	691 (23.6)	301 (30.0)
Physical abuse, n (%)	3932	45 (1.5)	37 (3.7)
Orphanage, n (%)	3932	15 (0.5)	3 (0.3)
Lack of attachment to mothers, median (IQR)	3932	8 (4)	8 (5)
Lack of attachment to fathers, median (IQR)	3932	10 (4)	10 (4)
Mother’s harsh punishment, median (IQR)	3932	2 (1)	2 (1)
Father’s harsh punishment, median (IQR)	3932	2 (2)	2 (2)
No adverse childhood experiences, n (%)	3932	999 (34.1)	262 (26.1)
Age, median (IQR)	3932	53.9 (10.1)	54.4 (9.9)
Ethnicity (white/non-white), n (%)	3932		
White		2773 (94.7)	920 (91.6)
Father's occupational grade (non-manual/manual), n (%)	3932		
Non-manual		1856 (63.4)	587 (58.5)
Employment grade, n (%)	3932		
Administrative (high grade)		1542 (52.7)	251 (25.0)
Professional/executive (intermediate grade)		1259 (43.0)	486 (48.4)
Clerical/support (low grade)		127 (4.3)	267 (26.6)
Medication, n (%)			
Beta-blocker	3924	104 (3.6)	49 (4.9)
ACE inhibitor	3924	81 (2.8)	23 (2.3)
Calcium channel blocker	3924	74 (2.5)	21 (2.1)
Diuretics	3924	54 (1.9)	52 (5.2)

Table 7-2. Average resting heart rate at baseline, 5 years, and 10 years follow-up by covariates

		rHR [mean (standard deviation)]					
		Baseline		5 years		10 years	
Sex	Men	68.86	(10.91)	67.37	(11.51)	66.90	(11.19)
	Women	70.76	(10.23)	67.91	(10.06)	68.54	(10.01)
Age	less than 50	69.11	(10.48)	67.05	(10.3)	66.55	(10.52)
	50-54	69.15	(10.4)	67.62	(10.51)	67.63	(11)
	55-59	68.33	(10.21)	66.48	(11.38)	66.44	(10.66)
	60-64	70.72	(11.48)	68.46	(11.87)	68.17	(10.96)
	65 and over	69.89	(12.07)	69.32	(13.81)	69.62	(12.83)
Ethnicity	White	69.31	(10.76)	67.50	(11.16)	67.25	(10.94)
	Non-white	69.92	(10.97)	67.68	(11.02)	68.29	(10.87)
Father's occupational grade	Non-manual	69.30	(10.9)	67.50	(11.27)	66.84	(10.95)
	Manual	69.43	(10.57)	67.52	(10.96)	68.11	(10.87)
Employment grade	Administrative (high grade)	68.12	(10.56)	66.78	(11.01)	66.39	(10.54)
	Professional/executive (intermediate grade)	70.29	(10.73)	68.34	(11.28)	68.24	(11.13)
	Clerical/support (low grade)	70.48	(11.26)	67.43	(11.03)	68.22	(11.86)
Medication	Beta-blocker	60.60	(9.21)	58.06	(9.71)	59.14	(9.42)
	ACE inhibitor	70.48	(13.36)	69.96	(12.11)	70.51	(12.17)
	Calcium channel blocker	69.26	(13.18)	69.27	(13.4)	69.38	(12.09)
	Diuretics	72.08	(11.52)	68.01	(13.29)	69.12	(10.76)

rHR: resting heart rate

Table 7-3. Average heart rate variability (SDNN) at baseline, 5 years, and 10 years follow-up by covariates

		SDNN [median (interquartile range)]					
		Baseline		5 years		10 years	
Sex	Men	35.16	(20.37)	33.84	(20.08)	30.51	(18.96)
	Women	33.72	(17.64)	34.83	(18.9)	29.65	(16.88)
Age	less than 50	39.32	(20.4)	37.39	(19.81)	33.78	(19.95)
	50-54	36.84	(18.73)	34.59	(18.07)	30.70	(17.03)
	55-59	33.37	(18.11)	33.55	(18.69)	29.58	(18.52)
	60-64	29.48	(18.62)	30.39	(17.58)	26.34	(15.44)
	65 and over	27.91	(17.66)	27.62	(24.63)	25.70	(22.86)
Ethnicity	White	34.73	(19.7)	34.15	(19.89)	30.23	(18.13)
	Non-white	33.84	(16.46)	34.62	(18.41)	31.68	(22.65)
Father's occupational grade	Non-manual	35.18	(20.13)	34.35	(19.3)	30.53	(18.68)
	Manual	33.85	(18.92)	33.86	(20.73)	29.69	(17.87)
Employment grade	Administrative (high grade)	35.18	(20.55)	34.37	(19.24)	30.71	(18.12)
	Professional/executive (intermediate grade)	34.83	(19.27)	33.70	(19.21)	30.15	(18.01)
	Clerical/support (low grade)	32.32	(18.19)	35.97	(20.99)	28.20	(20.52)
Medication	Beta-blocker	33.15	(17.37)	31.19	(19.62)	28.11	(14.82)
	ACE inhibitor	30.51	(16.89)	30.82	(19.3)	26.56	(17)
	Calcium channel blocker	27.51	(24.12)	29.96	(19.63)	25.34	(14.98)
	Diuretics	26.08	(18.01)	31.50	(21.69)	26.78	(17.42)

SDNN: standard deviation of normal-to-normal RR intervals

Table 7-4. Average heart rate variability (RMSSD) at baseline, 5 years, and 10 years follow-up by covariates

		RMSSD [median (interquartile range)]					
		Baseline		5 years		10 years	
Sex	Men	20.01	(16.1)	19.86	(15.73)	17.54	(15.22)
	Women	21.55	(16.11)	22.44	(18.61)	18.18	(15.25)
Age	less than 50	23.81	(17.02)	23.56	(18.28)	19.44	(15.07)
	50-54	21.45	(15.94)	19.95	(15.18)	17.78	(14.2)
	55-59	18.99	(14.38)	20.16	(15.19)	17.44	(15.37)
	60-64	17.39	(14.96)	18.74	(16.93)	15.99	(14.14)
	65 and over	16.41	(16.44)	17.21	(18.89)	14.72	(23.12)
Ethnicity	White	20.34	(15.99)	20.46	(16.45)	17.70	(14.89)
	Non-white	22.70	(18.18)	24.06	(25.23)	19.68	(18.95)
Father's occupational grade	Non-manual	20.66	(15.93)	20.60	(16.45)	18.00	(14.76)
	Manual	20.08	(16.41)	20.54	(17.5)	17.52	(15.67)
Employment grade	Administrative (high grade)	20.26	(15.77)	20.82	(16.06)	17.79	(14.35)
	Professional/executive (intermediate grade)	20.43	(15.82)	19.93	(16.46)	17.79	(15.81)
	Clerical/support (low grade)	20.71	(18.04)	22.53	(23.35)	18.10	(16.32)
Medication	Beta-blocker	25.12	(20.18)	24.84	(21.06)	20.51	(15.35)
	ACE inhibitor	19.01	(15.94)	18.72	(16.82)	15.70	(14.66)
	Calcium channel blocker	16.88	(20.04)	19.31	(16.19)	15.27	(14.52)
	Diuretics	13.73	(15.7)	20.26	(17.61)	16.12	(15.81)

RMSDD: square root of successive differences of normal-to-normal RR intervals

Table 7-5. Average heart rate variability (LF) at baseline, 5 years, and 10 years follow-up by covariates

		LF [median (interquartile range)]					
		Baseline		5 years		10 years	
Sex	Men	341.33	(463.47)	302.19	(399.05)	244.54	(360.01)
	Women	256.25	(330.96)	280.89	(353.85)	203.63	(297.25)
Age	less than 50	425.68	(494.21)	383.74	(504.96)	309.93	(442.21)
	50-54	387.11	(449.79)	304.56	(354.87)	238.46	(309.31)
	55-59	267.19	(341.12)	270.49	(339.48)	207.84	(295.57)
	60-64	219.65	(329.73)	232.65	(312.22)	167.66	(232.99)
	65 and over	186.60	(321.56)	184.10	(363.6)	138.26	(398.34)
Ethnicity	White	325.32	(435.35)	299.26	(393.95)	235.56	(339.38)
	Non-white	253.67	(308.98)	259.50	(311.41)	217.61	(396.01)
Father's occupational grade	Non-manual	326.58	(443.61)	299.92	(396.42)	238.49	(355)
	Manual	308.65	(404.83)	292.85	(384.39)	227.53	(326.69)
Employment grade	Administrative (high grade)	337.59	(464.66)	299.89	(390.48)	238.37	(352.02)
	Professional/executive (intermediate grade)	325.15	(419.48)	286.91	(364.58)	240.34	(341.23)
	Clerical/support (low grade)	252.09	(309.3)	294.25	(438.26)	184.45	(306.14)
Medication	Beta-blocker	259.05	(282.32)	204.90	(311.6)	168.32	(250.99)
	ACE inhibitor	187.72	(357.75)	213.46	(321.26)	159.87	(259.59)
	Calcium channel blocker	170.50	(277.17)	219.59	(353.27)	149.07	(223.42)
	Diuretics	156.64	(196.52)	222.66	(371.83)	170.26	(300.21)

LF: low-frequency power

Table 7-6. Average heart rate variability (HF) at baseline, 5 years, and 10 years follow-up by covariates

		HF [median (interquartile range)]					
		Baseline		5 years		10 years	
Sex	Men	123.14	(192.42)	105.65	(167.55)	84.04	(137.85)
	Women	153.23	(228.81)	150.84	(249.49)	101.67	(166.5)
Age	less than 50	184.70	(256.58)	155.58	(228.97)	117.16	(176.3)
	50-54	145.52	(219.36)	115.44	(164.22)	90.84	(139.93)
	55-59	116.73	(162.38)	108.65	(169.99)	79.75	(131.98)
	60-64	94.30	(151.81)	90.29	(155.12)	64.61	(100.34)
	65 and over	77.33	(155.21)	72.50	(144.4)	52.63	(160.46)
Ethnicity	White	127.74	(201.21)	113.68	(179.39)	89.07	(144.63)
	Non-white	142.44	(197.78)	141.20	(252.18)	97.97	(194.77)
Father's occupational grade	Non-manual	131.23	(204.13)	116.48	(180.81)	90.72	(153.19)
	Manual	122.06	(193.3)	111.10	(193.33)	88.93	(136.62)
Employment grade	Administrative (high grade)	127.80	(200.22)	115.55	(180.49)	91.50	(146.4)
	Professional/executive (intermediate grade)	129.69	(197.55)	110.91	(168.48)	86.41	(146.28)
	Clerical/support (low grade)	131.61	(223.61)	147.64	(267.31)	90.83	(154.08)
Medication	Beta-blocker	141.14	(240.14)	137.35	(223.34)	101.73	(142.37)
	ACE inhibitor	97.17	(166.49)	89.58	(153.46)	69.27	(111.34)
	Calcium channel blocker	84.61	(167.36)	90.70	(122.27)	62.99	(98.17)
	Diuretics	58.69	(123.92)	107.27	(170.28)	72.24	(126.25)

Table 7-7. Estimates from the models: resting heart rate (men)

rHR	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60 (year)	-0.146	[-0.190, -0.101]	-0.145	[-0.190, -0.100]	-0.160	[-0.205, -0.115]
Age squared (year^2)	0.005	[0.001, 0.009]	0.005	[0.001, 0.009]	0.004	[0.001, 0.007]
Age at baseline (year)	0.203	[0.126, 0.280]	0.199	[0.121, 0.277]	0.239	[0.163, 0.316]
Maternal separation 1yr+			0.557	[-0.928, 2.043]	0.206	[-1.254, 1.666]
Parental death			-0.562	[-2.205, 1.081]	-0.525	[-2.140, 1.089]
Hospitalisation 4wks+			0.531	[-0.690, 1.751]	0.285	[-0.916, 1.487]
Divorce			-0.306	[-3.308, 2.695]	-0.193	[-3.149, 2.763]
Mental illness and alcohol problems			-0.603	[-2.501, 1.295]	-0.482	[-2.343, 1.379]
Arguments between parents			0.215	[-0.914, 1.343]	0.275	[-0.834, 1.384]
Unemployment			1.120	[-0.277, 2.517]	0.792	[-0.586, 2.169]
Financial problems			-0.289	[-1.271, 0.692]	-0.380	[-1.351, 0.592]
Physical abuse			-0.749	[-3.957, 2.460]	-1.095	[-4.242, 2.052]
Orphanage			-4.310	[-9.658, 1.038]	-5.521	[-10.769, -0.273]
Lack of attachment to mothers			-0.074	[-0.256, 0.109]	-0.055	[-0.234, 0.123]
Lack of attachment to fathers			0.011	[-0.166, 0.188]	-0.004	[-0.179, 0.170]
Mother’s harsh punishment			-0.162	[-0.747, 0.424]	-0.145	[-0.719, 0.430]
Father’s harsh punishment			0.107	[-0.404, 0.619]	0.013	[-0.489, 0.516]
Ethnicity					0.102	[-1.664, 1.868]
Father's occupational grade					0.252	[-0.061, 0.565]
Employment grade (per grade?)					1.670	[1.035, 2.305]
Beta-blocker					-11.661	[-12.848,-10.475]
ACE inhibitor					1.841	[0.976, 2.705]
Calcium channel blocker					0.999	[-0.074, 2.073]
Diuretics					0.885	[-0.203, 1.972]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient

Table 7-8. Estimates from the models: resting heart rate (women)

rHR	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60	-0.145	[-0.223, -0.066]	-0.141	[-0.220, -0.063]	-0.163	[-0.239, -0.086]
Age squared	0.002	[-0.004, 0.009]	0.003	[-0.003, 0.009]	0.002	[-0.004, 0.007]
Age at baseline	0.256	[0.135, 0.378]	0.241	[0.117, 0.365]	0.277	[0.153, 0.400]
Maternal separation 1yr+			-0.606	[-2.588, 1.376]	-0.416	[-2.372, 1.540]
Parental death			-0.491	[-2.788, 1.806]	-0.722	[-2.945, 1.502]
Hospitalisation 4wks+			2.127	[0.335, 3.920]	1.618	[-0.114, 3.349]
Divorce			-1.460	[-5.475, 2.555]	-1.321	[-5.232, 2.591]
Mental illness and alcohol problems			-0.285	[-2.716, 2.146]	-0.491	[-2.859, 1.876]
Arguments between parents			-0.464	[-1.956, 1.029]	-0.727	[-2.176, 0.722]
Unemployment			-1.765	[-3.827, 0.298]	-2.004	[-4.007, -0.001]
Financial problems			1.278	[-0.130, 2.687]	1.462	[0.087, 2.837]
Physical abuse			1.524	[-1.526, 4.575]	2.468	[-0.493, 5.429]
Orphanage			-0.005	[-10.462, 10.452]	-0.577	[-10.747, 9.593]
Lack of attachment to mothers			0.081	[-0.152, 0.315]	0.104	[-0.124, 0.332]
Lack of attachment to fathers			0.055	[-0.177, 0.287]	0.035	[-0.190, 0.261]
Mother’s harsh punishment			-0.529	[-1.311, 0.254]	-0.595	[-1.354, 0.164]
Father’s harsh punishment			-0.804	[-1.482, -0.126]	-0.784	[-1.445, -0.124]
Ethnicity					0.077	[-2.086, 2.240]
Father's occupational grade					0.000	[-0.454, 0.454]
Employment grade					0.292	[-0.541, 1.126]
Beta-blocker					-12.573	[-14.214,-10.932]
ACE inhibitor					3.041	[1.554, 4.529]
Calcium channel blocker					-1.075	[-2.970, 0.821]
Diuretics					1.985	[0.520, 3.450]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient

Table 7-9. Estimates from the models: SDNN (men)

SDNN	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60	-1.339	[-1.577, -1.101]	-1.339	[-1.577, -1.101]	-1.138	[-1.388, -0.887]
Age squared	0.002	[-0.017, 0.021]	0.002	[-0.017, 0.021]	0.006	[-0.013, 0.025]
Age at baseline	-0.107	[-0.449, 0.234]	-0.119	[-0.464, 0.225]	-0.282	[-0.630, 0.067]
Maternal separation 1yr+			1.084	[-4.892, 7.060]	1.551	[-4.398, 7.500]
Parental death			1.993	[-4.546, 8.533]	1.718	[-4.778, 8.215]
Hospitalisation 4wks+			-0.969	[-5.806, 3.868]	-0.090	[-4.908, 4.727]
Divorce			0.565	[-11.097, 12.227]	0.315	[-11.300, 11.930]
Mental illness and alcohol problems			0.273	[-7.181, 7.727]	-0.294	[-7.684, 7.097]
Arguments between parents			-0.029	[-4.484, 4.427]	-0.027	[-4.451, 4.397]
Unemployment			-4.849	[-10.339, 0.641]	-4.906	[-10.381, 0.569]
Financial problems			1.005	[-2.853, 4.863]	0.995	[-2.862, 4.853]
Physical abuse			-0.389	[-12.913, 12.136]	-0.048	[-12.453, 12.358]
Orphanage			12.513	[-8.296, 33.322]	13.765	[-6.844, 34.374]
Lack of attachment to mothers			0.250	[-0.468, 0.968]	0.198	[-0.514, 0.910]
Lack of attachment to fathers			-0.169	[-0.867, 0.528]	-0.187	[-0.881, 0.507]
Mother’s harsh punishment			1.031	[-1.276, 3.339]	1.234	[-1.055, 3.524]
Father’s harsh punishment			-0.456	[-2.472, 1.560]	-0.567	[-2.569, 1.435]
Ethnicity					6.211	[-0.927, 13.349]
Father's occupational grade					-0.120	[-1.368, 1.127]
Employment grade					-3.374	[-6.026, -0.723]
Beta-blocker					1.926	[-3.976, 7.829]
ACE inhibitor					-5.835	[-10.319, -1.351]
Calcium channel blocker					-10.332	[-15.802, -4.862]
Diuretics					-5.050	[-10.723, 0.623]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient, interpreted as percent change in outcome

Table 7-10. Estimates from the model: SDNN (women)

SDNN	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60	-1.367	[-1.780, -0.953]	-1.386	[-1.801, -0.972]	-1.205	[-1.637, -0.773]
Age squared	0.002	[-0.030, 0.035]	0.004	[-0.028, 0.037]	0.011	[-0.022, 0.044]
Age at baseline	-0.239	[-0.795, 0.317]	-0.147	[-0.711, 0.417]	-0.252	[-0.841, 0.338]
Maternal separation 1yr+			-2.044	[-10.407, 6.319]	-2.328	[-10.944, 6.288]
Parental death			-2.823	[-12.473, 6.826]	-2.295	[-12.009, 7.418]
Hospitalisation 4wks+			-4.495	[-11.994, 3.004]	-4.598	[-12.112, 2.917]
Divorce			0.381	[-16.251, 17.013]	-0.204	[-16.974, 16.566]
Mental illness and alcohol problems			0.647	[-9.432, 10.726]	0.845	[-9.309, 10.999]
Arguments between parents			-0.877	[-7.044, 5.290]	-0.699	[-6.900, 5.501]
Unemployment			8.243	[-0.322, 16.807]	8.175	[-0.429, 16.779]
Financial problems			-6.048	[-11.877, -0.220]	-6.513	[-12.401, -0.624]
Physical abuse			-8.054	[-20.519, 4.411]	-7.286	[-19.796, 5.224]
Orphanage			47.606	[4.939, 90.274]	46.223	[3.324, 89.122]
Lack of attachment to mothers			0.278	[-0.693, 1.249]	0.252	[-0.730, 1.233]
Lack of attachment to fathers			-0.084	[-1.048, 0.880]	-0.139	[-1.108, 0.830]
Mother’s harsh punishment			1.833	[-1.391, 5.057]	1.917	[-1.320, 5.153]
Father’s harsh punishment			-0.361	[-3.157, 2.436]	-0.301	[-3.120, 2.517]
Ethnicity					2.762	[-6.515, 12.039]
Father's occupational grade					0.569	[-1.375, 2.512]
Employment grade					-0.204	[-3.910, 3.502]
Beta-blocker					5.593	[-2.892, 14.077]
ACE inhibitor					-5.616	[-13.465, 2.232]
Calcium channel blocker					-10.400	[-20.399, -0.401]
Diuretics					-6.359	[-13.990, 1.272]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient, interpreted as percent change in outcome

Table 7-11. Estimates from the models: RMSSD (men)

RMSSD	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60	-1.011	[-1.356, -0.665]	-1.009	[-1.355, -0.664]	-0.824	[-1.185, -0.463]
Age squared	0.029	[0.002, 0.057]	0.029	[0.002, 0.056]	0.033	[0.006, 0.060]
Age at baseline	-0.278	[-0.773, 0.216]	-0.298	[-0.797, 0.202]	-0.519	[-1.024, -0.015]
Maternal separation 1yr+			1.082	[-7.563, 9.726]	1.337	[-7.292, 9.965]
Parental death			6.353	[-3.076, 15.783]	5.123	[-4.262, 14.508]
Hospitalisation 4wks+			-1.692	[-8.658, 5.275]	-0.733	[-7.688, 6.221]
Divorce			-6.742	[-23.408, 9.924]	-8.325	[-24.944, 8.294]
Mental illness and alcohol problems			5.081	[-5.610, 15.773]	4.040	[-6.579, 14.658]
Arguments between parents			-2.517	[-8.925, 3.891]	-2.670	[-9.045, 3.705]
Unemployment			-5.001	[-12.888, 2.887]	-4.928	[-12.809, 2.952]
Financial problems			2.702	[-2.841, 8.245]	2.677	[-2.875, 8.229]
Physical abuse			-0.619	[-18.602, 17.364]	0.219	[-17.625, 18.063]
Orphanage			1.140	[-28.876, 31.155]	4.255	[-25.570, 34.080]
Lack of attachment to mothers			0.296	[-0.736, 1.327]	0.254	[-0.771, 1.279]
Lack of attachment to fathers			-0.263	[-1.265, 0.739]	-0.207	[-1.206, 0.792]
Mother’s harsh punishment			2.553	[-0.765, 5.871]	2.781	[-0.518, 6.079]
Father’s harsh punishment			-0.388	[-3.287, 2.510]	-0.568	[-3.453, 2.316]
Ethnicity					14.215	[3.908, 24.522]
Father's occupational grade					-0.291	[-2.087, 1.505]
Employment grade					-4.178	[-7.997, -0.359]
Beta-blocker					24.630	[16.079, 33.182]
ACE inhibitor					-7.565	[-14.080, -1.050]
Calcium channel blocker					-12.250	[-20.214, -4.286]
Diuretics					-2.395	[-10.653, 5.863]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient, interpreted as percent change in outcome

Table 7-12. Estimates from the models: RMSSD (women)

RMSSD	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60	-1.339	[-1.918, -0.761]	-1.357	[-1.937, -0.777]	-1.246	[-1.846, -0.647]
Age squared	0.045	[-0.000, 0.090]	0.048	[0.003, 0.093]	0.055	[0.010, 0.100]
Age at baseline	-0.260	[-1.041, 0.520]	-0.200	[-0.991, 0.592]	-0.246	[-1.066, 0.575]
Maternal separation 1yr+			1.188	[-10.458, 12.834]	-2.413	[-14.400, 9.573]
Parental death			0.815	[-12.645, 14.274]	0.939	[-12.585, 14.462]
Hospitalisation 4wks+			-3.335	[-13.803, 7.132]	-2.493	[-12.958, 7.972]
Divorce			0.379	[-22.891, 23.649]	-3.785	[-27.164, 19.595]
Mental illness and alcohol problems			6.646	[-7.454, 20.746]	8.169	[-5.984, 22.323]
Arguments between parents			-3.822	[-12.439, 4.795]	-2.783	[-11.422, 5.857]
Unemployment			12.206	[0.234, 24.177]	12.620	[0.631, 24.609]
Financial problems			-5.787	[-13.934, 2.360]	-6.688	[-14.894, 1.518]
Physical abuse			-5.052	[-22.476, 12.371]	-7.417	[-24.852, 10.017]
Orphanage			65.911	[6.117,125.705]	70.323	[10.456,130.189]
Lack of attachment to mothers			0.123	[-1.233, 1.480]	0.214	[-1.154, 1.581]
Lack of attachment to fathers			-0.454	[-1.800, 0.893]	-0.472	[-1.822, 0.878]
Mother's harsh punishment			4.168	[-0.341, 8.676]	4.129	[-0.382, 8.640]
Father's harsh punishment			1.376	[-2.534, 5.286]	1.478	[-2.450, 5.406]
Ethnicity					16.251	[3.324, 29.179]
Father's occupational grade					0.618	[-2.091, 3.326]
Employment grade					-1.939	[-7.101, 3.223]
Beta-blocker					35.069	[23.274, 46.864]
ACE inhibitor					-4.790	[-15.694, 6.114]
Calcium channel blocker					-12.691	[-26.579, 1.197]
Diuretics					-6.000	[-16.602, 4.602]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient, interpreted as percent change in outcome

Table 7-13. Estimates from the models: LF (men)

LF	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60	-3.494	[-4.002, -2.987]	-3.495	[-4.002, -2.987]	-3.007	[-3.541, -2.472]
Age squared	0.017	[-0.023, 0.058]	0.018	[-0.022, 0.058]	0.028	[-0.013, 0.068]
Age at baseline	-0.633	[-1.368, 0.102]	-0.661	[-1.403, 0.081]	-1.003	[-1.753, -0.254]
Maternal separation 1yr+			1.746	[-11.231, 14.723]	3.082	[-9.807, 15.971]
Parental death			-1.172	[-15.394, 13.051]	-0.694	[-14.791, 13.403]
Hospitalisation 4wks+			-0.970	[-11.492, 9.552]	0.543	[-9.912, 10.998]
Divorce			-1.643	[-27.105, 23.819]	-2.406	[-27.707, 22.895]
Mental illness and alcohol problems			-2.176	[-18.416, 14.065]	-3.214	[-19.279, 12.851]
Arguments between parents			2.358	[-7.341, 12.056]	2.631	[-6.976, 12.237]
Unemployment			-8.830	[-20.788, 3.128]	-8.640	[-20.536, 3.256]
Financial problems			0.943	[-7.460, 9.346]	0.848	[-7.534, 9.231]
Physical abuse			-1.516	[-28.830, 25.798]	-1.447	[-28.438, 25.543]
Orphanage			38.400	[-6.900, 83.701]	38.347	[-6.395, 83.089]
Lack of attachment to mothers			0.598	[-0.966, 2.161]	0.452	[-1.095, 1.998]
Lack of attachment to fathers			-0.782	[-2.301, 0.737]	-0.868	[-2.375, 0.640]
Mother’s harsh punishment			2.489	[-2.535, 7.513]	2.874	[-2.099, 7.847]
Father’s harsh punishment			-0.782	[-5.171, 3.607]	-0.814	[-5.163, 3.535]
Ethnicity					-0.243	[-15.732, 15.247]
Father's occupational grade					-0.332	[-3.042, 2.379]
Employment grade					-4.410	[-10.159, 1.338]
Beta-blocker					-7.952	[-20.618, 4.714]
ACE inhibitor					-15.470	[-25.066, -5.874]
Calcium channel blocker					-20.323	[-32.036, -8.610]
Diuretics					-9.693	[-21.824, 2.437]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient, interpreted as percent change in outcome

Table 7-14. Estimates from the models: LF (women)

LF	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60	-2.981	[-3.879, -2.082]	-3.014	[-3.914, -2.115]	-2.546	[-3.487, -1.606]
Age squared	0.011	[-0.060, 0.082]	0.015	[-0.056, 0.086]	0.028	[-0.043, 0.100]
Age at baseline	-0.747	[-1.980, 0.486]	-0.580	[-1.834, 0.673]	-1.003	[-2.312, 0.306]
Maternal separation 1yr+			-8.187	[-27.109, 10.734]	-4.402	[-23.782, 14.979]
Parental death			-2.315	[-24.112, 19.482]	-0.630	[-22.452, 21.191]
Hospitalisation 4wks+			-6.917	[-23.839, 10.006]	-7.476	[-24.345, 9.393]
Divorce			-5.721	[-43.284, 31.841]	-1.206	[-38.918, 36.505]
Mental illness and alcohol problems			6.622	[-16.091, 29.335]	5.599	[-17.183, 28.381]
Arguments between parents			3.024	[-10.886, 16.933]	2.520	[-11.395, 16.434]
Unemployment			9.942	[-9.369, 29.253]	9.556	[-9.750, 28.863]
Financial problems			-11.947	[-25.089, 1.195]	-12.787	[-25.999, 0.425]
Physical abuse			-20.611	[-48.708, 7.487]	-18.033	[-46.078, 10.011]
Orphanage			70.181	[-26.428,166.790]	63.010	[-33.745,159.764]
Lack of attachment to mothers			0.603	[-1.586, 2.792]	0.461	[-1.741, 2.663]
Lack of attachment to fathers			0.608	[-1.564, 2.781]	0.496	[-1.677, 2.668]
Mother’s harsh punishment			4.977	[-2.295, 12.250]	5.358	[-1.907, 12.624]
Father’s harsh punishment			-4.024	[-10.332, 2.284]	-4.216	[-10.543, 2.111]
Ethnicity					-16.830	[-37.659, 3.998]
Father's occupational grade					0.406	[-3.951, 4.763]
Employment grade					4.221	[-4.078, 12.520]
Beta-blocker					-0.824	[-19.547, 17.899]
ACE inhibitor					-13.755	[-31.084, 3.574]
Calcium channel blocker					-18.125	[-40.175, 3.924]
Diuretics					-15.241	[-32.087, 1.604]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient, interpreted as percent change in outcome

Table 7-15. Estimates from the models: HF (men)

HF	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60	-3.397	[-3.990, -2.805]	-3.400	[-3.992, -2.808]	-3.031	[-3.652, -2.410]
Age squared	0.050	[0.003, 0.096]	0.049	[0.002, 0.096]	0.057	[0.010, 0.104]
Age at baseline	-0.188	[-1.046, 0.670]	-0.224	[-1.091, 0.642]	-0.596	[-1.472, 0.280]
Maternal separation 1yr+			5.727	[-9.382, 20.835]	6.100	[-8.988, 21.189]
Parental death			7.656	[-8.871, 24.184]	6.115	[-10.352, 22.582]
Hospitalisation 4wks+			-1.814	[-14.036, 10.408]	0.075	[-12.135, 12.286]
Divorce			-12.384	[-41.808, 17.040]	-14.564	[-43.941, 14.814]
Mental illness and alcohol problems			6.317	[-12.501, 25.135]	4.600	[-14.109, 23.310]
Arguments between parents			-1.389	[-12.646, 9.867]	-1.759	[-12.969, 9.451]
Unemployment			-7.950	[-21.818, 5.918]	-8.228	[-22.098, 5.641]
Financial problems			2.163	[-7.584, 11.909]	2.170	[-7.603, 11.944]
Physical abuse			-7.479	[-39.124, 24.165]	-6.254	[-37.689, 25.180]
Orphanage			-7.619	[-60.306, 45.068]	-3.094	[-55.481, 49.293]
Lack of attachment to mothers			0.099	[-1.715, 1.912]	0.036	[-1.767, 1.839]
Lack of attachment to fathers			-0.240	[-2.002, 1.521]	-0.167	[-1.924, 1.591]
Mother’s harsh punishment			4.351	[-1.479, 10.182]	4.884	[-0.918, 10.686]
Father’s harsh punishment			-0.550	[-5.643, 4.542]	-0.872	[-5.945, 4.201]
Ethnicity					23.938	[5.852, 42.023]
Father's occupational grade					-0.515	[-3.676, 2.645]
Employment grade					-7.425	[-14.128, -0.723]
Beta-blocker					21.900	[7.093, 36.706]
ACE inhibitor					-13.339	[-24.572, -2.106]
Calcium channel blocker					-19.553	[-33.281, -5.826]
Diuretics					-6.907	[-21.121, 7.307]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient, interpreted as percent change in outcome

Table 7-16. Estimates from the models: HF (women)

HF	Model 1		Model 2		Model 3	
	b	95% CI	b	95% CI	b	95% CI
Age centred at 60	-3.826	[-4.844, -2.807]	-3.847	[-4.866, -2.828]	-3.524	[-4.582, -2.465]
Age squared	0.067	[-0.014, 0.147]	0.070	[-0.010, 0.151]	0.087	[0.006, 0.168]
Age at baseline	-0.190	[-1.576, 1.196]	-0.100	[-1.508, 1.307]	-0.178	[-1.645, 1.290]
Maternal separation 1yr+			-3.194	[-24.228, 17.839]	-6.921	[-28.527, 14.684]
Parental death			7.158	[-17.269, 31.585]	7.304	[-17.265, 31.873]
Hospitalisation 4wks+			0.374	[-18.682, 19.431]	1.105	[-17.986, 20.196]
Divorce			-11.755	[-54.062, 30.553]	-17.572	[-60.214, 25.069]
Mental illness and alcohol problems			10.934	[-14.857, 36.726]	13.131	[-12.850, 39.113]
Arguments between parents			0.324	[-15.478, 16.126]	1.706	[-14.172, 17.583]
Unemployment			16.750	[-5.128, 38.629]	17.160	[-4.826, 39.146]
Financial problems			-12.040	[-26.971, 2.890]	-13.473	[-28.552, 1.606]
Physical abuse			-13.046	[-45.280, 19.189]	-13.945	[-46.285, 18.394]
Orphanage			139.506	[30.548,248.464]	140.069	[30.613,249.526]
Lack of attachment to mothers			0.125	[-2.356, 2.605]	0.161	[-2.345, 2.668]
Lack of attachment to fathers			-0.319	[-2.785, 2.147]	-0.411	[-2.889, 2.067]
Mother’s harsh punishment			9.908	[1.636, 18.179]	9.912	[1.612, 18.213]
Father’s harsh punishment			-0.850	[-8.017, 6.317]	-0.481	[-7.704, 6.741]
Ethnicity					23.648	[-0.061, 47.358]
Father's occupational grade					1.658	[-3.331, 6.648]
Employment grade					-4.179	[-13.587, 5.229]
Beta-blocker					37.472	[16.499, 58.445]
ACE inhibitor					-11.335	[-30.538, 7.869]
Calcium channel blocker					-23.223	[-47.743, 1.296]
Diuretics					-16.476	[-35.216, 2.265]

Model 1: age adjusted, Model 2: + all 14 ACEs adjusted, Model 3: + other covariates adjusted
b is regression coefficient, interpreted as percent change in outcome

Chapter 8 Discussion

8.1. Introduction

The research questions of my PhD are whether ACEs increase the risk of developing CHD later in life, and whether the neuroendocrine and the autonomic nervous systems explain the association. In this chapter, I summarise key findings; discuss the findings; acknowledge and discuss the impact of the limitations; and highlight the implication for future research and for policy.

8.2. Summary of findings

Associations of ACEs with incident CHD later in life were not consistent across all individual ACEs. There was little evidence of a dose-response association between the count of ACEs and incident CHD. I estimated a 6.0% reduction in CHD in the absence of ACEs, but 95% confidence intervals included zero. There was also little evidence that number of ACEs were associated with the proposed mediating mechanisms. An increasing number of adversities had no association with AUC, or CAR. The cortisol values on wakening decreased and the diurnal slope became flatter as the number of ACEs increased but the effect was small. My findings also highlight a possible sex difference in cortisol secretion, which may shed light on physiological explanations for sexual differences in stress-related diseases. There

was no evidence that ACEs were associated with autonomic activity measured later in life.

8.3. Discussion

Overall, my research shows only weak evidence of a role of ACEs on incident CHD. The magnitude of any association appears to be small and is related to the count of ACEs rather than to particular individual adversities. Despite these findings, I did proceed to investigate the two proposed biological pathways (the neuroendocrine system and the autonomic nervous systems), given that evidence for an association of these systems with CHD has been increasingly accumulated.[75,140,166-168] Studying the association with these biological markers is also of interest in their own right in the understanding of biological changes that may result from ACEs. These pathways may also be relevant for other health outcomes such as mental illness, which is thought to be associated with the dysregulation of the HPA axis.[267]

8.3.1. Measurement and modelling of ACEs

There are multiple challenges in studying ACEs: measurement, setting (place and time), and modelling. There is no standardised questionnaire of ACEs, meaning that types of ACEs included in research differ across studies. Some studies have used

the questionnaires from CDC or WHO, while others used their own questionnaires. In historic cohort studies, where data collection took place before there was a recognition of the need to measure ACEs (i.e., a study conducted by Felitti in 1998[78]), researchers often identify questions from various sources in order to identify such experiences. The Whitehall II cohort study and the NCDS do not collect ACEs through standard questionnaires, but instead do have questions relating to adverse experiences in childhood. The items either taken from existing previous studies or designed by the Whitehall II or NCDS study teams. It is therefore challenging to compare findings across the studies because the types of ACEs included in counts and the method of collection (prospective or retrospective) are not consistent across studies.[268] Furthermore, the Whitehall II cohort study, which is the main source of data in my PhD, did not collect information relating to some important ACEs. For instance, there is a growing literature on the association of bullying with adverse mental health and behavioural problems.[269,270] My PhD is therefore limited to ACEs measured in the Whitehall II cohort study.

A review has documented apparent discrepancies in reported ACEs between prospective and retrospective assessment, but better agreement for factual ACEs such as parental separation.[109] Recall may be biased by health conditions, such as

depression, at the time of assessment. However, the prospective reporting can also be biased by, for example, stigma in a society (e.g., mental health problems) or shame or fear to talk about the experience (e.g., sexual abuse) at the time of occurrence. Studies which are old enough to be able to study the association of ACEs with incident CHD, which occurs at older ages, often rely on retrospective reporting of ACEs, such as in Whitehall II cohort study. Studies which have selected ACE questionnaires, therefore, are not yet old enough to consider CHD as an outcome, although they can consider CHD risk factors and potential mediators. Other older studies, including NCDS, derive ACE scores from questions which happened to be asked prospectively for other scientific reasons before ACEs were defined. It is therefore important to interpret the findings in the historical context in which ACEs were measured and whether they are collected prospectively or retrospectively.

ACEs are also interpreted differently according to generation and places. For instance, smacking children might have been accepted as a way of punishment decades ago, while smacking is now completely banned under law in Scotland and Wales (March 2022). This implies that what is recognised as an ACE can change across time (i.e., cohort effect). In some places, for instance where there are

Catholic religious beliefs, prevalence of parental divorce might be lower than other countries, and children experiencing their parental divorce may be stigmatised, while in other countries divorce may be highly prevalent. Given that the participants are currently around 70 to 90 years old in the Whitehall II cohort study and 64 years old in the NCDS, some ACEs may be more, or less, common than they are now, while there may be some impact from the period when they grew up, such as war (e.g., parental death). It is therefore necessary to interpret the findings in the circumstance in which participants grew up and it would be interesting to compare findings across generations.

Given that ACEs are likely to co-occur and cluster, there are several possible approaches to the analysis of ACEs when investigating their association with subsequent health. One of the analytical approaches, which I used in my PhD, is to regard ACEs as multiple exposures. One of the main advantages of this approach is that each ACE is allowed to contribute differently to the analysis. This is in contrast to the commonly used approach of an ACEs score, where individual ACEs are assumed to have equal effects on the outcome. However, there are still strong assumptions to the approach that I used, as it is assumed that all ACEs occurred at the same time, and that any one ACE does not lie on the causal pathway between

another ACE and the outcome. For example, having financial problems may lead to parental mental health problems such as anxiety and depression. Other possible approaches are to use exploratory factor analysis or latent class analysis. The exploratory factor analysis aims to identify the underlying structure of observed ACEs (a variable-centred), and the latent class analysis aims to separate people into similar clusters by their ACEs. These approaches might be suitable given that classifications of ACEs remain unclear. Such approaches have, however, been criticised due to their data driven nature and lack of theoretical basis.[116] As mentioned previously that there is no established classification of ACEs to date, confirmatory factor analysis might not be appropriate. During my PhD, I attempted to fit a model using latent class analysis in the Whitehall II cohort study, using Bayesian information criterion (BIC), Akaike information criterion (AIC), and theoretical interpretability to identify an optimal number of classes.[271] As the number of classes increased, the BIC and AIC declined, but there was no improvement in the interpretability of classes. Poor model fit and low interpretability have been previously found in other studies relating to ACEs[224] as well as childhood social risk factors (e.g., parental educational attainment).[272] I also fitted a principal component analysis (PCA) in the Whitehall II cohort study. Seven components among 14 ACEs were identified based on eigenvalues, scree plots, and interpretability after oblique

rotation, accounting for 66% of total variance. Some of the groups, however, included only one type of ACEs, or more than one but some of which had low factor loadings. Based on these findings, and their lack of interpretability, I therefore did not use these approaches to modelling ACEs in my PhD.

8.3.2. Explanation for findings

Despite largely positive findings reported in existing studies examining the association between ACEs and CHD, my study showed little evidence for this association. There are various possible explanations. Firstly, it may be due to the study population. I used the Whitehall II cohort study, which is a sample who were civil servants in London at the time of recruitment rather than a general population sample. They are, therefore, from an advantaged socioeconomic position than the general as they are employed in non-manual occupations. They are also likely to be healthier than the general population because they were an occupational cohort and were in work at recruitment (i.e., a healthy worker effect). For instance, a study which examined life course trajectory of systolic blood pressure from eight cohort studies within UK has documented that the Whitehall II cohort had a lower mean systolic blood pressure in mid to older ages than the general population from a similar generation.[273] The Whitehall II cohort, therefore, may be a group of people who have

had subsequent experiences which may have protected them against the harmful effects of ACEs. Further, assuming that ACEs are likely to be clustered and to be more severe among those in a disadvantaged socioeconomic position in childhood,[12] fewer people in the Whitehall II cohort study might have experienced critically harmful adversities in childhood, although the incidence itself is similar to general population.[80] There are fewer participants in the Whitehall II cohort study from a disadvantaged socioeconomic position in childhood compared with population-based studies of a similar age, such as the National Survey of Health and Development (1946 British Birth cohort).[274]

Secondly, measurement of ACEs. In the Whitehall II cohort study, most of questions measuring ACEs are binary (i.e., yes or no), meaning that there is no information available regarding the severity, duration, and timing (i.e., age when ACEs occurred). Timing of the exposure to ACEs, including during the prenatal period, may be associated with the HPA axis in adolescence,[275] mental illness,[276,277] and physical problems (e.g., heart complaints).[278] In contrast, a study from the National Survey of Midlife Development in the US argues that duration matters more than the timing, when examining the association with the cardiometabolic health.[214] Detailed information on severity, timing, and duration of ACEs is not available in the Whitehall

II cohort study or NCDS, and therefore research questions related to whether severity, timing, and duration of ACEs are important could not be addressed. As ACEs in the Whitehall II cohort study were collected retrospectively, mental health may have an impact on how ACEs are recalled and reported. I did not control for mental health conditions at the time when ACEs were collected, because mental illness may be one of the causal pathways between ACEs and CHD. Therefore, consideration as to whether there is a greater influence on health depending on how ACEs are perceived by people in adulthood requires further research.

Thirdly, study design. I applied longitudinal analysis, more specifically survival analysis with follow-up time from when ACEs were retrospectively assessed. This type of analysis considers the time each individual is at risk, while cross-sectional analysis (e.g., logistic regression), which the majority of existing studies used, considers that all individuals were at risk for the same length of time. A recent meta-analysis[96] included 38 studies in total, of which approximately 75% of the studies used a cross-sectional approach in their analyses. Moreover, following up people from the time ACEs were assessed minimises recall bias because people who have already had a CHD episode are excluded. Therefore, participants' recall of ACEs will not be influenced by the current health condition of CHD.

Fourthly, statistical approach. Along with the longitudinal analysis, I took into account of the potentially different effect sizes of individual ACEs. Most existing studies applied an “ACEs score”, which is a simple sum of number of ACEs reported. It is possible that application of the ACEs score may over/underestimate the effect of ACEs on the outcome by assuming that all ACEs have an equal effect size.[114,116]

Lastly, the existing literature may be affected by publication bias. A systematic review with meta-analysis published in 2018 reported possible publication bias.[96] The funnel plot in this review showed that positive findings were more likely to be published, of which the sample sizes were smaller than the publications reporting null findings. This highlights the importance of the open science agenda.[279] It has been gradually acknowledged that many findings in scientific publications are not reproducible, which has led to the movement of open science practices. As well as sharing data and analytical codes, preregistering studies (e.g., hypothesis, study design, analysis plan) and then submitting registered reports (i.e., peer-reviewed at a stage of research proposal, and after results are obtained) are crucial to convey all empirical evidence, including studies producing null findings such as those in this PhD.

8.3.3. Biological pathways

A large body of research has examined the association between ACEs and inflammatory markers. A meta-analysis has documented that childhood trauma was associated with slightly elevated CRP, IL-6, and TNF-α, although the effect size of these associations appears to depend on the type of trauma and type of inflammatory marker.[121,280] Inflammation, which is a response of the immune system, may be an underlying pathway between stress and CHD.[281] The neuroendocrine system and the autonomic nervous system are thought to be the upstream of the immune system in stress response, while there may be interplay between these systems.[282]

The autonomic nervous system provides the immediate response to stress exposure, and then the hypothalamus activates the HPA axis. Findings in my PhD provide a cross-sectional picture of differences in functioning of these two systems according to the experiences of adversities in middle age. A review describes that older people exhibit less dynamic change in cortisol than younger people.[249] Similarly, impaired vagal activity is shown to be associated with ageing.[283] My study of cortisol suggests that experience of adversities in childhood may be associated

with accelerated ageing. My studies, however, do not provide evidence of whether the ACEs have a direct effect on the dysfunction of the systems, and to what extent possible indirect pathways, such as behavioural and psychological factors, account for the total association of ACEs with the disruption of the systems. It would therefore be interesting to investigate these indirect paths, along with the biological pathways that I examined in my PhD, to elucidate possible causal explanation of the association. This would be useful to identify the extent to which intervention may be able to prevent people who had ACEs from developing impaired responses to stress with potential subsequent harmful effects on health.

High vagal activity is an indicator of successful regulation in stress response.[284] It has been documented that high vagal activity is associated with activity of the prefrontal cortex, a region in the brain that has an inhibitory function of the amygdala.[285] As described in Chapter 1, the amygdala initiates the HPA axis, of which the final effector is cortisol. An increase in prefrontal cortex activity inhibits the activation of the amygdala, reduces the HPA axis activation, and leads to stress adaptability. My studies do not provide evidence of a longitudinal interaction between the neuroendocrine system and the autonomic nervous systems in relation to the ACEs. However, given physiological evidence from existing studies,[40] I can

hypothesise a temporality as low vagal activity may indicate subsequent hypoactivity of prefrontal cortex. It leads to high activation of the HPA axis as acute response, but it can result in the wear-and-tear if the stress prolongs,[40] which the study examining the association between ACEs and cortisol might have captured.

I used salivary cortisol and HRV as markers of the neuroendocrine system, and of the autonomic nervous system, respectively. As I hypothesised that allostatic stress responses in people who had ACEs, cortisol is the main hormone reflecting the responses among those released in the HPA axis (CRH and ACTH) in the neuroendocrine system. Cortisol is observed in serum, saliva, urine, and hair. Assessment of cortisol in hair reflects accumulation of the cortisol exposure over time but does not measure circadian rhythm of the cortisol secretion.[286] Assessing cortisol in serum shows the total serum cortisol level including protein-bound and free cortisol. Therefore, levels of total serum cortisol are affected by changes in protein-bound cortisol, although free cortisol concentration is of interest. In addition, it is an invasive procedure to obtain blood samples which is therefore more challenging to collect. Thus, salivary and urinary cortisol measures are generally favoured in stress research, and in large population-based studies, because their

values are highly correlated with those of serum free cortisol as well as being non-invasive.[137]

There are various biomarkers which represent the autonomic nervous system, as described in Chapter 7. Given that HR is measured as beats per minute and HRV is variation in time between heart beats, HR is likely associated with HRV. However, a review has documented a nonlinear inverse relationship between HR and RR interval,[287] suggesting that HR should be treated as a different quantity from HRV. Existing studies have documented an inverse correlation between HR and the time domain of HRV. For instance, SDNN is known to increase as average HR decreases.[288,289] The frequency domain of HRV is also reported to be related to mean HR such that HF is inversely and LF is positively associated with average HR.[289] It is therefore important to correct HRV for average HR when comparing the HRV in individual levels.[288] Furthermore, it is reported that the HRV dependence of HR determines the predictive power of HRV for cardiac death, which differs by sex.[287] The more the HRV is dependent on HR, the higher the predictive power is for cardiac death among men, while the lower in women. It, however, remains questionable as to which quantity, HR or HRV, is a better predictor of cardiac mortality.[290] HR may be recorded relatively routinely in biomedical data collection in

studies, while HRV is less so as it requires special equipment and further analysis. Thus, it is crucial to assess both HR and HRV as independent markers of autonomic activity.

8.4. Strengths and Limitations

I highlight some overall strengths and limitations of my PhD, picking up on those highlighted within each chapter.

The studies in my PhD benefited from a large sample size, objectively measured incident CHD, repeated measurements of salivary cortisol and HRV, and longitudinal analyses. Sample size is important so as to obtain estimates of associations with small standard error. Most of the existing studies, particularly in work relating to cortisol, are drawn from specific study groups (e.g., people with underlying health conditions) and their sample sizes are small.[122,144] The study used in my PhD was based on a relatively large population-based study sample of men and women. Incident CHD in the Whitehall II cohort study has been identified through a combination of data collected during the medical examination, and linkage of study participants to records from the National Health Service.[176] My study therefore benefits from quality ascertainment of the main outcome. There are also two key

strengths in repeated measures of biomarkers in terms of measurement errors and assessment of longitudinal changes. Values of biomarkers are prone to measurement errors because they are influenced by other factors such as stressful events on the day of sample collection. By using the repeated measurements, it is possible to consider variation within individuals as well as the variance between individuals. The effect of ACEs on changes over time could also be estimated in analyses employing the repeated measures of HR and HRV. I used longitudinal analysis across all studies in my PhD such that the ACEs were measured prior to the outcome. Therefore, as highlighted in section 8.3.2 above, reporting of ACEs cannot be influenced by the occurrence of the outcome.

Epidemiological studies generally aim to estimate the causal effect of the exposure on the outcome, however due to challenges with confounding, it is often argued that only associations can be estimated. A study design considered the gold standard for estimating causal effects is the randomised controlled trial (RCT), as it is considered not to have biases from confounding and selection. There are, however, also limitations in RCTs,[217,291] such as loss to follow-up and unblinding so that participants are aware of the treatment and can change behaviour.[291] Importantly, an RCT is not always feasible due to ethical (e.g., harmful exposure) or financial

reasons (e.g., cost of exposure such as treatment). An RCT is clearly not a possible study design to address the question posed in my PhD as it would not be possible to randomise to ACEs, therefore I had to use observational studies. Methodologies to draw causal inference in observational studies have been of interest among researchers,[217] so that causal inference can be drawn from multiple sources (i.e., various study design and statistical analyses), each of which has strengths and weaknesses.[292] I conducted counterfactual analysis, which is a statistical analysis to quantify the difference between what actually happened (i.e., observed) and what would have happened in the absence of the exposure (i.e., hypothetical scenario), with adjustment for appropriate confounders in the study of ACEs with CHD (Chapter 5). As far as I know, this is the first longitudinal study to estimate to what extent the elimination of ACEs can be beneficial for CHD prevention, assuming that my model is correct.

Both studies used in my PhD were drawn from the UK population. This can affect the generalisability of the findings because prevalence and patterns of ACEs may be different in the UK compared to other contexts and ACEs may be differently related to SEP. Differences across countries is highlighted by, for example, a study in Saudi Arabia which reported that one fifth of the study sample experienced physical abuse,

while the prevalence in the Whitehall II study was 2.6%. This demonstrated how the prevalence of different types of ACEs can differ across settings due to various reasons (e.g., cultural background, social welfare system), making it difficult to apply findings from one country to another.

Homogeneity of study populations is observed in existing studies. Most of the studies to date, including the studies in my PhD, have been conducted in western high-income countries, in which data are likely available, such as UK and USA, but few studies from low- and middle-income countries, and non-western countries.[80,293] Firstly, this means that the impact of ACEs around the globe remains unclear. The similarity in existing study setting and populations used will mean that all studies are likely to have similar confounding structures, i.e., that ACEs will be strongly patterned according to socioeconomic position, and therefore could mean that there may remain residual confounding or unidentified confounders. This could result in spurious associations. It is therefore possible that there are unobserved or unidentified confounders or residual confounding, which may have affected the findings in my studies.

The Whitehall II cohort study, with baseline age of 35 to 55, has had now three decades of follow-up time, accumulating first episodes of CHD, but ACEs were asked retrospectively. I therefore also used NCDS, which has ACEs collected prospectively, in the study examining ACEs and salivary cortisol to compare the findings with those of the Whitehall II cohort study. Nevertheless, comparability of the results from these two cohort studies was challenging due to the measurement of cortisol being different. There are six saliva samples over a day in the Whitehall II cohort study, whereas the NCDS has collected only two saliva samples in a day. One of the samples, peak value in a day (i.e., 30 min after awakening in the Whitehall II cohort study, 45 min after awakening in the NCDS) was approximately equivalent across studies, although the value depends on the time of sample collection which varied between the two studies, and a single measure does not provide information of intra-individual variability. It was also not possible to assess overall cortisol secretion (i.e., AUC) in NCDS, because two saliva samples do not provide precise values of secretion over a day. I did not use NCDS to examine the association of ACEs with CHD because participants were still too young to have CHD at the time of assessment (i.e., age 44 to 45), as well as CHD was self-reported.

Another limitation is that dynamic interaction between the neuroendocrine and the autonomic nervous systems across time was not examined in my PhD. Salivary cortisol was measured six times over a day at phases 7 and 9 in the Whitehall II cohort study. These measures can exhibit diurnal patterns, but I did not assess changes with age because the analysis of change with only two measures will almost always produce inaccurate effect estimates due to random variation.[294] On the other hand, the HRV was assessed once in a day at phases 5, 7, and 9 over 10 years, exhibiting somewhat a longitudinal illustration of the system activity. These differences in time points of assessment made it challenging to investigate the interaction between the systems, limiting to interpretation of individual systems and of both systems in a cross-sectional way. Furthermore, the stress response in the body is thought to involve more systems, such as the immune system, than only these two systems examined in my PhD. However, current technology in biomedicine has not been able to measure all systems simultaneously yet.[40]

Lastly, as described in Chapter 4, I cannot rule out a possibility of selection bias due to attrition in my studies, given that I have performed complete case analyses in my PhD. In all of my analyses I aimed to estimate 14 effect sizes for the ACEs simultaneously as well as adjusting for covariates and, therefore, models need to

estimate a large number of parameters. Although it is recommended that the number of imputations required to examine an association be calculated using a linear[295] or quadratic rule,[296] the large number of variables in my models makes application of multiple imputation more challenging. It is a further methodological research question as to how many imputations should be created for a model in which there are many quantities of scientific interest to obtain robust estimates, and whether it is practically plausible. I therefore performed only complete case analyses. The findings in my studies are largely null, but it might be because people included as analytical sample are more likely to be healthy, possibly resulting in biasing the associations towards the negative findings.

8.5. Implications for future research and policy

My PhD has highlighted further key research questions which still need to be addressed. First, how the neuroendocrine and the autonomic nervous systems interact with each other, and how these are jointly dependent on early life exposures and experiences and stress. In other words, to what extent these systems, and other systems such as immune system, function independently from each other (i.e., does dysfunction of one system determine subsequent disruption of another system?), in stress response over the life course. The chain of events is important to understand

in order to be able to identify earlier points for intervention, but repeated measures of the multiple systems are required over the life course. Some studies have examined how biological systems respond to chronic stress including ACEs,[117,132] as well as what profile of biomarkers predict adverse outcome such as mortality.[297,298] This cumulative burden of chronic stress (i.e., allostatic load) is often quantified by measuring a range of biomarkers and creating a score in individual level summing the number of markers that deviate from the values within the sample distribution.[117,132,297,298] This approach has several issues. Like ACEs score, there is assumption that each biomarker has an equal effect on outcomes, which is unlikely. A series of biomarkers is determined by data availability, limiting aspects of the systems to be examined. Furthermore, categorisation of abnormal values based on the sample distribution is not recommended because of reproducibility in other populations and of the clinical implication.[180] Although addressing all these issues at once is difficult, it would be interesting to examine networks of these systems, using continuous scales of values, to better understand how they interact with each other, cross-sectionally and longitudinally across the life course.

Of key relevance for prevention of disease and promotion of healthy ageing is the identification of factors which can mitigate the effect of early life stress, and how

such factors could modify the effect (i.e., which part of the causal pathway is modified?). It has been documented that positive affect is a protective factor of adverse health outcomes.[299] Given that poor mental health is commonly observed in those who had ACEs, activities to promote positive affective states might block the pathway from ACEs to poor health through improving mental health and possibly through promoting better health risk behaviours as well. An example of such activities may be physical activity,[300] which is shown to be also protective against CVD.[300,301] Further, positive experiences co-occurring in childhood, such as "being able to talk to family about feelings", that counteract ACEs have been shed light on in recent years.[302,303] Having the positive experiences may also lead to lifelong resilience among people who had ACEs,[304]and lower risk of having mental health problems in adulthood.[302] Although measuring these experiences is complex, similar to the challenges in ACEs, assessing impact of positive childhood experiences may be beneficial for policy development.

My findings on the association between ACEs and the development of CHD did not support the existing studies reporting a strong association, while the studies examining the association of ACEs with the neuroendocrine system showed some associations, but not with the autonomic nervous system. Given that the

neuroendocrine system has been shown to be associated with the development of CHD,[281] an association of ACEs with CHD remains plausible. Also, there is accumulated evidence that ACEs are related to mental health later in life.[80] It is therefore important, even if the association of ACEs with CHD is unlikely or is small, to reduce the occurrence of ACEs where possible. Some ACEs have detrimental short-term impacts on children (e.g., injury or death due to physical abuse), while others are more likely to have long-term negative effect (e.g., parental mental health problems). As smacking was recently completely banned under law in Wales and Scotland, policy against a specific ACE may be important to particularly prevent its adverse short-term consequences irrespective of evidence of any longer-term impacts on health. Furthermore, policies and interventions need to recognise that ACEs are clustered.

8.6. Conclusion

The studies in my PhD showed null association between ACEs and incident CHD, but positive associations between ACEs and the neuroendocrine systems. There are possible explanations as to why my study showed null effects, such as study population, study design, ACEs measurement, statistical approach, and publication bias, while majority of existing studies have reported largely positive associations.

Despite some limitations, my research benefited from large-scale long running cohort studies with repeated measures of biomarkers and the statistical approach to address methodological issues seen in existing studies. Given that ACEs are more likely to be co-occurring, holistic approach to people who have had ACEs over the life course, as well as policies against a specific type of ACE are required. Research to identify factors which mitigate the effect of ACEs on lifelong health will be beneficial moving forward in order to develop effective intervention for those who have ACEs.

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Appendices

Appendix 1. SHRs and 95% CIs of CVD mortality in three models (Analysis 2)

	Adjusted for					
	Sex and age		+ Demography and health behaviours		+ Health status	
n=8791	SHR	95% CI	SHR	95% CI	SHR	95% CI
Response status						
Response		*ref.*		*ref.*		*ref.*
Withdrawal	1.28	(0.89-1.84)	1.14	(0.79-1.65)	1.21	(0.84-1.75)
Non-response	1.82	(1.37-2.41)	1.49	(1.10-2.01)	1.53	(1.13-2.06)
Sex						
Men		*ref.*		*ref.*		*ref.*
Women	0.78	(0.62-0.98)	0.57	(0.43-0.75)	0.54	(0.40-0.71)
Age in years						
39 and below		*ref.*		*ref.*		*ref.*
40 - 44	1.58	(1.01-2.47)	1.59	(1.01-2.49)	1.50	(0.95-2.36)
45 - 49	3.29	(2.17-5.00)	3.20	(2.09-4.88)	2.79	(1.83-4.25)
50 and over	7.33	(5.03-10.66)	7.20	(4.92-10.54)	6.02	(4.12-8.81)
Ethnicity						
White				*ref.*		*ref.*
Non-white			1.49	(1.08-2.05)	1.42	(1.02-1.96)
Marital status						
Married/cohabit				*ref.*		*ref.*
Single			1.46	(1.10-1.92)	1.47	(1.12-1.95)
Divorced/widowed			0.96	(0.67-1.39)	0.95	(0.66-1.37)
Employment grade						
High				*ref.*		*ref.*
Intermediate			1.07	(0.82-1.40)	1.05	(0.81-1.38)
Low			1.50	(1.05-2.14)	1.47	(1.03-2.11)
Smoking habit						
Never-smoker				*ref.*		*ref.*
Ex-smoker			1.11	(0.87-1.42)	1.10	(0.86-1.41)
Current smoker			1.62	(1.23-2.14)	1.54	(1.17-2.03)
Alcohol drinking						
<14 units per week				*ref.*		*ref.*
≥14 units per week			0.90	(0.69-1.16)	0.90	(0.70-1.16)
Physical activity						
High				*ref.*		*ref.*
Intermediate			0.97	(0.69-1.36)	0.95	(0.68-1.34)
Low			1.34	(1.00-1.78)	1.31	(0.98-1.74)
SF-36: PCS						
Q4 (best)						*ref.*
Q3					1.61	(1.01-2.35)
Q2					1.42	(0.97-2.09)
Q1 (worst)					2.39	(1.68-3.40)
SF-36: MCS						
Q4 (best)						*ref.*
Q3					0.84	(0.63-1.11)
Q2					0.72	(0.53-0.96)
Q1 (worst)					0.74	(0.56-0.98)

Appendix 2. SHRs and 95% CIs of non-CVD mortality in three models (Analysis 2)

		Adjusted for				
	Sex and age		+ Demography and health behaviours		+ Health status	
n=8791	SHR	95% CI	SHR	95% CI	SHR	95% CI
Response status						
Response		*ref.*		*ref.*		*ref.*
Withdrawal	1.75	(1.46-2.11)	1.72	(1.43-2.08)	1.77	(1.47-2.13)
Non-response	1.65	(1.40-1.95)	1.62	(1.36-1.92)	1.59	(1.34-1.89)
Sex						
Men		*ref.*		*ref.*		*ref.*
Women	0.94	(0.83-1.07)	0.95	(0.81-1.11)	0.90	(0.76-1.05)
Age in years						
39 and below		*ref.*		*ref.*		*ref.*
40 - 44	1.30	(1.04-1.63)	1.30	(1.04-1.64)	1.29	(1.03-1.63)
45 - 49	2.25	(1.82-2.79)	2.33	(1.88-2.89)	2.22	(1.78-2.77)
50 and over	4.56	(3.78-5.50)	4.76	(3.93-5.77)	4.45	(3.65-5.42)
Ethnicity						
White				*ref.*		*ref.*
Non-white			0.75	(0.60-0.94)	0.69	(0.55-0.87)
Marital status						
Married/cohabit				*ref.*		*ref.*
Single			1.00	(0.84-1.20)	0.97	(0.82-1.16)
Divorced/widowed			1.05	(0.86-1.28)	1.02	(0.84-1.24)
Employment grade						
High				*ref.*		*ref.*
Intermediate			1.02	(0.88-1.17)	0.99	(0.86-1.15)
Low			0.89	(0.73-1.09)	0.83	(0.68-1.02)
Smoking habit						
Never-smoker				*ref.*		*ref.*
Ex-smoker			1.09	(0.94-1.25)	1.06	(0.92-1.22)
Current smoker			2.04	(1.75-2.37)	1.91	(1.64-2.23)
Alcohol drinking						
<14 units per week				*ref.*		*ref.*
≥14 units per week			1.10	(0.96-1.26)	1.10	(0.96-1.26)
Physical activity						
High				*ref.*		*ref.*
Intermediate			0.81	(0.68-0.97)	0.80	(0.67-0.96)
Low			0.96	(0.83-1.12)	0.92	(0.79-1.07)
SF-36: PCS						
Q4 (best)						*ref.*
Q3					1.20	(0.97-1.49)
Q2					1.42	(1.16-1.74)
Q1 (worst)					2.04	(1.69-2.46)
SF-36: MCS						
Q4 (best)						*ref.*
Q3					1.01	(0.85-1.20)
Q2					1.18	(0.99-1.39)
Q1 (worst)					1.32	(1.12-1.56)

Appendix 3. Marginal log-hazard for counterfactual ACEs intervention and Marginal log-hazard for counterfactual ACEs intervention by ACE count group

This material was written by Owen Nicholas.

Marginal log-hazard for counterfactual ACEs intervention

Let the ith participants covariate values be denoted $(x)_i$, and let their counterfactual covariate values be denoted $(x')_i$,

For the population, the marginal log-hazard for the counterfactual ACEs intervention is

$$\frac{1}{N}\sum_i \beta^T((x')_i - (x)_i) = \beta^T \frac{1}{N}\sum_i ((x')_i - (x)_i)$$

where the sum is over the N participants. Note that in the sum, for any non-ACE variable x_j, $(x_j')_i - (x_j)_i = 0$, so the only variables which contribute to the margin are the ACE variables, and the only coefficients the corresponding ACE coefficients. Thus, we set all the non-ACE variables equal to zero, before running the margins command in Stata.

As a first approximation, marginal log-hazards can be turned in to marginal hazards by exponentiating the margins.

Marginal log-hazard for counterfactual ACEs intervention by ACE count group

If, in the previous section, only those participants who had a specific number of ACEs had been selected, the same logic applies.

Constant of proportionality

Those who have an ACE count of zero have no change to their ACE variables made by the counterfactual intervention. They are a reference group, and the marginal log-hazards of the intervention is zero for them. For groups with higher number of ACEs, we want to estimate how their marginal log-hazard increases in proportion to the number of ACEs.

Let z_k be the average covariates for the subgroup of participants with k ACEs, and z_k' be the average intervened upon covariates (as described above). Then, for the kth group, the marginal log-hazard is

$$\beta^T(z_k' - z_k)$$

The estimate for the constant of proportionality between the above expression and k is given by

$$\frac{\sum_k k\beta^T(z_k' - z_k)}{\sum_k k^2} = \beta^T \frac{\sum_k k(z_k' - z_k)}{\sum_k k^2}$$

It is a linear combination of the coefficients β, and therefore using β's estimate and covariance we estimated the constant of proportionality between the number of ACEs and the marginal log-hazards for the related subgroup.

Again, the only variables which contribute to this calculation are the ACE variables, and their counterfactual values, as an average.

Appendix 4. Coefficients and 95% confidence intervals (CIs) for the association of adverse childhood experiences with area under the curve (AUC) and cortisol awakening response (CAR) of the salivary cortisol by sex

	AUC (n = 3232) a b (95% CI)		**CAR** (n = 2950) a b (95% CI)	
	Men (n = 2467)	Women (n = 765)	Men (n = 2257)	Women (n = 693)
Adverse childhood experiences				
Maternal separation 1yr+	-3.242 (-10.317, 3.834)	12.33 (1.666, 22.994)	1.025 (-1.68, 3.729)	1.168 (-2.158, 4.495)
Parental death	7.561 (-0.386, 15.508)	8.656 (-4.488, 21.801)	3.529 (0.587, 6.47)	1.289 (-2.62, 5.199)
Hospitalisation 4wks+	-0.412 (-6.242, 5.419)	14.316 (3.936, 24.696)	-1.007 (-3.147, 1.134)	1.396 (-1.837, 4.629)
Divorce	2.034 (-13.235, 17.303)	1.698 (-19.701, 23.098)	1.158 (-4.842, 7.158)	-2.872 (-9.452, 3.707)
Mental illness and alcohol problems	4.411 (-4.683, 13.505)	-7.293 (-20.51, 5.923)	2.141 (-1.301, 5.583)	-3.585 (-7.636, 0.466)
Arguments between parents	-3.857 (-9.423, 1.709)	5.842 (-2.277, 13.961)	-0.902 (-2.982, 1.177)	0.416 (-2.121, 2.954)
Unemployment	4.857 (-1.834, 11.547)	0.509 (-11.116, 12.135)	2.484 (-0.028, 4.995)	-0.269 (-3.841, 3.303)
Financial problems	-3.317 (-8.212, 1.577)	2.648 (-5.151, 10.447)	0.514 (-1.333, 2.36)	0.627 (-1.806, 3.061)
Physical abuse	-0.576 (-17.13, 15.977)	-5.753 (-22.526, 11.02)	0.141 (-6.454, 6.736)	-2.736 (-8.073, 2.601)
Orphanage	9.846 (-20.469, 40.161)	-34.938 (-85.363, 15.486)	-3.206 (-14.607, 8.196)	-2.733 (-17.819, 12.354)
Lack of attachment to mothers	-0.881 (-1.778, 0.016)	0.703 (-0.547, 1.954)	-0.245 (-0.582, 0.092)	-0.035 (-0.424, 0.353)
Lack of attachment to fathers	0.276 (-0.595, 1.147)	-1.037 (-2.3, 0.226)	0.118 (-0.209, 0.445)	-0.011 (-0.408, 0.387)
Mother's harsh punishment	-1.775 (-4.646, 1.095)	-3.252 (-7.589, 1.085)	-0.397 (-1.477, 0.683)	-0.736 (-2.096, 0.625)
Father's harsh punishment	0.521 (-2.012, 3.054)	1.421 (-2.377, 5.219)	0.067 (-0.883, 1.016)	0.62 (-0.595, 1.835)
Covariates				
Age in years	0.559 (0.235, 0.883)	-0.138 (-0.682, 0.407)	-0.032 (-0.154, 0.09)	-0.202 (-0.371, -0.033)
Ethnicity	-10.782 (-20.629, -0.935)	-21.565 (-32.909, -10.22)	-2.811 (-6.607, 0.985)	-2.231 (-5.895, 1.433)
Childhood socioeconomic position	1.168 (-0.399, 2.734)	-1.85 (-4.423, 0.722)	0.26 (-0.333, 0.853)	0.294 (-0.502, 1.09)
Adult socioeconomic position	0.371 (-3.069, 3.811)	1.425 (-3.435, 6.285)	0.192 (-1.099, 1.483)	1.371 (-0.158, 2.9)
Smoking on the day of saliva sampling	3.001 (-5.489, 11.491)	12.812 (-0.816, 26.439)	0.938 (-2.139, 4.015)	-0.165 (-4.387, 4.057)
Awakening time	-7.587 (-9.319, -5.855)	-8.211 (-11.023, -5.4)	-0.874 (-1.543, -0.205)	-0.623 (-1.522, 0.276)
Intercept	4.915 (4.643, 5.187)	5.366 (4.945, 5.786)	15.199 (4.907, 25.491)	23.196 (10.117, 36.275)

a Log-transformed values (ln(nmol/l)*hr) are presented for intercept, otherwise % in AUC, while CAR is presented in an original scale (nmol/l).

Appendix 5. Estimates and 95% Confidence Intervals (CIs) of diurnal slope in the association with adverse childhood experiences of log cortisol

	b (95% CI)	
	Men (n = 2586)	**Women (n = 814)**
Fixed part: reference trajectory		
Intercept: log salivary cortisol at awakening (ln(nmol/l))	2.937(2.606, 3.267)	2.935(2.441, 3.429)
Time since awakening (linear, hr)	-19.092 (-22.542, -15.642)	-14.246 (-20.034, -8.459)
Time since awakening (quadratic, hr^2)	0.115 (0.071, 0.158)	0.254 (0.176, 0.332)
Fixed part: intercept		
Adverse childhood experiences; binary (ref. no experience)		
Maternal separation 1yr+	-2.493 (-11.099, 6.113)	8.895 (-3.668, 21.458)
Parental death	4.905 (-4.471, 14.281)	-1.194 (-15.647, 13.259)
Hospitalisation 4wks+	3.345 (-3.603, 10.293)	13.246 (0.981, 25.511)
Divorce	0.302 (-18.581, 19.185)	12.957 (-12.316, 38.23)
Mental illness and alcohol problems	-4.585 (-15.618, 6.447)	5.847 (-9.391, 21.086)
Arguments between parents	-2.906 (-9.618, 3.807)	-3.816 (-13.348, 5.717)
Unemployment	3.474 (-4.656, 11.603)	7.308 (-6.313, 20.93)
Financial problems	-5.981 (-11.919, -0.043)	3.752 (-5.481, 12.985)
Physical abuse	-5.097 (-25.011, 14.816)	-16.816 (-36.289, 2.658)
Orphanage	12.514 (-23.542, 48.571)	-17.52 (-71.731, 36.691)
Adverse childhood experiences; ordinal		
Lack of attachment to mothers	-0.907 (-1.991, 0.177)	1.593 (0.109, 3.076)
Lack of attachment to fathers	0.717 (-0.337, 1.772)	-0.9 (-2.395, 0.595)
Mother's harsh punishment	0.303 (-3.166, 3.772)	-3.613 (-8.7, 1.473)
Father's harsh punishment	-0.745 (-3.809, 2.319)	0.585 (-3.883, 5.053)
Covariates		
Awakening time	-3.17 (-5.295, -1.046)	-1.56 (-4.851, 1.731)
Age in years	-0.209 (-0.6, 0.182)	-0.364 (-1.008, 0.279)
Ethnicity: non-White (ref. White)	-12.186 (-24.045, -0.328)	-39.729 (-53.218, -26.24)
Childhood socioeconomic position	1.662 (-0.235, 3.56)	-2.164 (-5.196, 0.868)
Smoking	-8.247 (-18.415, 1.92)	-10.708 (-26.68, 5.264)

Adulthood socioeconomic position	-2.745 (-6.902, 1.411)	-1.141 (-6.861, 4.579)
Fixed part: slope		
Adverse childhood experiences; binary (ref. no experience)		
Maternal separation 1yr+	0.084 (-0.784, 0.951)	0.307 (-1.109, 1.723)
Parental death	0.174 (-0.777, 1.124)	0.726 (-0.928, 2.38)
Hospitalisation 4wks+	-0.29 (-0.996, 0.416)	0.071 (-1.304, 1.446)
Divorce	0.044 (-1.861, 1.949)	-0.836 (-3.696, 2.024)
Mental illness and alcohol problems	1.008 (-0.099, 2.116)	-0.839 (-2.571, 0.892)
Arguments between parents	0.116 (-0.561, 0.792)	1.126 (0.052, 2.2)
Unemployment	0.278 (-0.546, 1.102)	-0.832 (-2.359, 0.694)
Financial problems	0.205 (-0.395, 0.806)	-0.791 (-1.831, 0.249)
Physical abuse	0.136 (-1.838, 2.111)	1.343 (-0.847, 3.533)
Orphanage	0.176 (-3.451, 3.802)	-2.578 (-8.664, 3.509)
Adverse childhood experiences; ordinal		
Lack of attachment to mothers	0.035 (-0.075, 0.144)	-0.118 (-0.286, 0.05)
Lack of attachment to fathers	-0.1 (-0.206, 0.006)	-0.007 (-0.176, 0.162)
Mother's harsh punishment	-0.262 (-0.613, 0.088)	-0.255 (-0.83, 0.32)
Father's harsh punishment	0.297 (-0.012, 0.607)	0.14 (-0.362, 0.643)
Covariates		
Interaction of awakening time with time since awakening	-0.428 (-0.643, -0.214)	-0.627 (-0.996, -0.258)
Interaction of age in years with time since awakening	0.126 (0.087, 0.166)	0.043 (-0.029, 0.115)
Interaction of ethnicity with time since awakening	0.773 (-0.432, 1.977)	2.571 (1.05, 4.093)
Interaction of childhood socioeconomic position with time since awakening	-0.023 (-0.215, 0.168)	0.139 (-0.2, 0.479)
interaction of smoking with time since awakening	1.69 (0.654, 2.727)	2.754 (0.963, 4.544)
Interaction of adulthood socioeconomic position with time since awakening	0.469 (0.051, 0.888)	0.542 (-0.1, 1.184)
Random part		
Variance: individual level intercept	0.094(0.076, 0.117)	0.024(0.007, 0.082)
Variance: individual level slope (linear time since awakening, hr)	0.001(0.001, 0.001)	0.001(0.001, 0.001)
Covariance: individual level intercept and slope change	0(-0.002, 0.001)	0.004(0.001, 0.006)
Variance: measurement occasions in a day level intercept	0.456(0.442, 0.47)	0.448(0.423, 0.474)

[a] Adjusted for age in years, ethnicity, childhood socioeconomic position, adult socioeconomic position, and awakening time and smoking on the day of saliva sampling

Appendix 6. Coefficients and 95% confidence intervals (CIs) for the association of cumulative score of adverse childhood experiences with area under the curve (AUC) and cortisol awakening response (CAR) of the salivary cortisol

	AUC (n = 3232) a b (95% CI)		**CAR** (n = 2950) a b (95% CI)	
	Sex and age	All covariates	Sex and age	All covariates
Adverse childhood experiences				
Cumulative score	-0.647 (-1.681, 0.387)	-0.64 (-1.675, 0.396)	0.161 (-0.208, 0.531)	0.113 (-0.263, 0.489)
Covariates				
Sex	-5.946 (-9.797, -2.095)	-5.514 (-9.547, -1.482)	1.256 (-0.124, 2.637)	1.12 (-0.343, 2.583)
Age in years	0.449 (0.177, 0.722)	0.46 (0.19, 0.729)	-0.046 (-0.144, 0.052)	-0.05 (-0.148, 0.049)
Ethnicity		-12.49 (-19.867, -5.114)		-1.759 (-4.518, 1.001)
Childhood socioeconomic position		0.577 (-0.742, 1.896)		0.382 (-0.098, 0.862)
Adult socioeconomic position		0.097 (-2.662, 2.855)		0.387 (-0.618, 1.391)
Smoking on the day of saliva sampling		5.254 (-1.946, 12.454)		0.597 (-1.94, 3.134)
Awakening time		-7.698 (-9.176, -6.221)		-0.782 (-1.333, -0.231)
Intercept	4.427 (4.247, 4.608)	4.943 (4.735, 5.15)	9.667 (3.197, 16.137)	14.132 (6.57, 21.694)

a Log-transformed values (ln(nmol/l)*hr) are presented for intercept, otherwise % in AUC, while CAR is presented in an original scale (nmol/l).

Appendix 7. Estimates[a] and 95% Confidence Intervals (CIs) of diurnal slope of log cortisol by cumulative score of adverse childhood experiences (n = 3400)

	b (95% CI)			
	Model 1	**Model 2**	**Model 3**	**Model 4**
Fixed part: reference trajectory				
Intercept: log salivary cortisol at awakening (ln(nmol/l))	2.529 (2.507, 2.552)	2.546 (2.516, 2.575)	2.742 (2.613, 2.87)	2.906 (2.656, 3.157)
Time since awakening (linear, hr)	-14.032 (-14.672, -13.393)	-14.154 (-14.825, -13.483)	-10.783 (-12.312, -9.253)	-18.526 (-21.232, -15.821)
Time since awakening (quadratic, hr^2)	0.16 (0.122, 0.198)	0.16 (0.122, 0.198)	0.145 (0.107, 0.183)	0.148 (0.11, 0.186)
Fixed part: intercept				
Adverse childhood experiences				
Cumulative score		-1.044 (-2.264, 0.176)	-0.998 (-2.216, 0.221)	-0.745 (-1.982, 0.492)
Covariates				
Awakening time			-2.877 (-4.681, -1.072)	-2.757 (-4.555, -0.958)
Sex				-6.061 (-10.931, -1.192)
Age in years				-0.189 (-0.515, 0.137)
Ethnicity: non-White (ref. White)				-21.986 (-30.884, -13.088)
Childhood socioeconomic position				0.858 (-0.733, 2.449)
Smoking				-8.423 (-17.036, 0.189)
Adulthood socioeconomic position				-2.384 (-5.709, 0.941)
Fixed part: slope				
Adverse childhood experiences				
Cumulative score		0.076 (-0.051, 0.204)	0.082 (-0.045, 0.208)	0.031 (-0.097, 0.159)
Covariates				
Awakening time			-0.458 (-0.645, -0.27)	-0.477 (-0.663, -0.291)
Sex				0.049 (-0.455, 0.553)
Age in years				0.107 (0.073, 0.14)
Ethnicity: non-White (ref. White)				1.689 (0.764, 2.614)

Childhood socioeconomic position				-0.001 (-0.165, 0.163)
Smoking				1.943 (1.046, 2.84)
Adulthood socioeconomic position				0.434 (0.09, 0.777)
Random part				
Variance: individual level intercept	0.087 (0.071, 0.106)	0.087 (0.071, 0.106)	0.085 (0.07, 0.105)	0.08 (0.065, 0.099)
Variance: individual level slope (linear time since awakening, hr)	0.001 (0.001, 0.001)	0.001 (0.001, 0.001)	0.001 (0.001, 0.001)	0.001 (0.001, 0.001)
Covariance: individual level intercept and slope change	0 (-0.001, 0.001)	0 (-0.001, 0.001)	0 (-0.002, 0.001)	0 (-0.001, 0.002)
Variance: measurement occasions in a day level intercept	0.454 (0.442, 0.467)	0.454 (0.442, 0.467)	0.455 (0.442, 0.467)	0.455 (0.442, 0.467)

[a] In fixed part, log-transformed values (ln(nmol/l)*hr) are presented for intercept, otherwise %.

Appendix 8. Relative changes in diurnal cortisol slope with 95% confidence intervals by cumulative score of adverse childhood experiences (n = 3400)