Maternal separation in childhood and the role of biomarkers of stress in its association with mental health

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Declarations

No part of this thesis has been submitted for another degree and all the work in this thesis is original and my own.

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Abstract:

Parent-child separation has been shown to increase the risk of depressive symptoms later in life. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis may help to explain this association. However, few studies have examined the effects of separation on cortisol in late adulthood. This thesis aims to investigate the interplay between these social, psychological and biological factors.

Data were from phases 7 (2002-04) to 11 (2012-13) of the Whitehall II study, when participants were aged 60 to 70 years, on average. The first two empirical chapters focus on establishing the association between maternal separation in childhood and cortisol, measured in saliva and hair. The third empirical chapter examines the association between long-term depressive symptoms and hair cortisol. Analyses were conducted using linear regression and multilevel mixed modelling.

Participants who reported separation during childhood showed flatter diurnal cortisol slopes and higher hair cortisol concentrations (HCC), compared to their non-separated counterparts. Associations between separation and cortisol awakening response (CAR) and area under the curve (AUC) were not significant. An association between maternal separation and salivary cortisol was observed at phase 7 of Whitehall II but not phase 9 (2007-09). Diurnal cortisol slopes became flatter from phase 7 to 9 across all participants but the degree of change was lower in participants reporting separation. Current and recurrent, but not past, depressive symptoms were positively associated with HCC. An interaction effect between maternal separation in childhood and depressive symptoms was not significant.

Maternal separation in childhood and adult depressive symptoms were independently associated with cortisol in saliva and hair in late adulthood. No evidence was found for mediating or moderating effects, suggesting that early-life stress and depression may have distinct effects on the HPA axis in later life.

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Abbreviations:

ACEs	Adverse childhood experiences
ANS	Autonomic nervous system
AUC	Area under the curve
AUCg	Area under the curve with respect to the ground
AVP	Arginine vasopressin
BMI	Body mass index
CAR	Cortisol awakening response
CBG	Cortisol-binding globulin
CES-D	Center for Epidemiological Studies Depression scale
CIS-R	Revised Clinical Interview Schedule
CLIA	Chemiluminescence Immunoassay
CVD	Cardiovascular disease
FMI	Fraction of missing information
GC	Glucocorticoids
GR	Glucocorticoid receptor
HCC	Hair cortisol concentrations
HPA axis	Hypothalamus-pituitary-adrenal axis
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MICE	Multiple imputation by chained equations
MR	Mineralocorticoid receptor
PVN	Paraventricular nucleus
SD	Standard deviation
UFC	Urinary free cortisol

1 Introduction:

Early life stress has been shown to have a detrimental impact on both physical and mental health outcomes in adulthood (Nelson et al., 2020). In particular, studies have reported an increased risk of depression in individuals that experienced parental loss during childhood (SIMBI et al., 2020). Dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis may help to explain how the effects of early life stress 'get under the skin' (Koss and Gunnar, 2018). The HPA axis is the body's stress response system that produces the stress hormone cortisol (Smith and Vale, 2006). A well-established literature on cortisol and depression also exists (Belvederi Murri et al., 2014; Stetler and Miller, 2011). However, the relationship between early life, psychological factors, and biological stress markers remains to be fully elucidated. This thesis aims to address this gap by examining the associations between maternal separation in childhood, cortisol, and depressive symptoms in later life. Attention is paid to the role of potential mediating and moderating effects that may help to explain the interplay between these factors.

The remainder of this chapter is structured as follows: First, the theoretical framework is presented. Second, an overview of maternal separation in childhood is presented, along with the literature on its association with depression. The HPA axis is then introduced with a discussion regarding the measurement of cortisol. This is followed by studies examining the role of cortisol in early life stress and adult depression. Third, a summary of the main research aims that are addressed in the empirical chapters. Fourth, the chapter ends with an overview of the Whitehall II study, including the variables used and the phases of data collection they were collected in.

1.1 Theoretical framework:

The analyses in this thesis are situated primarily in the theory of life course epidemiology. This provides a theoretical framework for understanding how early life exposures may impact biology and health across the life course. In its initial development, two broad models were proposed: critical or sensitive periods and accumulation models (Kuh et al., 2003). The critical periods model posits that exposures during a specific period can have long-lasting or permanent effects on a biological or health outcome. A well-known example of this model is the fetal origins of adult disease hypothesis (Barker et al., 2002). This model was later extended to consider the modifying effects of subsequent exposures. Critical and sensitive periods are similar but contain an important distinction. The former suggests that the effect of an exposure can only occur during a specific point in time. The latter refers to a period when an exposure has a greater chance of affecting health outcomes (Kuh et al., 2003).

The accumulation model shares similarities with the concept of allostatic load (McEwen, 2000) and focuses on the accumulative effect of exposures on disease risk. These exposures may independently affect outcomes or reflect pathways in what is known as chains of risk. Here, one exposure may increase the likelihood of another and so forth. Although they were initially thought of as distinct models, Ben-Shlomo et al. (2016) now suggest that critical and sensitive periods should be more accurately described as sub-classes of the accumulative model. This is best portrayed when considering the accumulative effects of the same exposure over time. In this context, sensitive models describe a setting where the effect of exposures still accumulate over time but have differential impacts during certain periods. The critical period model can then be considered as a specific type of

sensitive model whereby the effects of the exposure are either all or none (Ben-Shlomo et al., 2016).

Alongside the life course perspective, insights are also drawn from the fields of neurobiology and psychoneuroendocrinology regarding theories on the effects of chronic stress during critical periods of HPA axis development in childhood and adolescence (Heim et al., 2008; Koss and Gunnar, 2018).

1.2 Maternal separation in childhood:

1.2.1 Overview:

Maternal separation has long been used in animal studies to model the effects of early-life stress (Tractenberg et al., 2016). This refers to a physical separation between mother and offspring during the early stages of life. Much of the research using this model has been conducted in rodents and non-human primates. Studies have reported significant associations between maternal separation and a range of outcomes, including cognitive impairment (Aisa et al., 2007), behavioural abnormalities (Jin et al., 2018), and HPA axis dysfunction (Feng et al., 2011).

Maternal separation in humans is a complex event that is strongly influenced by the social environment and requires consideration of the historical, social, and cultural context in which it takes place. Here, I define maternal separation as a period of separation occurring between mother and child for an extended period. This goes beyond a physical separation and refers to an event which causes an interpersonal relationship to become severely limited. Parent-child separation can occur for several reasons, ranging from parental death to adoption. Moreover, the causes of separation may vary across time or cultures. A significant cause of separation in recent history was the evacuation of children during the Second World War.

Similarly, separation may occur in some societies as a result of parents migrating from rural to urban environments to seek employment (Wang and Mesman, 2015).

1.2.2 Maternal separation in childhood and adult depression:

Studies have shown an increased risk of depressive disorders in adulthood among individuals with a history of childhood separation (Amato, 2016; Coffino, 2009; Foster et al., 2003; SIMBI et al., 2020). However, the effects may differ by the type of separation experienced. Research on female (Kendler et al., 1992) and male (Otowa et al., 2014) twin studies show an increased risk of depression in individuals with a history of parent-child separation but not parental death. In contrast, Tyrka et al. (2008) found an increased risk of depressive and anxiety disorders for both parental separation and death but the association with the former was explained by a family history of depressive and anxiety disorders. Therefore, the type of separation experienced may not only have a differential impact on outcomes but also differ in terms of relevant confounding factors. This highlights the importance of considering the pre-separation environment. Some studies have suggested that the effect may also depend on whether an individual experiences maternal or paternal separation but the findings are mixed. Pesonen et al. (2007) found that individuals who were evacuated during the Second World War had higher depressive symptoms than those who were not evacuated but no differences were found for those who were separated only from their fathers. Otowa et al. (2014), however, found maternal and paternal separation to have broadly similar effects on a range of psychopathological outcomes. Pesonen et al. (2007) suggest that their findings may reflect a protective factor by the mother. However, the higher depressive symptoms seen in individuals separated from both parents may instead reflect the experience of evacuation itself.

Various pathways have been proposed for how early life stress may impact psychological health (Kuhlman et al., 2017). However, perhaps the most prominent of these has been the HPA axis.

1.3 HPA axis:

1.3.1 Overview:

The HPA axis is a neuroendocrine system composed of the hypothalamus, pituitary gland, and adrenal glands and is responsible for the body's capacity to respond to stress. Following signals from the autonomic nervous system (ANS), corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) are secreted by the paraventricular nucleus (PVN) of the hypothalamus. These hormones stimulate the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland, prompting the adrenal cortex to produce and release glucocorticoid (GC) hormones. Cortisol is the principal GC in humans. Once released, cortisol acts upon cells throughout the body by binding to mineralocorticoid (MR) and glucocorticoid (GR) receptors.

Regulation of the HPA axis is mediated by a negative feedback mechanism whereby cortisol inhibits further CRH and ACTH secretion via activation of MR and GR in regions of the brain. MRs have higher binding affinity and help maintain optimum cortisol levels under basal conditions. GRs have higher binding affinity and are involved in inhibiting the stress response (Herman et al., 2016). The functioning of this system is vital for the body to respond to and successfully recover from stressors and maintain homeostasis. However, prolonged activation, particularly during periods of critical development, can result in dysregulation of the HPA axis leading to excessive cortisol levels (Van Bodegom et al., 2017). Attention has focused mainly on GR impairment (Pariante, 2006) but research suggests that MR plays an equally

vital role in maintaining HPA axis functioning (ter Heegde et al., 2015). In turn, HPA axis dysfunction has been associated with various adverse health outcomes (Maniam et al., 2014).

1.3.2 Measurement of cortisol:

There are several ways to measure cortisol in humans. These include serum, urine, saliva and hair. Traditionally, serum has been the standard method used to measure cortisol. However, there are well-known limitations to this method. Cortisol follows a diurnal rhythm, and this is reflected in serum cortisol. As a result, single measures provide only a limited snapshot of HPA axis activity (Russell et al., 2012). Multiple measurements are required to capture diurnal patterns but this is not feasible in large-scale studies due to cost and unnecessary participant burden. Second, serum reflects total cortisol levels, which consists of protein-bound cortisol and unbound (free) cortisol. Various studies have demonstrated that variation in cortisol-binding globulin (CBG), which a number of different factors can cause, can have significant effects on serum total cortisol levels (Dhillo et al., 2002; Le Roux et al., 2003). Third, evidence suggests that the data collection method may raise cortisol levels in some participants due to the distress associated with venepuncture procedures (Weckesser et al., 2014).

Urine is another data collection method, which has a few distinct advantages. First, it requires a less invasive sampling procedure compared to serum. However, it is still quite labour-intensive and thus may increase levels of non-response (Lee et al., 2015). Second, urine reflects levels of urinary free cortisol (UFC), which is not affected by diurnal variation. Samples are often collected over a 12 or 24-h period and then pooled together to provide average cortisol levels. As a result, UFC is not

affected by the range of factors that are known to influence CBG levels (Ifedayo and Olufemi, 2013). Despite this, measurements can still be affected by other external factors, such as excessive fluid intake, which has been shown to increase levels of UFC (Mericq and Cutler, 1998).

Saliva has been a common method of cortisol sampling in recent times, particularly in large-scale observational studies. This is partly due to its reduced cost and relative ease of data collection procedures. Salivary cortisol is usually conducted in one of two ways in research: measurements are taken to examine the responsiveness of the HPA axis in cortisol reactivity tests, or multiple measurements are taken over the course of a day to examine the diurnal rhythm. Like UFC, salivary cortisol reflects free cortisol and displays diurnal variation like serum. The diurnal rhythm of cortisol follows a similar pattern in most healthy people. Approximately 30 to 45 minutes after waking, an increase in cortisol levels is observed, known as the cortisol awakening response (CAR). This is followed by a gradual decline over the the day, until reaching a nadir around bedtime (Adam and Kumari, 2009). This decline is commonly referred to as the diurnal cortisol slope. In healthy individuals, the slope is relatively steep. Dysregulation of the HPA axis can result in a flattening of the slopes, and studies have found associations between this and a range of adverse health outcomes (Adam et al., 2017). An additional measure of salivary cortisol that can be computed is the area under the curve, which reflects total cortisol output over the day (Pruessner et al., 2003). Salivary cortisol is influenced by various situational and environmental factors (Stalder et al., 2012). As a result, studies have shown low levels of stability in cortisol, meaning that multiple days of sampling may be required to attain reliable estimates (Ross et al., 2014; Segerstrom et al., 2014).

A common limitation among cortisol measured in bodily fluids is that it reflects acute cortisol levels and thus may not be suited for examining the effects of chronic stress. Hair is a more recent sampling method which partially overcomes this limitation. Cortisol measured in hair reflects total cortisol output over a period of several months, depending on the length of the sample taken (Sauvé et al., 2007). Researchers are still unsure about how cortisol is transmitted to hair but it has been hypothesised that hair cortisol concentration (HCC) reflects concentrations of the free cortisol component in serum (Russell et al., 2012). Further research is needed to clarify this. Unlike other measurements of cortisol, hair samples are relatively non-invasive in comparison. Additionally, it is not affected by the situational and environmental influences that can impact saliva measurements. Despite this, some evidence suggests that hair washing and treatment procedures may impact HCC (Abell et al., 2016b; Sauvé et al., 2007). Also, it is an unsuitable data collection method for individuals suffering from baldness.

1.3.3 Adult cortisol and maternal separation in childhood:

Extensive research has been conducted on the association between early life stress and cortisol (Bunea et al., 2017; Fogelman and Canli, 2018), but few studies have focused on the effects of parent-child separation. Of the studies that have been conducted, the findings have been mixed with evidence of both elevated (Kumari et al., 2013; Luecken, 2000; Luecken and Appelhans, 2006; Nicolson, 2004; Pesonen et al., 2010) and decreased (Hengesch et al., 2018; Kraft and Luecken, 2009; Meinlschmidt and Heim, 2005; Tyrka et al., 2008a) cortisol levels. Many existing studies have been conducted using small samples and focused only on cortisol in serum and saliva. Evidence regarding the associations with hair is currently lacking.

The mechanisms linking separation to HPA axis dysregulation in adulthood are not clear. Studies have shown that the HPA axis is particularly sensitive to the effects of chronic stress during childhood (Agorastos et al., 2019). Chronic stress experienced as a result of separation may dysregulate the HPA axis in childhood, with the effects persisting into adulthood. Alternatively, something specific within the experience of separation may impact the HPA axis. Research shows that parents have a regulatory and stress-buffering effect on their children's HPA axis, although this diminishes during puberty (Gunnar and Hostinar, 2015). Therefore, the effect of separation may reflect this loss of buffering. This is likely also affected by the care provided in the post-separation environment. Luecken (2000), for example, found that parental loss during childhood was positively associated with cortisol, but this effect was present only within participants who also reported low levels of parental care. Studies have reported links between the relationship quality between parent and child and cortisol profiles in childhood (Kopala-Sibley et al, 2017; Barrios et al, 2017) and young adulthood (Lucas-Thompson, 2013; Luecken et al, 2016).

Other life course factors, such as health behaviours, may also partially explain the effects of childhood separation on adult cortisol levels. Kumari et al. (2013) found that smoking status explained a significant proportion of the association between maternal separation in childhood and CAR. Previous research has established links between separation in childhood and the risk of adverse health behaviours. Lacey et al. (2018) found that parental absence was associated with an increased risk of having smoked or consumed alcohol before adolescence. Similarly, Martindale and Lacey (2017) found that individuals whose parents separated when they were children were more likely to be a current or ex-smoker in mid-adulthood versus having never smoked. The relationship between smoking and higher cortisol has

previously been established, but this effect is limited to current smokers (Badrick et al., 2007; Steptoe and Ussher, 2006).

1.3.4 HPA axis and depression:

The HPA axis has also been implicated in the pathophysiology of depression (Nandam et al., 2020). Studies have reported associations between depression and cortisol in both basal levels (Belvederi Murri et al., 2014; Knorr et al., 2010; Stetler and Miller, 2011) and cortisol reactivity (Burke et al., 2005; Zorn et al., 2017). Findings have been mixed in terms of the effect size and direction, but hypercortisolism in depressed patients versus controls has been a reasonably consistent outcome reported in several meta-analytic studies (Belvederi Murri et al., 2014; Stetler and Miller, 2011). Classical theories suggest that HPA axis hyperactivity may arise due to impaired negative feedback (Pariante and Lightman, 2008). However, support has also been found for decreased MR expression (ter Heegde et al., 2015) and adrenal enlargement, resulting in elevated and irregular basal cortisol (Carroll et al., 2012).

Fewer studies have been conducted on the association between depression and cortisol in hair compared to bodily fluids. Here, the findings are mixed with reports of higher (Dettenborn et al., 2012; Duncko et al., 2019; Iob et al., 2019; Song et al., 2019) and lower (Gerber et al., 2013; Pochigaeva et al., 2017) HCC. In a recent meta-analysis and systematic review of the literature, Psarraki et al. (2021) suggest that the heterogeneity in the findings may be explained by factors such as the recurrence of symptoms, medication use, and the role of early life stress.

1.3.5 Interplay between maternal separation in childhood, cortisol, and depression:

HPA axis dysregulation has been associated with early-life stress and depressive disorders, but the relationship between them is still not fully understood. Evidence of both mediating and moderating effects has been reported. Iob et al. (2021) show that adverse childhood experiences (ACEs) were positively associated with depressive symptoms in early adulthood. This was partly mediated by lower cortisol levels, measured at age 11, and was robust to genetic confounding. In contrast, Cantave et al. (2019) failed to find evidence for a mediating effect of cortisol on the association between childhood maltreatment and depressive symptoms and instead found support for moderation. Childhood maltreatment was associated with a higher risk of depressive symptoms in a sample of men aged between 18 and 35 y, but only amongst those with a high cortisol response to a cognitive stress test. Low-to-moderate cortisol responses were not statistically significant. Support for both mediating (Ju et al., 2020) and moderating (Goldman-Mellor et al., 2012) effects has been reported elsewhere. Despite this, most studies examining the joint effects of early-life stress and cortisol on depression have failed to see an association (Cantave et al., 2022; Oresta et al., 2021; Power et al., 2012; Scarth et al., 2022). It is not yet clear whether HPA axis abnormalities precede the onset of depression. Two recent meta-analyses have attempted to provide further understanding of this by focusing on prospective associations. Assessing studies on adolescents and adults, Kennis et al. (2019) found that cortisol was prospectively associated with a small, increased risk of depression but the effect was attenuated after adjusting for baseline clinical diagnosis. In contrast, Zajkowska et al. (2022) found that morning cortisol predicted an increased risk of major depressive disorder (MDD) in

adolescents and young adults. Therefore, the prospective association between cortisol and depression may depend on study participants' age.

1.4 Research aims:

This thesis aims to examine the associations between maternal separation in childhood, cortisol, and depressive symptoms in late adulthood. To investigate this, analyses will be conducted in a cohort using data from the Whitehall II study across three empirical chapters. The aims of these chapters are discussed below.

Aim 1: Examine the association between maternal separation in childhood and cortisol, measured in saliva and hair, in late adulthood.

Evidence suggests that early life stress is associated with HPA axis dysregulation, and this effect may persist throughout the life course. However, few studies have examined the effects of maternal separation. Chapters 2 and 3 are dedicated to establishing the relationship between separation and cortisol over three phases of data collection in the Whitehall II study (phases 7, 9, and 11). Associations are examined in both salivary and hair cortisol. Existing literature suggests that the effect of separation on cortisol may be explained by factors over the life course. Similarly, circumstances within the pre-separation environment may confound this relationship. To account for this, various childhood, socioeconomic, and health-related factors are adjusted for and examined to see whether they explain any of the associations.

Aim 2: Examine the association between maternal separation in childhood and depressive symptoms in late adulthood.

Studies have shown that parent-child separation is associated with an increased risk of depression in adulthood. Chapter 4 will examine whether similar associations exist in the Whitehall II cohort.

Aim 3: Examine the association between depressive symptoms and cortisol in late adulthood.

The literature on depression and cortisol is extensive, but still, gaps in the knowledge remain. Most of the research has been conducted using serum and salivary cortisol. Few studies have examined the association between depression and cortisol in hair, showing mixed findings. Chapter 4 aims to address some of those gaps by examining the association between long-term depressive symptoms and HCC. Recurrent symptoms, measured over three phases of data, and long-term antidepressant use are investigated to see whether they account for some of the variability in this relationship.

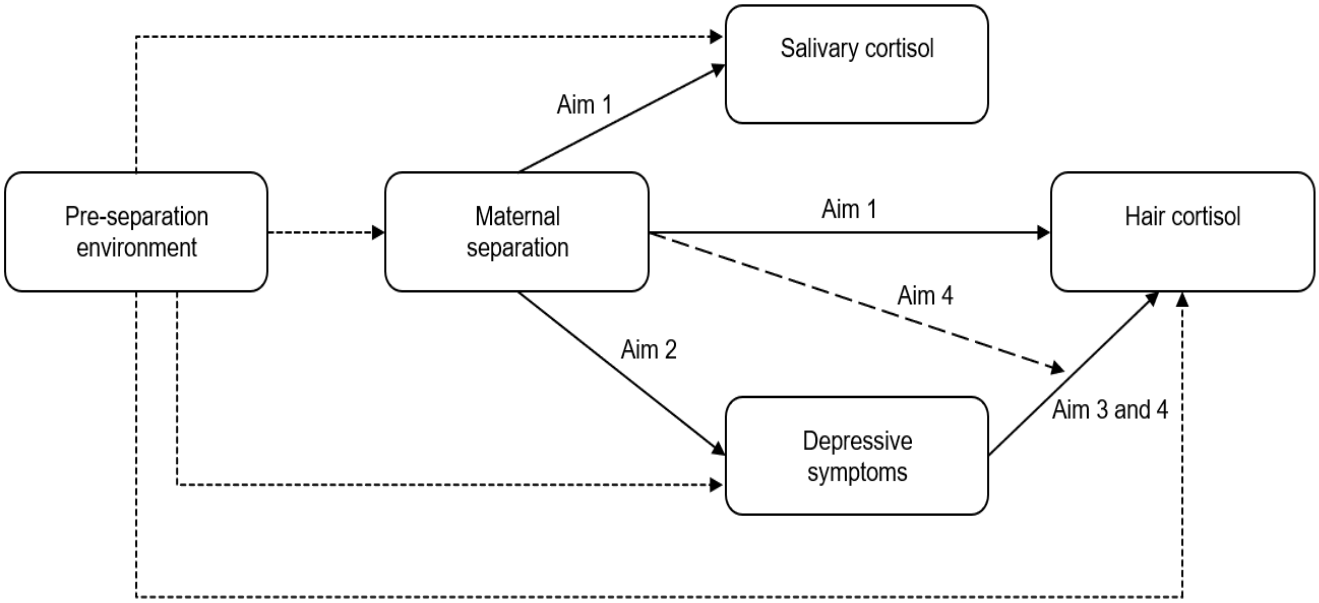
Aim 4: Investigate the interplay between maternal separation in childhood, depressive symptoms and cortisol in late adulthood.

Another potential source of variability in the association between depression and cortisol is the role of early life stress. Previous research has shown mixed findings with evidence of both mediating and moderating effects. The final aim of this thesis is to investigate the relationship between these three variables. This will be carried out across all three empirical chapters. Despite extensive research, the pathways have not been fully explained. Therefore, two possibilities will be examined. The first seeks to establish whether cortisol mediates the association between maternal separation in childhood and depressive symptoms in later life. The second examines the potential moderating effect of maternal separation on the association between cortisol and depressive symptoms. An important caveat regarding mediation must be highlighted. Based on the existing literature, it is still not clear whether cortisol abnormalities precede the onset of depressive symptoms in adulthood (Kennis et al.,

2019). However, studies suggest that HPA axis dysregulation and depression may begin relatively early in the life course (Agorastos et al., 2019; Maughan et al., 2013). Since this thesis focuses on late adulthood, it is difficult to determine the correct temporal ordering. This is a vital assumption in mediation analysis (Fairchild and McDaniel, 2017; Gelfand et al., 2009) and cannot be confidently met in the analyses. As a result, the focus will be on examining whether the association between maternal separation and depressive symptoms is independent of cortisol rather than a formal mediation analysis.

A conceptual framework is shown in Figure 1.1 below. Factors originating within the pre-separation environment are displayed on the left-hand side of the framework. Dotted arrows from this box towards maternal separation, depressive symptoms and salivary and hair cortisol represent possible confounding. Solid arrows represent the associations tested between exposure and outcome. The dashed arrow line from maternal separation to the line between depressive symptoms and hair cortisol represents a potential interaction effect.

Figure 1.1: Conceptual framework



1.5 Overview of the Whitehall II study

This section provides a brief overview of the Whitehall II study and discusses the phases of data used in the analytic chapters.

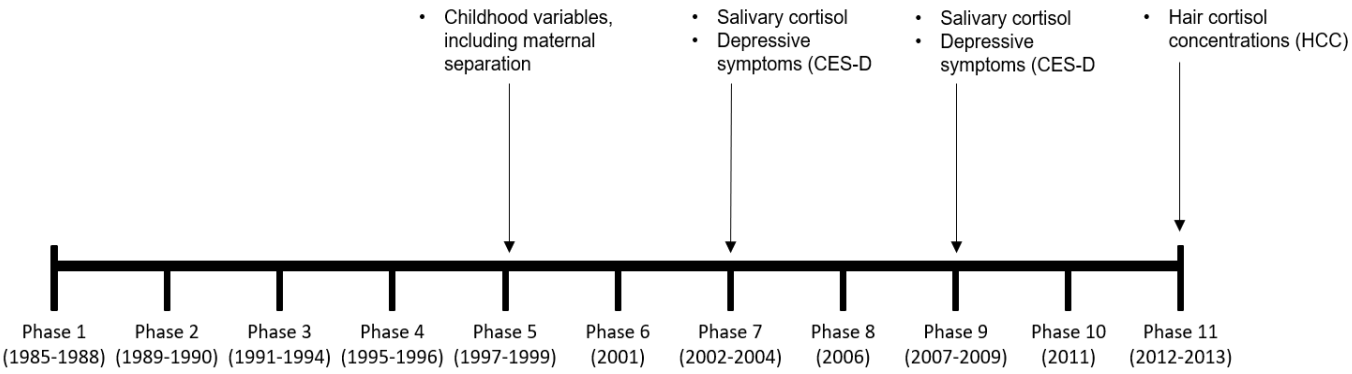
Whitehall II is an occupational cohort study of British civil servants employed in 20 London-based civil service offices. Recruitment began between 1985 and 1988 (phase 1), and since then, a further 11 phases of data have been collected, with clinical screening taking place every other phase.

The analyses in this thesis use data from phases 5 (1997-1999), phase 7 (2002-2004), phase 9 (2007-2009), and phase 11 (2012-2013). Data were made available through a data application (number: 209). Phase 5 is when retrospective measures regarding childhood were collected (Appendix 1.A and 1.B), including maternal separation in childhood.

Cortisol was measured at three different phases. Saliva was collected at phases 7 and 9, and hair was collected at phase 11. Chapter 2 focuses on salivary cortisol at phases 7 and 9 while making use of the childhood measures collected at phase 5. Chapters 3 and 4 focus on hair cortisol at phase 11.

Finally, depressive symptoms were measured at phases 7, 9 and 11. Chapter 4 focuses on hair cortisol at phase 11 as an outcome variable but utilises measures of depressive symptoms from phases 7 to 11, and the childhood variables from phase 5. A timeline of the Whitehall II study is shown in Figure 1.2, along with the phases at which maternal separation, depressive symptoms and salivary and hair cortisol were measured.

Figure 1.2: Timeline of the Whitehall II study



2 Maternal separation in childhood and change in diurnal salivary cortisol in late adulthood

2.1 Background:

There is a well-established link between early life stress and hypothalamic-pituitary-adrenal (HPA) axis functioning (Koss and Gunnar, 2018; Van Bodegom et al., 2017). The HPA axis is a neuroendocrine system responsible for responding to psychological and physiological stress and maintaining homeostasis (Smith and Vale, 2006), of which the end product is cortisol. With chronic stress, the HPA axis can become dysregulated resulting in a flattening of the diurnal cortisol slope (Miller et al., 2007). Moreover, chronic stress may pose an additional risk to HPA axis functioning during periods of critical development, such as childhood (Agorastos et al., 2019). In turn, flatter diurnal slopes are associated with a range of poor physical and mental health outcomes, including diabetes (Hackett et al., 2014), cardiovascular mortality (Kumari et al., 2011), and anxiety and depression (Doane et al., 2013).

The effect of early life stress on long-term HPA axis functioning may differ by adversity subtype (Kuhlman et al., 2015). In this paper, we focus our attention on maternal separation, which Kuhlman et al. (2017) identify as belonging to a disrupted caregiving dimension. Experience of this during childhood may impair down-regulation of cortisol production and impact the negative feedback system and overall regulation of the HPA axis.

Studies have shown that separation from one or both parents during childhood is associated with disrupted cortisol reactivity across different ages-groups, including childhood and adolescence (Zhang et al., 2021), and young (Kraft and Luecken,

2009; Luecken, 2000; Luecken and Appelhans, 2006; Meinlschmidt and Heim, 2005), middle (Nicolson, 2004), and late adulthood (Pesonen et al., 2010). Other studies, focusing on basal diurnal cortisol, have also shown an association between separation and flatter diurnal slopes in both childhood (Isenhour et al., 2021; Koss et al., 2014) and adulthood (Kumari et al., 2013). As such, it is possible that the effects of maternal separation may persist throughout the life-course, although its effect may be moderated by timing (Bosch et al., 2012; Pesonen et al., 2010) and the post-separation environment (Lee Raby et al., 2020; Luecken and Appelhans, 2006). However, much of the research on adults has been cross-sectional, and there is currently a lack of evidence on whether these effects persist in the same individuals or groups longitudinally.

Few studies have had the data available to assess cortisol levels over long periods. Observing general age-related trends in diurnal salivary cortisol, Miller et al. (2016) found that cortisol gradually increases during adolescence, and then becomes relatively stable across adulthood until around 50 when it starts to increase again. Similar patterns have been observed in 24-hour urinary free cortisol to creatinine ratio (UFC/CR; Moffat et al., 2020). Wang et al. (2014) examined repeat salivary cortisol measures over a six-year period in a sample of adults aged 48-87 years. Throughout the study period, wake-up cortisol and the area under the curve (AUC) became higher, diurnal slopes became flatter, and the cortisol awakening response (CAR) decreased. This flattening of slopes in older adults has also been observed in other studies (Adam et al., 2006; Heaney et al., 2012) and is often characterised by an increase in cortisol late in the day (van den Beld et al., 2018). Age-related increases in cortisol may be explained by a reduction in the absolute glucocorticoid (GR) and mineralocorticoid (MR) receptors (Gupta and Morley, 2014). However, this

may also be affected by other age-related factors, such as disrupted sleep (Abell et al., 2016; van den Beld et al., 2018).

Although these studies offer insight into how diurnal cortisol changes with age, there is still limited understanding of whether certain groups, such as those exposed to maternal separation, demonstrate more stable patterns of change. Ross et al. (2014) suggest that some sub-groups may experience less cortisol plasticity than others. However, this has not been confirmed regarding experiences of early life stress, particularly separation.

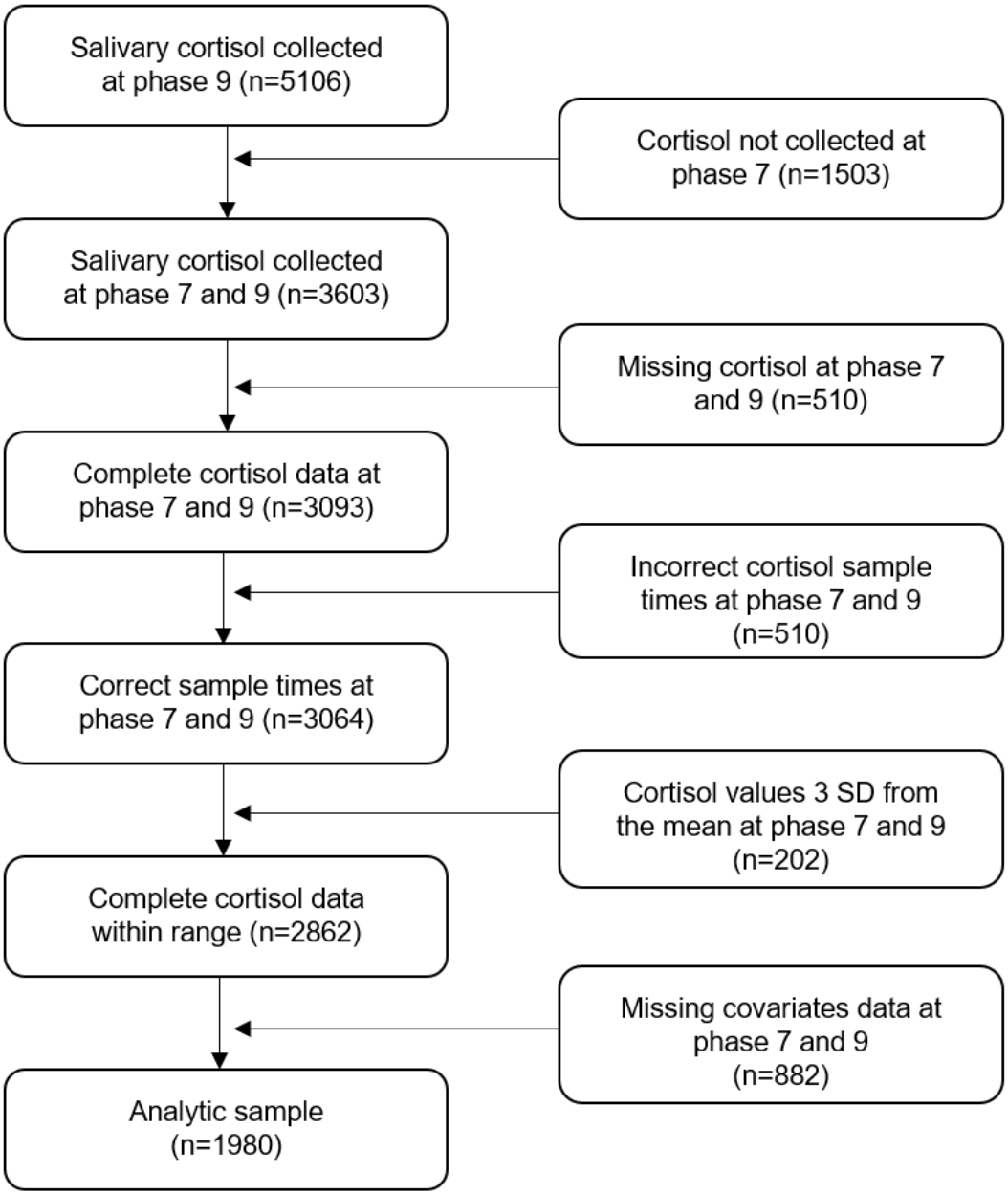
Using data from the Whitehall II study, Kumari et al. (2013) report an association between maternal separation in childhood and flatter diurnal cortisol slopes in phase 7 (2002-2004). In this study, we investigate whether these associations are still present in the same group of participants four to five years later in phase 9 (2007-2009). Additionally, we also investigate differences in the cortisol awakening response (CAR) and area under the curve (AUC). Following this, we then examine whether the change in diurnal slope from baseline (phase 7) to follow-up (phase 9) differs by maternal separation status. To our knowledge, this is the only study to date that has examined the association between separation in childhood and diurnal salivary cortisol at more than one time point in late adulthood.

2.2 Methods

2.2.1 Data

We use data from phases 5 (1997-1999), 7 (2002-2004) and 9 (2007-2009) in the Whitehall II study. An initial cohort of 10,308 British civil servants from 20 London-based offices was recruited between 1985 and 1988 (phase 1). Salivary cortisol collection was initiated partway through phase 7 (baseline) with data collected from 4608 participants. This was followed up at phase 9 where data was collected from 5106 participants, of which approximately 71% (n=3603) also had cortisol samples at baseline. Of those, 3093 had complete information on cortisol values and sample times at both baseline and follow-up. Participants that provided incorrect sample times were removed (n=29); for example, the sample time recorded for sample 1 was earlier than the recorded time of waking. After removing outliers (n=202) and limiting the analytic sample to participants with complete cases on covariates at baseline and follow-up (n=882), we were left with a final sample of 1980 (Figure 2.1). Ethical approval for the Whitehall II study was obtained from the University College London Medical School committee on the ethics of human research. Informed consent was gained from every participant.

Figure 2.1: Flow diagram of the analytic sample



2.2.2 Cortisol collection and analysis

The procedure for salivary cortisol collection has been reported previously for phase 7 (Kumari et al., 2013) and phase 9 (Abell et al., 2016a). Eligible participants who had agreed to take part provided six saliva samples in salivettes at waking (sample

1), +30min (sample 2), +2.5 h (sample 3), +8 h (sample 4), +12 h (sample 5), and at bedtime (sample 6) on a normal weekday. Participants were asked to record the time each sample was taken in a logbook diary. They were instructed to record waking as the time they first woke and not when they had risen from bed. Additionally, they were asked to refrain from eating, drinking, or brushing their teeth for 15 minutes prior to sample collection. The salivettes and logbook diary were returned by post. Salivettes were centrifuged at 3000 rpm for 5 minutes, resulting in a clear supernatant of low viscosity. Salivary cortisol levels were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL-Hamburg, Hamburg, Germany). The limit of detection (LOD) was 0.44 nmol/l; intra- and inter-assay coefficients of variance were less than 8%. Any sample over 50 nmol/l was remeasured.

2.2.3 Maternal separation

Maternal separation was measured at phase 5 (1997-1999), alongside other retrospective questions regarding childhood conditions. Participants were asked: “Were you ever separated from your mother for a year or more as a child (that is, up until you were 16)?” Further information was provided on the age they were separated and the reason this occurred. Reasons included parents separated/divorced, mother died, mother ill, adoption, evacuation, other. Evacuation refers to Second World War evacuation.

2.2.4 Covariates

Waking time was recorded on the day saliva samples were collected at baseline and follow-up. This was categorised as waking before 6 am, between 6 am and 9:59 am,

and 10 am and after. An indicator variable measuring whether the participants took the saliva sample after the requested time was included for samples one (waking) and two (+30 min). This was categorised into 5-minute intervals (0-4 minutes, 5-9 minutes, 10-14 minutes, 15-19 minutes, 20 minutes and over). Additional covariates were measured at baseline and follow-up, other than childhood measures which were collected at phase 5. Age, sex, and ethnicity were assessed by questionnaire. Childhood covariates refer to ages up until 16 years. Adverse childhood experiences (ACEs) were captured using a three-item scale: “Your parent(s) were mentally ill or drank so often that it caused family problems”, “You were physically abused by someone close to you”, “Your parents often argued or fought”. Material disadvantage used a four-item scale: “Your family had continuing financial problems”, “Your family/household did not have an inside toilet”, “Your father/mother was unemployed when they wanted to be working”, “Your family/household did not own a car”. Perceived parenting was measured by using selected items from the Midlife Development in the United States (MIDUS) study (Brim et al., 1996; Shaw et al., 2004). Participants were asked: “Please show how you remember your mother (or the woman who cared for you) during the years you were growing up.” The same questions were asked about their fathers/male carers. Three separate dimensions were identified: parental warmth (four items, Cronbach’s alpha=0.89), parental strictness (two items, Cronbach’s alpha=0.7), and parental expectations (one item, Cronbach’s alpha=0.73). Higher scores reflect greater warmth, strictness, and expectations.

Marital status was categorised as: married/cohabiting, single, divorced/separated, and widowed. Smoking status measured whether participants currently smoked cigarettes/ pipe/cigars versus having never smoked/given up. Adult socioeconomic

position was measured using current or last known civil service employment grade: administrative, professional/executive, clerical/support. Administrative is the highest grade. Employment status was self-reported and categorised as follows: working, retired, other (unemployed, long-term sick, caring). To assess the effect of sleep patterns, sleep disturbance and average hours sleep were included. Sleep disturbance was measured using the four-item Jenkins sleep scale (Jenkins et al., 1988). An additional question asked: “how often in the past month did you have disturbed or restless sleep?” Response categories for each question were coded as: not at all, 1-3 days, 4-7 days, 8-14 days, 15-21 days, 22-31 days. Responses were summed and higher values reflect greater sleep disturbance. Average hours sleep on a weeknight was categorised as: 5 hours or less, 6 hours, 7 hours, 8 hours, 9 hours or more. Body mass index (BMI) was measured using height and weight measurements collected by a trained interviewer or nurse. Current use of cardiovascular (CVD) medication and local and systemic cortico-steroids were recorded during the clinical assessment. Diabetes was defined by using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006). Depression was defined as a score of ≥16 (Radloff, 1977) on The Center for Epidemiologic Studies Depression scale (CES-D), or self-reported use of antidepressants.

2.2.5 Statistical analysis

Analysis of variance (ANOVA) and Pearson’s chi-square tests were used for descriptive purposes to compare the means and proportions of the characteristics between participants reporting maternal separation during childhood versus no reported separation. Extreme values (3 standard deviations (SD) above the mean)

are often reported in cortisol studies (Adam and Kumari, 2009). It is unknown precisely what causes these, but it has been suggested they may be a result of sample contamination, clinical conditions, or antibody cross-reactivity with the assay (Miller et al., 2013; Schlotz, 2011). Removing values 3 SD from the mean is a commonly employed approach. However, this method still left extreme values in the data. To remedy this, cortisol values >500 nmol/l (n=3) were removed first, followed by values 3 SD from the mean (n=199). Visual inspection revealed that the data were still skewed and thus they were logarithmically transformed prior to analysis.

The CAR, peak in cortisol 30-45 minutes after waking, was calculated by subtracting cortisol at time 1 (waking) from cortisol at time 2 (+30 minutes). Area under the curve with respect to the ground (AUCg) was calculated using the trapezoid formula, as has been previously recommended (Pruessner et al., 2003). This reflects total cortisol output.

The diurnal slope can be interpreted as the decline in cortisol over the day. Multilevel growth curve models were used to account for the nesting of cortisol samples within participants. Log cortisol values were regressed on hours since awakening. A random intercept and slope were included to enable us to estimate the subject-specific effects. Both a linear and quadratic term for the hours since awakening variable was included. However, the variance estimates for the quadratic term were very close to zero. Therefore, in the final models presented below, hours since awakening is included as linear only. Sample 2 (+30 minutes) was not included as the CAR is believed to be regulated by a different mechanism from the diurnal slope (Clow et al., 2009). An interaction term between maternal separation and hours since awakening was included to test whether the diurnal cortisol slope differed by

separation status. Models were run separately with cortisol at baseline and follow-up as outcomes, adjusting for all covariates at the respective phase of data collection.

Following this, the diurnal slope for each participant was extracted from a basic model (at baseline and follow-up), adjusting only for waking time and the late indicator for sample 1. Greater negative values indicate a steeper diurnal slope and values closer to zero reflect a flatter slope. To examine the change in diurnal slope between the two time points, a difference score was calculated by subtracting the slope at baseline from follow-up. This has been shown to be a suitable approach in non-randomised settings when differences occur between groups at baseline (Allison, 1990; Farmus et al., 2019). Because the slope values are negative, a positive difference score means that the slope has become flatter between the two time-points (closer to zero). A multiple linear regression model was fitted to estimate the association between maternal separation and a change in slopes, adjusting for all covariates at baseline.

Models were estimated using ordinary least squares (CAR, AUCg, difference score) and maximum likelihood (diurnal slope) with Huber-White robust standard errors reported. In total, four series of models were tested separately for each cortisol outcome. For the CAR, AUCg, and diurnal slope, models were estimated at baseline and then follow-up, adjusting for covariates from the corresponding phase of data collection. For the difference score of the diurnal slope, covariates were adjusted at baseline.

Model 1 is adjusted for demographic covariates (age, sex, ethnicity) and use of corticosteroid (local and systemic) medication. Model 2 is further adjusted for childhood covariates (ACEs, material disadvantage, parental warmth, parental

strictness, parental expectations), measured at phase 5. Model 3 includes marital and smoking status, civil service employment grade, employment status, and sleep variables. Model 4 adjusts for health-related covariates (BMI, CVD medication, diabetes, depression). A nonlinear effect was found for BMI in the multilevel model at baseline and so a quadratic term was included. However, this effect was not significant at follow-up and so BMI was left as linear. All analyses were carried out using Stata (Version 16.1, StataCorp, College Station, Texas).

2.2.5.1 Sensitivity analysis

In studies on cortisol, it is common practice to remove participants taking corticosteroid medication (Masharani et al., 2005). Similarly, participants that took their first sample later than 15 minutes after waking are also sometimes removed, due to its effect on the CAR (Dockray et al., 2008; Griefahn and Robens, 2011). We chose to adjust for these in the analyses in order to preserve the sample size. To assess whether the model estimates were affected by these decisions, we ran sensitivity analyses that removed participants on corticosteroid medication and were more than 15 minutes late taking their first sample and then compared the results to the original models.

2.3 Results

2.3.1 Summary statistics

Table 2.1 shows the characteristics of the separated and non-separated participants between baseline and follow-up. At baseline, participants that reported separation during childhood had lower morning cortisol and AUCg, higher CAR and evening cortisol, and a flatter diurnal slope. However, only the latter two were statistically significant. By follow-up, morning and evening cortisol was higher in the separated

group, the CAR and AUCg were lower, and the diurnal slope steeper, none of the differences were statistically significant at the 5% level. Over 60% of samples at time 1 (waking) and time 2 (+30 minutes) were taken within 5 minutes of the specified times at both baseline and follow-up. Regarding the covariates, the separated group were older, less likely to be white, and more likely to report taking local corticosteroid medication. They reported higher ACEs and material disadvantage, lower parental warmth and expectations, were less likely to be married or cohabiting (due to higher rates of widowhood), and more likely to smoke, be in the lowest civil service employment grade, and be retired.

Table 2.2 shows the differences between the analytic sample and excluded observations at baseline. This refers to participants that provided a cortisol sample free of outliers (as they may reflect technical error) but were excluded due to incomplete information on cortisol values, sample times, and covariates at baseline and/or follow-up. Cortisol levels and waking time were similar, but participants in the analytic sample were more likely to complete samples 1 and 2 within 5 minutes of the specified times. They were also younger, more likely to be male and white, and less likely to report using local corticosteroid medication. Childhood covariates were similar between the analytic sample and excluded participants, but the former were less likely to report maternal separation during childhood (9.8% vs 13.2%, p=0.004). They were also more likely to be married or cohabiting, in the highest civil service grade, still in work, and less likely to smoke. They reported lower levels of sleep disturbance and were less likely to sleep 5 hours or less or 9 hours or more. Finally, regarding health, they had a lower BMI, were less likely to be on CVD medication, and had lower rates of diabetes and depression.

Table 2.1: Summary statistics by maternal separation status at baseline and follow-up (n=1980)

		Baseline			Follow-up		
		Not separated (n=1785)	Separated (n=195)	p-value	Not separated (n=1785)	Separated (n=195)	p-value
Morning cortisol (nmol/l)		16.350 (8.083)	15.465 (7.716)	0.15	14.883 (7.311)	15.187 (7.710)	0.58
CAR (nmol/l)		7.189 (11.284)	7.682 (10.285)	0.56	5.985 (10.596)	5.486 (10.307)	0.53
Evening cortisol (nmol/l)		2.207 (2.578)	2.574 (2.633)	0.06	2.463 (2.245)	2.510 (2.312)	0.78
AUCg (nmol/l)		118.435 (45.743)	117.317 (42.143)	0.74	112.368 (43.269)	110.467 (42.246)	0.56
Diurnal slope (nmol/l/h)		-0.133 (0.023)	-0.128 (0.023)	0.008	-0.116 (0.018)	-0.117 (0.019)	0.7
Waking time							
	<6am	340 (19.0%)	29 (14.9%)	0.32	277 (15.5%)	31 (15.9%)	0.55
	6am-9:59am	1,433 (80.3%)	164 (84.1%)		1,494 (83.7%)	161 (82.6%)	
	>=10:00am	12 (0.7%)	2 (1.0%)		14 (0.8%)	3 (1.5%)	
Late for cortisol 1							
	0-4mins	1245 (69.7%)	130 (66.7%)	0.63	1227 (68.7%)	135 (69.2%)	0.59
	5-9mins	227 (12.7%)	27 (13.8%)		277 (15.5%)	24 (12.3%)	
	10-14mins	93 (5.2%)	14 (7.2%)		102 (5.7%)	12 (6.2%)	
	15-19mins	79 (4.4%)	11 (5.6%)		74 (4.1%)	8 (4.1%)	
	20mins and over	141 (7.9%)	13 (6.7%)		105 (5.9%)	16 (8.2%)	
Late for cortisol 2							
	30-34mins	1126 (63.1%)	126 (64.6%)	0.56	1164 (65.2%)	130 (66.7%)	0.74
	35-39mins	277 (15.5%)	24 (12.3%)		300 (16.8%)	28 (14.4%)	
	40-44mins	122 (6.8%)	15 (7.7%)		117 (6.6%)	12 (6.2%)	
	45-49mins	93 (5.2%)	14 (7.2%)		85 (4.8%)	8 (4.1%)	
	50mins and over	167 (9.4%)	16 (8.2%)		119 (6.7%)	17 (8.7%)	
Age		60.54 (5.84)	63.78 (5.98)	<0.001	65.55 (5.86)	68.85 (6.03)	<0.001
Sex							
	Female	387 (21.7%)	47 (24.1%)	0.44	-	-	
	Male	1398 (78.3%)	148 (75.9%)		-	-	

Ethnicity							
	Non-white	64 (3.6%)	19 (9.7%)	<0.001	-	-	
	White	1721 (96.4%)	176 (90.3%)		-	-	
Systemic steroids							
	No	1783 (99.9%)	195 (100.0%)	0.64	1785 (100%)	195 (100.0%)	
	Yes	2 (0.1%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Local steroids							
	No	1707 (95.6%)	178 (91.3%)	0.007	1691 (94.7%)	178 (91.3%)	0.047
	Yes	78 (4.4%)	17 (8.7%)		94 (5.3%)	17 (8.7%)	
ACEs		0.26 (0.55)	0.41 (0.71)	<0.001	-	-	
Material disadvantage		1.10 (1.06)	1.39 (1.19)	<0.001	-	-	
Parental warmth		22.18 (4.69)	20.14 (5.51)	<0.001	-	-	
Parental strictness		9.36 (2.35)	9.45 (2.50)	0.62	-	-	
Parental expectations		5.91 (1.42)	5.63 (1.51)	0.008	-	-	
Marital status							
	Married/cohabiting	1425 (79.8%)	149 (76.4%)	<0.001	1407 (78.8%)	147 (75.4%)	0.008
	Single	206 (11.5%)	18 (9.2%)		203 (11.4%)	17 (8.7%)	
	Divorce/separated	109 (6.1%)	12 (6.2%)		102 (5.7%)	13 (6.7%)	
	Widow	45 (2.5%)	16 (8.2%)		73 (4.1%)	18 (9.2%)	
Smoking status							
	No	1630 (91.3%)	167 (85.6%)	0.009	1673 (93.7%)	178 (91.3%)	0.19
	Yes	155 (8.7%)	28 (14.4%)		112 (6.3%)	17 (8.7%)	
Employment grade							
	Administrative	880 (49.3%)	82 (42.1%)	0.003	886 (49.6%)	82 (42.1%)	0.004
	Professional/executive	789 (44.2%)	88 (45.1%)		779 (43.6%)	88 (45.1%)	
	Clerical/support	116 (6.5%)	25 (12.8%)		120 (6.7%)	25 (12.8%)	
Employment status							
	Working	965 (54.1%)	78 (40.0%)	<0.001	553 (31.0%)	40 (20.5%)	0.01
	Retired	730 (40.9%)	107 (54.9%)		1177 (65.9%)	148 (75.9%)	
	Other	90 (5.0%)	10 (5.1%)		55 (3.1%)	7 (3.6%)	
Jenkins sleep scale		11.58 (5.31)	12.61 (6.10)	0.011	11.66 (5.29)	11.76 (5.84)	0.79

Average hours sleep							
	<=5 hrs	104 (5.8%)	19 (9.7%)	0.15	116 (6.5%)	22 (11.3%)	0.097
	6 hrs	543 (30.4%)	61 (31.3%)		508 (28.5%)	47 (24.1%)	
	7 hrs	817 (45.8%)	81 (41.5%)		768 (43.0%)	79 (40.5%)	
	8 hrs	290 (16.2%)	33 (16.9%)		359 (20.1%)	44 (22.6%)	
	>=9 hrs	31 (1.7%)	1 (0.5%)		34 (1.9%)	3 (1.5%)	
BMI		26.33 (3.90)	26.49 (3.56)	0.57	26.38 (4.02)	26.59 (3.78)	0.49
CVD medication							
	No	1311 (73.4%)	132 (67.7%)	0.086	855 (47.9%)	83 (42.6%)	0.16
	Yes	474 (26.6%)	63 (32.3%)		930 (52.1%)	112 (57.4%)	
Diabetes[a]							
	No	1691 (94.7%)	179 (91.8%)	0.089	1592 (89.2%)	171 (87.7%)	0.53
	Yes	94 (5.3%)	16 (8.2%)		193 (10.8%)	24 (12.3%)	
Depression[b]							
	No	1554 (87.1%)	163 (83.6%)	0.18	1571 (88.0%)	172 (88.2%)	0.94
	Yes	231 (12.9%)	32 (16.4%)		214 (12.0%)	23 (11.8%)	

Notes: Mean(sd)/n(%). Cortisol values are displayed in nmol/l. CAR = cortisol awakening response, AUCg = area under the curve with respect to the ground, ACEs = adverse childhood experiences. [a] Diabetes was defined using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006). [b] Depression was defined using a cut-off score of >=16 on Center for Epidemiologic Studies Depression Scale (CES-D) or use of antidepressants. Time-invariant covariates (sex, ethnicity, childhood conditions) are shown at baseline only.

Table 2.*2*: Summary statistics by included and excluded participants at baseline

		Excluded[a] (n=1245)	Included[b] (n=1980)	p-value
Morning cortisol (nmol/l)		15.897 (8.484)	16.263 (8.050)	0.22
CAR (nmol/l)		6.589 (11.334)	7.237 (11.188)	0.11
Evening cortisol (nmol/l)		2.451 (2.846)	2.243 (2.585)	0.034
AUCg (nmol/l)		117.200 (46.308)	118.325 (45.392)	0.5
Diurnal slope (nmol/l/h)		-0.131 (0.024)	-0.132 (0.023)	0.2
Waking time				
	<6am	202 (16.4%)	369 (18.6%)	0.29
	6am-9:59am	1017 (82.8%)	1597 (80.7%)	
	>=10:00am	9 (0.7%)	14 (0.7%)	
Late for cortisol 1				
	0-4mins	807 (65.4%)	1375 (69.4%)	0.007
	5-9mins	155 (12.6%)	254 (12.8%)	
	10-14mins	62 (5.0%)	107 (5.4%)	
	15-19mins	74 (6.0%)	90 (4.5%)	
	20mins and over	136 (11.0%)	154 (7.8%)	
Late for cortisol 2				
	30-34mins	722 (58.8%)	1252 (63.2%)	<0.001
	35-39mins	179 (14.6%)	301 (15.2%)	
	40-44mins	76 (6.2%)	137 (6.9%)	
	45-49mins	73 (5.9%)	107 (5.4%)	
	50mins and over	177 (14.4%)	183 (9.2%)	
Age		61.57 (6.03)	60.86 (5.93)	<0.001
Sex				
	Female	381 (30.6%)	434 (21.9%)	<0.001
	Male	864 (69.4%)	1,546 (78.1%)	
Ethnicity				
	Non-white	122 (9.8%)	83 (4.2%)	<0.001
	White	1123 (90.2%)	1897 (95.8%)	
Systemic steroids				
	No	1241 (100.0%)	1978 (99.9%)	0.26
	Yes	0 (0.0%)	2 (0.1%)	
Local steroids				
	No	1160 (93.5%)	1885 (95.2%)	0.036
	Yes	81 (6.5%)	95 (4.8%)	
Maternal separation				
	Not separated	957 (86.8%)	1785 (90.2%)	0.004
	Separated	146 (13.2%)	195 (9.8%)	
ACEs		0.30 (0.60)	0.27 (0.57)	0.19
Material disadvantage		1.19 (1.06)	1.13 (1.07)	0.15
Parental warmth		21.99 (5.07)	21.98 (4.82)	0.95
Parental strictness		9.39 (2.48)	9.37 (2.36)	0.79
Parental expectations		5.90 (1.49)	5.88 (1.43)	0.78
Marital status				
	Married/cohabiting	874 (70.5%)	1574 (79.5%)	<0.001

	Single	195 (15.7%)	224 (11.3%)	
	Divorce/separated	105 (8.5%)	121 (6.1%)	
	Widow	66 (5.3%)	61 (3.1%)	
Smoking status				
	No	1069 (86.1%)	1797 (90.8%)	<0.001
	Yes	172 (13.9%)	183 (9.2%)	
Employment grade				
	Administrative	461 (37.0%)	962 (48.6%)	<0.001
	Professional/executive	585 (47.0%)	877 (44.3%)	
	Clerical/support	199 (16.0%)	141 (7.1%)	
Employment status				
	Working	595 (47.9%)	1043 (52.7%)	0.027
	Retired	575 (46.3%)	837 (42.3%)	
	Other	73 (5.9%)	100 (5.1%)	
Jenkins sleep scale		12.43 (5.97)	11.68 (5.40)	<0.001
Average hours sleep				
	<=5 hrs	109 (8.8%)	123 (6.2%)	0.001
	6 hrs	400 (32.5%)	604 (30.5%)	
	7 hrs	477 (38.7%)	898 (45.4%)	
	8 hrs	221 (17.9%)	323 (16.3%)	
	>=9 hrs	25 (2.0%)	32 (1.6%)	
BMI		27.31 (4.82)	26.34 (3.87)	<0.001
CVD medication				
	No	822 (66.2%)	1443 (72.9%)	<0.001
	Yes	419 (33.8%)	537 (27.1%)	
Diabetes[c]				
	No	1121 (90.0%)	1870 (94.4%)	<0.001
	Yes	124 (10.0%)	110 (5.6%)	
Depression[d]				
	No	928 (78.4%)	1717 (86.7%)	<0.001
	Yes	255 (21.6%)	263 (13.3%)	

Notes: Cortisol values are displayed in nmol/l. CAR = cortisol awakening response, AUCg = area under the curve with respect to the ground, ACEs = adverse childhood experiences. [a] Excluded refers to participants that provided a cortisol sample at *baseline* that was free of outliers but were excluded due to incomplete information on cortisol values, sample time, or covariates and baseline and/or follow-up. [b] Included refers to participants with complete information on all relevant variables (described above) at both baseline and follow-up. [c] Diabetes was defined using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006). [d] Depression was defined using a cut-off score of >=16 on Center for Epidemiologic Studies Depression Scale (CES-D) or use of antidepressants. All variables are at baseline.

2.3.2 Association between maternal separation and CAR and AUCg at baseline and follow-up

Table 2.3 shows the effect of maternal separation on CAR and AUCg at baseline and follow-up. At baseline, the CAR is higher among participants reporting separation, compared to their non-separated counterparts. However, at follow-up, the CAR is lower. After full adjustment (model 4), the AUCg is lower for participants reporting separation, compared to no separation, at both baseline and follow-up, but the regression coefficient is substantially larger at follow-up. At both time-points, the effect of maternal separation is not statistically significant for either CAR or AUCg and the confidence intervals are wide, particularly for AUCg.

Table *2.*3: Estimates (95% CI) from linear regression models of cortisol awakening response (CAR) and area under the curve with respect to the ground (AUCg) at baseline and follow-up (n=1980)

	Baseline		Follow-up	
	CAR (nmol/l)	AUCg (nmol/l)	CAR (nmol/l)	AUCg (nmol/l)
Model 1[a]	0.827	0.681	-0.441	-2.846
	(-0.73,2.38)	(-5.66,7.02)	(-1.94,1.06)	(-9.12,3.43)
Model 2[b]	0.700	0.610	-0.418	-2.302
	(-0.89,2.29)	(-5.75,6.97)	(-1.96,1.12)	(-8.63,4.03)
Model 3[c]	0.413	-0.257	-0.483	-2.717
	(-1.18,2.01)	(-6.73,6.22)	(-2.04,1.07)	(-9.07,3.63)
Model 4[d]	0.424	-0.330	-0.505	-2.602
	(-1.17,2.02)	(-6.78,6.12)	(-2.06,1.05)	(-8.98,3.77)

Notes: [a] Model 1 adjusts for age, sex, ethnicity, wake time, late indicator, systemic & local steroids. [b] Model 2 adjusts for Model 1 + childhood (adverse childhood experiences, material disadvantage, parental warmth, parental strictness, parental expectations). [c] Model 3 adjusts for Model 2 + marital status, smoking status, employment grade, employment status, Jenkins sleep scale, average hours sleep. [d] Model 4 adjusts for Model 3 + BMI, cardiovascular disease medication, diabetes, depression.

2.3.3 Association between maternal separation and log-transformed diurnal cortisol slopes (nmol/l) at baseline and follow-up

Tables 2.4 and 2.5 and Figure 2.1 display the results of the multilevel models that examine the association between maternal separation and the diurnal cortisol slopes at baseline and follow-up. At baseline (Table 4), the main effect for maternal separation is not statistically significant, but the interaction between maternal separation and the hours since awakening variable is. This suggests the presence of a crossover interaction (Loftus, 1978). The effect estimates remain largely unchanged after full adjustment. At follow-up (Table 2.5), neither the main effect nor interaction for maternal separation is statistically significant, and the effect estimate for the latter is near zero. Figure 2.1 helps to illustrate the interaction effects, showing the diurnal cortisol slope by separation status for each phase of data collection. The diurnal slope is flatter in participants reporting separation versus no separation at baseline. This is mostly characterised by differences in evening cortisol. As shown in table 2.1, morning cortisol is slightly lower in the separated group, leading to a slight crossover effect. At follow-up, the diurnal slopes become flatter in both groups, compared to baseline, however, there is no longer any discernible difference between them.

Table 2.4: Estimates (95% CI) from multilevel models of log diurnal cortisol slope (nmol/l) at baseline (n=1980)

	Model 1[a]	Model 2[b]	Model 3[c]	Model 4[d]
Hours since awakening	-0.136***	-0.136***	-0.136***	-0.136***
	(-0.14, -0.13)	(-0.14, -0.13)	(-0.14, -0.13)	(-0.14, -0.13)
Maternal separation[e]	-0.062	-0.059	-0.062	-0.062
	(-0.13,0.01)	(-0.13,0.01)	(-0.13,0.01)	(-0.13,0.01)
Maternal separation x	0.014**	0.014**	0.014**	0.014**
hours since awakening[f]	(0.01,0.02)	(0.01,0.02)	(0.01,0.02]	(0.01,0.02)
Random effects[g]				
var(hours since awakening)	0.001***	0.001***	0.001***	0.001***
	(0.00,0.00)	(0.00,0.00)	[0.00,0.00]	(0.00,0.00)
var(intercept)	0.013***	0.013***	0.015***	0.014***
	(0.00,0.08)	(0.00,0.08)	[0.00,0.08]	(0.00,0.08)

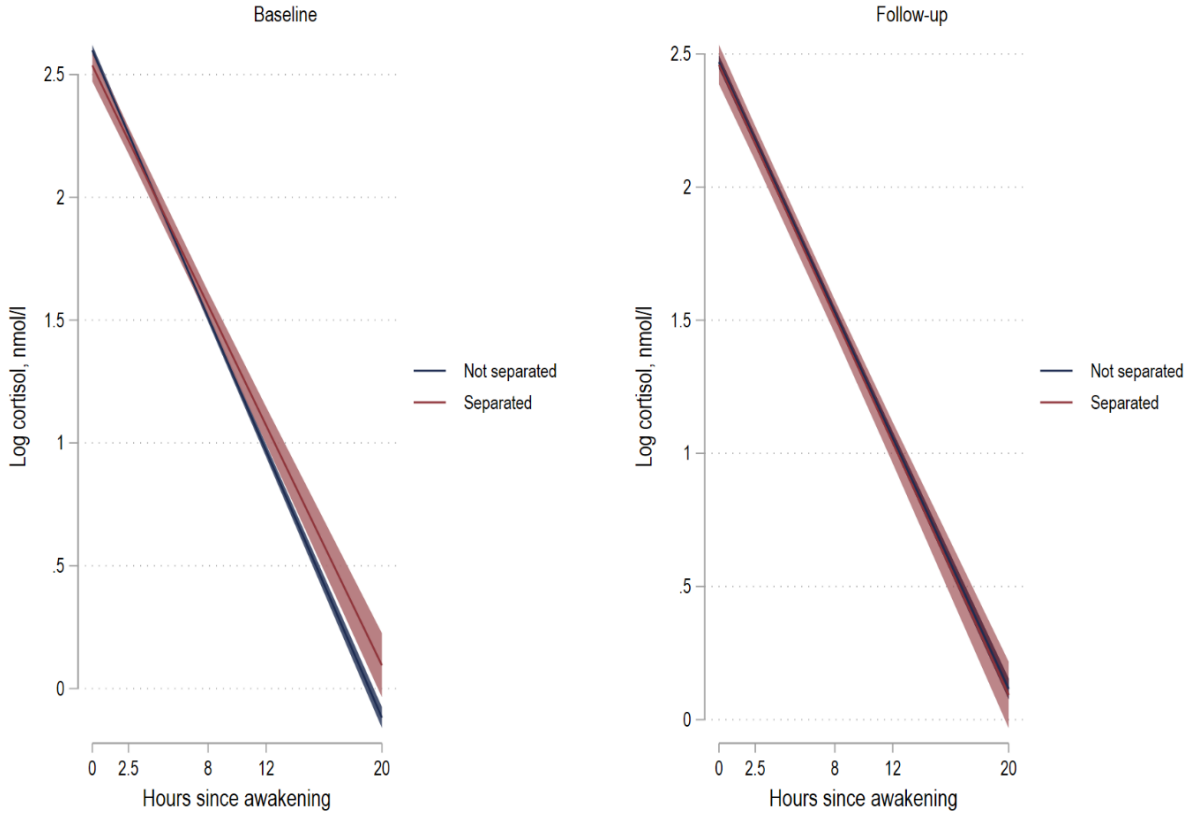
Notes: * p<0.05, ** p<0.01, *** p<0.001. [a] Model 1 adjusts for age, sex, ethnicity, wake time, late indicator, systemic & local steroids. [b] Model 2 adjusts for Model 1 + childhood (adverse childhood experiences, material disadvantage, parental warmth, parental strictness, parental expectations). [c] Model 3 adjusts for Model 2 + marital status, smoking status, employment grade, employment status, Jenkins sleep scale, average hours sleep. [d] Model 4 adjusts for Model 3 + BMI^2, CVD medication, diabetes, depression. [e] Maternal separation ref: not separated. [f] Interaction term between maternal separation and hours since awakening. [g] Random effects are shown as variance estimates (standard errors). Covariates are adjusted at baseline.

Table 2.5: Estimates (95% CI) from multilevel models of log diurnal cortisol slope (nmol/l/h) at follow-up (n=1980)

	Model 1[a]	Model 2[b]	Model 3[c]	Model 4[d]
Hours since awakening	-0.118***	-0.118***	-0.118***	-0.118***
	(-0.12, -0.12)	(-0.12, -0.12)	(-0.12, -0.12)	(-0.12, -0.12)
Maternal separation[e]	-0.014	-0.009	-0.01	-0.01
	(-0.09,0.06)	(-0.09,0.07)	(-0.09,0.07)	(-0.09,0.07)
Maternal separation x	-0.001	-0.001	-0.001	-0.001
hours since awakening[f]	(-0.01,0.01)	(-0.01,0.01)	(-0.01,0.01)	(-0.01,0.01)
Random effects[g]				
var(hours since awakening)	0.001***	0.001***	0.001***	0.001***
	(0.00,0.00)	(0.00,0.00)	(0.00,0.00)	(0.00,0.00)
var(intercept)	0.056***	0.056***	0.057***	0.056***
	(0.03,0.10)	(0.03,0.10)	(0.03,0.10)	(0.03,0.10)

Notes: * p<0.05, ** p<0.01, *** p<0.001. [a] Model 1 adjusts for age, sex, ethnicity, wake time, late indicator, systemic & local steroids. [b] Model 2 adjusts for Model 1 + childhood (adverse childhood experiences, material disadvantage, parental warmth, parental strictness, parental expectations). [c] Model 3 adjusts for Model 2 + marital status, smoking status, employment grade, employment status, Jenkins sleep scale, average hours sleep. [d] Model 4 adjusts for Model 3 + BMI, CVD medication, diabetes, depression. [e] Maternal separation ref: not separated. [f] Interaction term between maternal separation and hours since awakening. [g] Random effects are shown as variance estimates (standard errors). Covariates are adjusted at follow-up.

Figure 2.2: Estimates (95% CI) of log diurnal cortisol slope (nmol/l/h) by maternal separation status at baseline and follow-up (n=1980)



Notes: Models adjusted for age, sex, ethnicity, wake time, late indicator, systemic and local steroids, childhood (adverse childhood experiences, material disadvantage, parental warmth, parental strictness, parental expectations), marital status, smoking status, employment grade, employment status, Jenkins sleep scale, average hours sleep, BMI, CVD medication, diabetes, depression. Covariates are adjusted at baseline and follow-up, respectively.

2.3.4 Association between maternal separation and change in log-transformed diurnal cortisol slopes (nmol/l) from baseline to follow-up

Table 2.6 displays the results from the model that describes the change in average diurnal slope from baseline to follow-up. This model adjusts for the same covariates as the previous models at baseline. The effect of maternal separation in this model can be interpreted as the difference between the change in diurnal slope from baseline to follow-up for participants reporting separation versus no separation. In the fully adjusted model, the average change in diurnal slope from baseline to follow-up was smaller in participants that reported separation during childhood, compared to their non-separated counterparts. (β=-0.005, 95% CI -0.009,-0.001, p=0.008). A difference in change was also observed for smoking status and average hours sleep. Smokers showed less change than non-smokers (β=-0.006, 95% CI -0.010, -0.002, p=0.006). Compared to participants sleeping 7 hours, on average, in a normal week, those reporting either 6 hours or 5 hours or less also showed a smaller degree of change in diurnal slopes (β=-0.005, 95% CI -0.008, -0.003, p<0.001; β=-0.008, 95% CI -0.013,-0.003, p=0.003). No significant associations were observed for measures of health, including BMI, CVD medication, diabetes, and depression.

Table 2.6: Estimates (95% CI) from linear regression model of log diurnal cortisol slope (nmol/l/h) difference score (n=1980)

	Model 1[a]	Model 2[b]	Model 3[c]	Model 4[d]
Maternal separation[e]	-0.006** (-0.01, -0.00)	-0.006** (-0.01, -0.00)	-0.005** (-0.01, -0.00)	-0.005** (-0.01, -0.00)
Age	0.000 (-0.00,0.00)	0.000 (-0.00,0.00)	0.000 (-0.00,0.00)	0.000 (-0.00,0.00)
Sex[f]	-0.002 (-0.00,0.00)	-0.002 (-0.00,0.00)	-0.001 (-0.00,0.00)	-0.001 (-0.00,0.00)
Ethnicity[g]	0.002 (-0.00,0.01)	0.002 (-0.00,0.01)	0.002 (-0.00,0.01)	0.002 (-0.00,0.01)
Systemic steroids	0.012*** (0.01,0.02)	0.013*** (0.01,0.02)	0.014** (0.00,0.02)	0.014* (0.00,0.03)
Local steroids	0.003 (-0.00,0.01)	0.003 (-0.00,0.01)	0.003 (-0.00,0.01)	0.003 (-0.00,0.01)
ACEs		-0.001 (-0.00,0.00)	-0.001 (-0.00,0.00)	-0.001 (-0.00,0.00)
Material disadvantage		0.001 (-0.00,0.00)	0.001 (-0.00,0.00)	0.001 (-0.00,0.00)
Parental warmth		0.000 (-0.00,0.00)	0.000 (-0.00,0.00)	0.000 (-0.00,0.00)
Parental strictness		0.000 (-0.00,0.00)	0.000 (-0.00,0.00)	0.000 (-0.00,0.00)
Parental expectations		0.000 (-0.00,0.00)	0.000 (-0.00,0.00)	0.001 (-0.00,0.00)
Smoker			-0.006** (-0.01, -0.00)	-0.006** (-0.01, -0.00)
Marital status[h]				
Single			-0.002 (-0.01,0.00)	-0.002 (-0.01,0.00)
Divorce/separated			-0.002 (-0.01,0.00)	-0.002 (-0.01,0.00)
Widow			0.005 (-0.00,0.01)	0.005 (-0.00,0.01)
Employment grade[i]				
Professional/executive			0.003* (0.00,0.00)	0.002 (-0.00,0.00)
Clerical/support			0.005* (0.00,0.01)	0.004 (-0.00,0.01)
Employment status[j]				
Retired			0.001 (-0.00,0.00)	0.001 (-0.00,0.00)
Other			0.000 (-0.01,0.00)	0.000 (-0.01,0.00)
Jenkins sleep scale			0.000 (-0.00,0.00)	0.000 (-0.00,0.00)
Average hours sleep[k]				

<=5 hrs	-0.008** (-0.01, -0.00)	-0.008** (-0.01, -0.00)
6 hrs	-0.005*** (-0.01, -0.00)	-0.005*** (-0.01, -0.00)
8 hrs	-0.001 (-0.00,0.00)	-0.001 (-0.00,0.00)
>=9 hrs	-0.002 (-0.01,0.01)	-0.002 (-0.01,0.01)
BMI		0.000 (-0.00,0.00)
CVD medication		-0.001 (-0.00,0.00)
Diabetes[l]		-0.004 (-0.01,0.00)
Depression[m]		0.001 (-0.00,0.00)

Notes: * p<0.05, ** p<0.01, *** p<0.001. [a] Model 1 adjusts for age, sex, ethnicity, wake time, late indicator, systemic & local steroids. [b] Model 2 adjusts for Model 1 + childhood (adverse childhood experiences, material disadvantage, parental warmth, parental strictness, parental expectations). [c] Model 3 adjusts for Model 2 + marital status, smoking status, employment grade, employment status, Jenkins sleep scale, average hours sleep. [d] Model 4 adjusts for Model 3 + BMI^2, CVD medication, diabetes, depression. [e] Maternal separation ref: not separated. [f] Sex ref: female. [g] Ethnicity ref: non-white. [h] Marital status ref: married/cohabiting. [i] Employment grade ref: administrative. [j] Employment status: working. [k] Average hours sleep ref: 7 hours. [l] Diabetes was defined using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006). [m] Depression was defined using a cut-off score of >=16 on Center for Epidemiologic Studies Depression Scale (CES-D) or use of antidepressants. Covariates are adjusted at baseline.

2.3.5 Sensitivity analyses

The results from the sensitivity analyses are shown in Appendix tables 2.A to 2.C. Participants who reported taking corticosteroid medication and were >15 minutes late taking the first saliva sample were removed from the analyses and the model estimates were compared to those from the full analytic sample. Appendix Table 2.A shows the results from the CAR and AUCg models. At baseline, the regression coefficients for the CAR and AUCg become larger. At follow-up, the direction of effect changes in both the CAR and AUCg models (negative to positive). However, the confidence intervals remain wide and still cross zero. Appendix Table 2.B shows the sensitivity checks for the multilevel models. Removing participants taking corticosteroid medication and >15 minutes late on the first saliva sample had a negligible effect on the estimates and their interpretation. Similarly, the estimates between sensitivity models on the change in diurnal slope are near the same, as shown in Appendix Table 2.C.

2.4 Discussion

The findings from this study show that compared to their non-separated counterparts, participants who reported separation during childhood had flatter diurnal slopes at phase 7 but this was no longer present when reassessed four to five years later at phase 9. Overall, the diurnal cortisol slopes of the separated and non-separated groups both became flatter from baseline to follow-up. However, the degree of change was greater in the non-separated group. This finding was explained, to some extent, by changes in evening cortisol. At baseline, there was a distinct difference between evening cortisol levels in the separated and non-separated groups, but by follow-up, this difference was negligible. Maternal

separation was not associated with CAR or AUCg at either time point. The findings remained relatively unchanged in all models after full adjustment of covariates.

Our findings are, therefore, only partly in accordance with the literature. Kumari et al. (2013) report that maternal separation was associated with a larger CAR at phase 7 in WHII, but this effect became fully attenuated after adjustment. Similarly, we do not find support for an association between maternal separation and CAR at either baseline or follow-up. To our knowledge, this is the first paper to examine the association of maternal separation in relation to AUCg and so our finding of a null effect is difficult to compare with other studies. We also did not find support for a flatter diurnal slope for the separated group in phase 9 (follow-up) of the study when participants were aged 66 years, on average. Pesonen et al. (2010b) found that separation from both parents was associated with higher cortisol levels in a sample of older adults that had experienced evacuation during the Second World War. However, this study focused on cortisol reactivity and a comparison between reactivity and basal diurnal cortisol is difficult.

There are two main possible reasons why we fail to observe an association of maternal separation on diurnal slopes in a later phase of the study. First, it is plausible that differences in diurnal cortisol are longer present in older age groups. Similar findings of narrowing or converging differences in health with age have also been reported in the literature on socioeconomic differences in health, known commonly as the age-as-a-leveller hypothesis (Herd, 2016; House et al., 2005). We show that the diurnal slopes of both the separated and non-separated groups become flatter between phases. This finding accords with the literature that suggests that slopes become flatter, and overall HPA axis functioning worsens, with age (Adam et al., 2006; Heaney et al., 2012).) Similar to Wang et al. (2014), we also

found that the CAR decreased from baseline to follow-up. However, in contrast with their findings, waking cortisol and AUCg decreased as well. Instead, the flattening of the slopes between phases appears to be explained by changes occurring in evening cortisol, which increased in the non-separated from baseline to follow-up, but not in the separated. This finding of increased evening cortisol has been reported elsewhere (van den Beld et al., 2018). It is difficult to assess the effect that ageing is having in our models, and this is partly due to the difficulty in separating age and cohort effects. In the model assessing a change in diurnal cortisol slopes from baseline to follow-up, age was not statistically significant. This refers to the effect of an increase in age at baseline on the change in slope between both time points. However, in the cross-sectional multilevel models, examining diurnal slopes at baseline and follow-up, age was found to be statistically significant, and an increase in age was associated with flatter diurnal slopes. Studies examining the age-as-a-leveller hypothesis have suggested that this observed narrowing of differences with age may be a result of morbidity compression among advantaged groups, or selection and mortality effects (Dupre, 2007; House et al., 2005). In this study, the analytic sample remained the same, meaning that the models examining the effect of maternal separation on diurnal cortisol at follow-up contained the same sample of participants as at baseline. We, therefore, cannot explain the difference in findings because of attrition.

Second, the failure to detect an effect for maternal separation at follow-up may be a result of variability in the saliva samples. In Whitehall II, salivary cortisol was measured on a single day at each phase of data collection. Salivary cortisol can affected by a range of daily, situational factors. These include, but are not limited to, sleep (Van Lenten and Doane, 2016), physical activity (Hill et al., 2008), and daily

stressors (Stawski et al., 2013). Several studies have reported relatively low short- and long-term stability in salivary cortisol (Kuhlman et al., 2019; Ross et al., 2014; Wang et al., 2014). As a result, multiple days of sample collection to help increase reliability in the estimates have been recommended (Segerstrom et al., 2014). We may have observed a different result if we had saliva samples collected over a greater number of days. However, while this could likely lead to differences between cortisol levels at the individual level, it seems unlikely that this would then relate to group differences.

Ross et al. (2014) suggest that some sub-groups may experience greater stability in cortisol compared to others. In this study, we found that the separated group showed a smaller degree of change in diurnal slopes from baseline to follow-up, compared to participants that reported no separation. A similar effect was found for smokers and participants with short average sleep duration. Studies have suggested that changes in cortisol in late adulthood may be partly influenced by age-related changes in sleeping patterns (van den Beld et al., 2018). We adjusted for sleep disturbance and average hours slept per week, but it did not attenuate the effect of maternal separation on change. This group may reflect more stable patterns of cortisol over time. However, further repeat measures would be needed to support this. Moreover, multiple indicators of diurnal cortisol across days would have allowed for the estimation of latent difference score models which have been shown to perform better than traditional difference score models used in this study (Klopack and Wickrama, 2020).

A strength of this paper is that salivary cortisol was measured at two time points, allowing us to examine the change in cortisol between study phases. To our knowledge, this is the first study to examine predictors of change in adult cortisol

levels in relation to childhood separation. The sample size used was relatively large, which is an additional strength. This provided the necessary statistical power to examine a range of covariates, which may not be possible using a smaller data set. However, as previously discussed, Whitehall II is a cohort of relatively healthy, white-collar, civil service employees and is, therefore, not nationally representative. Restricting the analyses to participants with complete information on cortisol, sample times, and covariates at baseline and follow-up further exacerbated this. Overall, participants in the analytic sample were healthier and less likely to have reported separation, compared to participants who provided cortisol samples but had incomplete information on the included variables. Although sample attrition could not be held responsible for explaining the difference in association found between the effect of maternal separation on diurnal slopes at baseline and follow-up, we remain aware that the participants in this analytic sample are likely to have experienced separation differently from those who have dropped out of the study, or who were not present to begin with. Further research using a more representative sample is required to see if similar associations are observed.

Maternal separation, and other childhood covariates, were collected using retrospective self-reports. Previous research has highlighted the risk of recall bias associated with such measures (Newbury et al., 2018; Sheikh, 2018). Despite this, Reuben et al. (2016) reported strong agreement between retrospective and prospective accounts of parent-child separation in middle-aged adults, suggesting that maternal separation may pose less risk of bias, compared to other retrospective reports. Moreover, we lack information regarding separation from fathers. However, some cases of reported separation would have inevitably led to separation from both parents, such as evacuation during the war. Lastly, despite adjusting for a range of

childhood covariates, we cannot rule out residual confounding. The maternal separation variable in this study is highly heterogeneous, due to several listed reasons for separation. Moreover, it is likely the selection effects differ by the type of separation experienced.

To summarise, we find that maternal separation is associated with flatter diurnal slopes when participants were aged 60 years, on average, but this association did not remain when assessed four to five years later. Overall, diurnal slopes became flatter baseline to follow-up in both groups. However, participants that reported separation during childhood displayed more stable change, compared to those who reported no separation. Further research with salivary cortisol measured at multiple time points is required to assess change over a longer period.

3 Maternal separation in childhood and hair cortisol concentrations in late adulthood

3.1 Background

Separation from one or both parents during childhood has been shown to have a detrimental impact on long-term physical and mental health outcomes. Studies on separation during childhood have reported associations with cardiovascular disease and mortality (Alastalo et al., 2012), type 2 diabetes (Alastalo et al., 2009), and anxiety and depression (Räikkönen et al., 2011; Berg et al., 2016).

Hypothalamic-pituitary-adrenal (HPA) axis dysregulation has been proposed as a biological mechanism that may help explain how early life stress affects later health (Maniam et al., 2014). This may be particularly relevant during periods of early development when neurobiological systems are characterised by high plasticity and subject to maladaptive alterations (Agorastos et al., 2019). Caregivers have been shown to play an important role in the development and regulation of the HPA axis in early life (Gunnar et al., 2015). Parent-child separation may disrupt HPA axis functioning by removing the regulatory and stress-buffering effects that parents provide (Hostinar et al., 2014). This may be limited to early and middle childhood as studies have shown that the buffering effect provided by caregivers diminishes during puberty (Doom et al., 2015).

Animal studies show a well-established link between maternal separation and cortisol (Feng et al., 2011; Nishi et al., 2014), but few studies have examined the long-term effects in humans. Among these, the results have often been contradictory with evidence of both increased (Kumari et al., 2013; Luecken, 2000; Luecken and Appelhans, 2006; Nicolson, 2004; Pesonen et al., 2010) and decreased (Tyrka et al.,

2008; Meinlschmidt and Heim, 2005; Kraft and Luecken, 2009; Hengesch et al., 2018) basal or stress-induced cortisol levels being reported. This could be due to several reasons. First, timing of separation may play an important role. Research suggests that early childhood and puberty reflect distinct sensitive periods of HPA axis development, which may have long-term effects on functioning (Kuhlman et al., 2017). The former is characterised by a hyporesponsive period, possibly in part due to the buffering effect of caregivers. The latter is characterised by a hyperresponsive period, during which caregivers no longer exert a regulatory influence on the HPA axis (Agorastos et al., 2019). Currently, there is limited evidence of sensitive periods leading to differential cortisol levels in the literature. Bosch et al. (2012) found that adversity experienced during middle childhood was associated with higher cortisol levels, while adversity during early-to-mid adolescence was associated with lower cortisol in a sample of adolescents aged 16 years. Pesonen et al. (2010b) found that separation as a result of evacuation during WWII had a stronger effect in early childhood, compared to infancy or school age, in late adulthood. Other studies on separation found no effect for age of separation (Tyrka et al., 2008; Kumari et al., 2013).

Similarly, the amount of time elapsed since the onset of the stressor may also influence findings. It has been hypothesised that chronic stress may result initially in HPA axis hyperactivity, but then transition into a state of hypocortisolism (Fries et al., 2005). Support for this hypothesis was reported in an influential meta-analysis by (Miller et al., 2007)However, Kumari et al. (2013) and Pesonen et al. (2010b) both found higher cortisol levels among individuals separated during childhood in late-adulthood. An alternative hypothesis focuses on the interaction between early life and current stress, known as the double-hit or sensitisation model. This suggests

that early life stress may dysregulate the HPA axis which then increases vulnerability to the effects of stressors throughout the life course (Koss and Gunnar, 2018). Recent studies found evidence for this in diurnal cortisol patterns (Young et al., 2019), but not cortisol reactivity (Young et al., 2020).

Second, the direction of association may depend on the nature of separation. Luecken (2000) and Luecken and Appelhans (2006) found that individuals who reported either low levels of care or high levels of abuse and conflict following the death of a parent had higher cortisol levels. In contrast, Tyrka et al. (2008) report that individuals who experienced parental desertion during childhood along with low levels of care displayed decreased cortisol concentrations. The demographics of the sample population may also affect the role that different mediating factors across the life course play. In a study of young adults, Kraft and Luecken (2009) report that the effect of parental divorce on cortisol was explained partly by family income. Kumari et al. (2013) found that marital and smoking status largely explained the association between maternal separation and diurnal cortisol slopes in late adulthood.

Third, limitations in the measurement of salivary and plasma cortisol may also lead to conflicting results. While both are commonly used, the measurements obtained using either method are susceptible to environmental and situational influences (Stalder et al., 2017). Furthermore, they measure acute cortisol levels and so are limited in their ability to capture long-term HPA axis activity. Hair cortisol, on the other hand, is a relatively novel biomarker of the HPA axis that is thought to provide a measure of cortisol output over several months (Sauvé et al., 2007) and has been shown to have high test-retest stability (Short et al., 2016). Evidence for the relationship between early adversity and hair cortisol concentrations (HCC) has been mixed. White et al. (2017) and Schalinski et al. (2019) both report lower HCC among

individuals reporting maltreatment and neglect in childhood, respectively. Other studies have instead found no significant association between early adversity and HCC (Bossé et al., 2018; Iob et al., 2020; Pittner et al., 2020). Moreover, a meta-analysis on adverse life events across various ages found support for both higher and lower HCC (Khoury et al., 2019). As a result, the use of cortisol measurement is unlikely to explain all the variation in the findings on maternal separation and cortisol.

In this study, we examined the relationship between maternal separation for one year or longer during childhood and HCC in late adulthood in a cohort of British civil service workers. We hypothesised that participants who reported maternal separation during childhood would have higher expected HCC, compared to their non-separated counterparts. Previous studies have reported mediating effects by childhood and adult factors and so we adjusted for a range of these in our analyses. Finally, we examined whether age at onset and reason for separation were associated with HCC among those reporting maternal separation during childhood.

3.2 Methods

3.2.1 Data

This study uses data from the Whitehall II study (WHII). This is an occupational cohort of British civil servants employed in 20 London-based civil service offices. Recruitment began between 1985 and 1988 (phase 1) with 10308 participants. Since then, the study has collected a further 11 phases of data. We focused on data primarily from phase 11 (2012-13) when hair samples were collected by a trained interviewer or nurse. Retrospective measures on childhood were used from phase 5 (1997-99) and civil service employment grade used information from all available phases. The number of participants in phase 11 was 6308 and 5660 of these took part in the clinical screening. In total, 4460 samples were used for lab analysis, excluding those who did not provide consent (n=172), did not have sufficient hair (n=997), and had a severe head tremor (n=31). Ethical approval for WHII was obtained from the University College London Medical School committee on the ethics of human research. Informed consent was obtained from every participant.

3.2.2 Hair cortisol concentrations

Hair samples were collected by nurses who used scissors to cut hair strands from the posterior vertex region of the head and as close to the scalp as possible. Steroid hormone concentrations were determined from the 3cm hair segment closest to the scalp. It is estimated that hair grows approximately 1cm per month and so the 3cm segment proximal to the scalp is assumed to reflect cortisol secretion over 3 months prior to sample collection (Wennig, 2000). A column switching LC-APCI-MS/MS assay was used to analyse the samples. Hair samples were washed and steroids

extracted by following a protocol that has been previously described (Gao et al., 2013; Stalder et al., 2012). Minor changes were performed to allow for analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS). Intra- and inter-assay coefficients of variation ranged between 3.7% and 8.8% and the limit of quantification (LOQ) was 0.09 (pg/mg). Eleven samples were not analysed due to lab error. Samples that were <7.5mg in weight were removed from the analytic sample (n=467), as well as samples that could not be determined as from the scalp end (n=7).

3.2.3 Maternal separation

In phase 5 of the study, participants were asked a series of retrospective measures regarding their childhood. Maternal separation was measured by asking participants: “Were you ever separated from your mother for a year or more as a child (that is, up until you were 16)?” If participants answered yes, they were asked to provide further information on the age they were separated and the reason this occurred. Age of separation was categorised as follows: early childhood (0-5 years), middle childhood (6-11 years), and adolescence (12-16 years). Reasons included parents separated/divorced, mother died, mother ill, adoption, evacuation, other.

3.2.4 Covariates

Age, sex, ethnicity, and use of local and systemic corticosteroid medication were assessed at phase 11. Childhood factors were assessed at phase 5 of the study and refer to ages up until 16. These include adverse childhood experiences (ACEs), material disadvantage, and parenting dimensions. ACEs were captured using a three-item scale: “Your parent(s) were mentally ill or drank so often that it caused

family problems", "You were physically abused by someone close to you", "Your parents often argued or fought". Material disadvantage used a four-item scale: "Your family had continuing financial problems", "Your family/household did not have an inside toilet", "Your father/mother was unemployed when they wanted to be working", "Your family/household did not own a car". Participants responded either yes or no and responses were summed so that higher scores reflect higher ACEs and disadvantage. Parenting dimensions involved selected items from the Midlife Development in the United States (MIDUS) study (Brim et al., 1996; Shaw et al., 2004). Participants were asked: "Please show how you remember your mother (or the woman who cared for you) during the years you were growing up." The same was asked regarding fathers. Participants were then presented with seven questions (for each parent) that related to aspects of parenting and were asked to answer these according to the following scores: a great deal, quite a lot, a little, not at all. Three separate dimensions were examined: parental warmth (four items, Cronbach's alpha=0.89), parental strictness (two items, Cronbach's alpha=0.7), and parental expectations (one item, Cronbach's alpha=0.73). Items were reverse coded and then summed so that higher scores reflect greater warmth, strictness, and expectations.

Marital status was assessed at phase 11. Married and cohabiting participants were combined in a single category. Other categories include single/never married, divorced/separated, and widowed. To assess adult socioeconomic position, current or last civil service grade was used. Since most of the participants were retired by phase 11, employment grade was defined using their last known grade before leaving the civil service. Categories included administrative, professional/executive, clerical/support, with administrative being the highest grade. Smoking status at phase 11 measured whether participants currently smoked cigarettes versus having

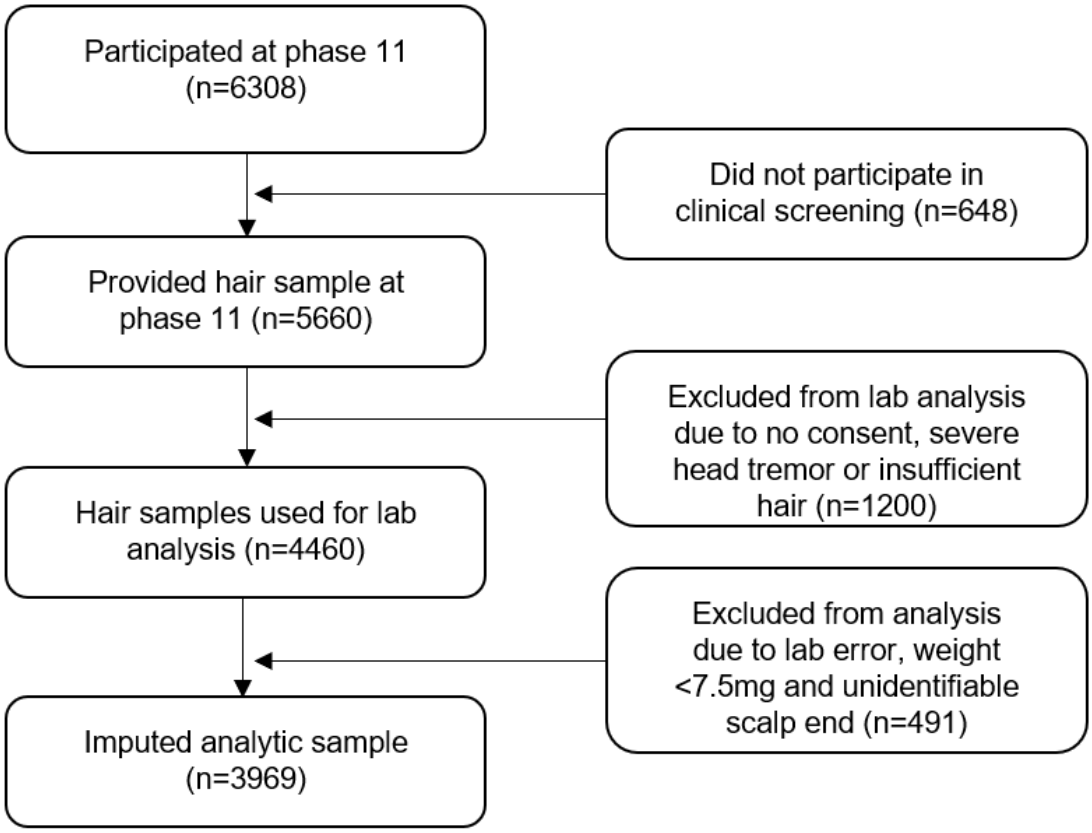
given up/never smoked. Waist and hip circumference were measured by a trained interviewer or nurse as standard in WHII. Waist-to-hip ratio was calculated by dividing waist (cm) by hip (cm), measured at phase 11. Measures of health status included use of CVD medication and prevalent diabetes assessed at phase 11. Diabetes was defined by using self-reported diabetes, or diabetic medication, or fasting glucose ≥7, or HbA1c ≥6.5% (WHO, 2006). Current mental health was examined using The Center for Epidemiologic Studies–Depression scale (CES–D). A score of ≥16, as previously reported (Radloff, 1977), or self-reported use of antidepressants was used to define depression.

3.2.5 Statistical analysis

A visual inspection of HCC values revealed that the data were positively skewed. To establish a normal distribution, HCC was log-transformed. The log values were used in subsequent analysis, but summary statistics show HCC in original units (pg/mg). Outlier HCC (pg/mg) values were winsorised to 3SD from the mean (n=26). The association between maternal separation and log-transformed HCC was estimated using linear regression. Four models in total were tested. Model 1 adjusts for age, sex, ethnicity, and use of local and systemic steroid medication. Model 2 includes covariates from model 1 as well as ACEs, material disadvantage, and parenting dimensions. Model 3 further adjusts for marital status, civil service employment grade, smoking status, and waist-to-hip ratio. Model 4 includes adjustment for CVD medication, diabetes, and depressive symptoms. The association between age at onset and reason for separation and log-transformed HCC was restricted to participants who had reported maternal separation during childhood. The non-linear association of age of separation and HCC was first tested using linear and quadratic

terms. Neither reached statistical significance. The final model included age of separation as a categorical variable. This model adjusted for age, sex, ethnicity, and use of local and systemic steroid medication. Age at onset and reason for separation were tested simultaneously to adjust for each other. Unstandardised regression coefficients (B) with 95% confidence intervals (CI) are presented throughout the results section and tables. To further aid interpretation of the results, we also show in text the effect of maternal separation on HCC in model 4 in terms of percentage change. This was achieved by exponentiating the coefficient (exp(B) *100). In the analytic sample, missingness among the sample individuals ranged from 0.1% to 17.7% of the variables with missing data. These missing values were imputed using multiple imputation by chained equations (MICE) following the best-practice (StataCorp, 2017a) to ensure it was working effectively. First, all the variables included in the analyses, along with a range of hair questionnaire variables were included in the imputation model. Second, a visual inspection of the distributions for observed and imputed values showed strong similarities, as required. Third, trace plots were visually examined to evaluate the convergence of the algorithm. Finally, based on the results of these plots and inspection of fraction of missing information (FMI) values, we imputed 20 datasets for eligible participants (i.e., those who had hair samples of an identifiable scalp end and of a sufficient weight). The final analytic sample was 3969 participants (Figure 3.1). All analyses were carried out using Stata (Version 15.1, StataCorp, College Station, Texas).

Figure 3.1: Flow diagram of the analytic sample



3.2.5.1 Sensitivity analysis

In total, three sensitivity analyses were run. The first model was carried out using complete cases. The second model removed participants who reported taking corticosteroid medication at phase 11. The third model included measures from the accompanying hair questionnaire. These included hair color, use of hair dye, whether hair was treated, and hair washing frequency. These variables were left out of the main models as they were not found to be associated with maternal separation, once age, sex, and ethnicity were controlled for.

3.3 Results

3.3.1 Summary statistics

Table 3.1 shows that 11.13% of the sample reported maternal separation during childhood. The separated group had higher mean values for HCC (pg/mg), ACEs, and material disadvantage, but lower parental warmth and expectations. They were also more likely to be non-white, in the lowest civil service employment grade, and less likely to be married. Characteristics by reason for separation can be found in Appendix Table 3.A. Table 3.2 shows a comparison between the analytic sample and excluded observations. Participants included in the analytic sample were more likely to be female (32.4% versus 24.2%, $p<0.001$) and less likely to report separation (11.1% versus 13.4%, $p=0.01$). They reported a higher proportion of ACEs, but lower material disadvantage. They were also less likely to smoke and to have worked in the lowest civil service grade. Regarding physical health, they had a slightly lower waist-to-hip ratio and were less likely to be on CVD medication. Overall, the analytic sample were slightly healthier and more advantaged than those not included in the analysis. A comparison of the distribution of maternal separation and other early life variables between phase 5 (when early life measures were first recorded) and the analytic sample in phase 11 showed few differences (Appendix Table 3.B).

Table 3.1. Summary statistics showing characteristics by maternal separation status

		Not separated (n=3169)	Separated (n=397)	p-value
HCC (pg/mg)		14.76 (63.95)	26.10 (102.63)	<0.01
Age when separated				
	0-5y		154 (40.4%)	
	6-11y		177 (46.5%)	
	12-16y		50 (13.1%)	
Age (years)		69.59 (5.77)	72.76 (6.07)	<0.001
Sex				<0.001
	Female	968 (30.5%)	154 (38.8%)	
	Male	2201 (69.5%)	243 (61.2%)	
Ethnicity				<0.001
	Non-white	147 (4.6%)	46 (11.6%)	
	White	3021 (95.4%)	351 (88.4%)	
ACEs		0.29 (0.57)	0.42 (0.69)	<0.001
Material disadvantage		1.14 (1.05)	1.46 (1.11)	<0.001
Parental warmth		22.20 (4.84)	20.24 (5.47)	<0.001
Parental strictness		9.40 (2.37)	9.31 (2.58)	0.52
Parental expectations		5.94 (1.44)	5.68 (1.53)	<0.01
Marital status				<0.01
	Married/cohabiting	2323 (74.1%)	272 (69.4%)	
	Single	373 (11.9%)	39 (9.9%)	
	Divorce/separated	193 (6.2%)	30 (7.7%)	
	Widow	248 (7.9%)	51 (13.0%)	
Civil service employment grade				<0.001
	Administrative	1526 (48.2%)	136 (34.3%)	
	Professional/executive	1355 (42.8%)	180 (45.3%)	
	Clerical/support	288 (9.1%)	81 (20.4%)	
Current smoker (cigarettes)				0.07

	No	2926 (97.0%)	359 (95.2%)	
	Yes	91 (3.0%)	18 (4.8%)	
Waist-to-hip ratio		0.92 (0.09)	0.92 (0.09)	0.67
CVD medication				
	No	1296 (40.9%)	152 (38.3%)	0.32
	Yes	1873 (59.1%)	245 (61.7%)	
Diabetes[a]				
	No	2791 (88.1%)	339 (85.4%)	0.12
	Yes	378 (11.9%)	58 (14.6%)	
CES-D >=16 or antidepressants				
	No	2572 (85.4%)	313 (83.7%)	0.39
	Yes	441 (14.6%)	61 (16.3%)	
Local steroids				
	No	2884 (91.0%)	348 (87.7%)	0.03
	Yes	285 (9.0%)	49 (12.3%)	
Systemic steroids				
	No	3112 (98.2%)	381 (96.0%)	<0.01
	Yes	57 (1.8%)	16 (4.0%)	

Notes: Mean(sd)/n(%). HCC= hair cortisol concentrations. [a] Diabetes was defined using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006). CES-D= Center for Epidemiologic Studies Depression Scale.

Table 3.2. Comparison of included versus not included in analysis

		Excluded n=2339[a]	Included n=3969	p-value	Missing %[b]
HCC (pg/mg)		22.92 (100.17)	16.38 (71.42)	0.07	0
Maternal separation				0.01	10.2
	No	1732 (86.6%)	3169 (88.9%)		
	Yes	268 (13.4%)	397 (11.1%)		
Maternal separation age				0.45	10.6
	0-5y	93 (35.8%)	154 (40.4%)		
	6-11y	127 (48.8%)	177 (46.5%)		
	12-16y	40 (15.4%)	50 (13.1%)		
Age (years)		69.80 (5.91)	69.83 (5.85)	0.83	0
Sex				<0.001	0
	Female	565 (24.2%)	1284 (32.4%)		
	Male	1774 (75.8%)	2685 (67.6%)		
Ethnicity				<0.001	0.1
	Non-white	228 (9.8%)	237 (6.0%)		
	White	2104 (90.2%)	3729 (94.0%)		
ACEs		0.26 (0.56)	0.30 (0.59)	0.02	13.1
Material disadvantage		1.24 (1.09)	1.17 (1.06)	0.03	13.8
Parental warmth		21.99 (4.93)	22.00 (4.94)	0.91	17.7
Parental strictness		9.27 (2.34)	9.39 (2.39)	0.08	17.0
Parental expectations		5.86 (1.44)	5.91 (1.45)	0.22	16.9
Marital status				0.19	1.0
	Married/cohabiting	1747 (75.3%)	2887 (73.5%)		
	Single	238 (10.3%)	450 (11.5%)		
	Divorce/separated	164 (7.1%)	262 (6.7%)		
	Widow	171 (7.4%)	330 (8.4%)		
Civil service employment grade				<0.01	0
	Administrative	998 (42.7%)	1797 (45.3%)		

	Professional/executive	1016 (43.4%)	1735 (43.7%)		
	Clerical/support	325 (13.9%)	437 (11.0%)		
Current smoker (cigarettes)				0.03	5.9
	No	2048 (95.5%)	3611 (96.7%)		
	Yes	96 (4.5%)	125 (3.3%)		
Waist-to-hip ratio		0.94 (0.08)	0.92 (0.09)	<0.001	0.7
CVD medication				0.03	0
	No	881 (37.7%)	1610 (40.6%)		
	Yes	1454 (62.3%)	2359 (59.4%)		
Diabetes[c]				0.19	0
	No	2019 (86.3%)	3472 (87.5%)		
	Yes	320 (13.7%)	497 (12.5%)		
CES-D >=16 or antidepressants				0.19	5.8
	No	1796 (83.3%)	3163 (84.6%)		
	Yes	360 (16.7%)	576 (15.4%)		
Local steroids				0.10	0
	No	2145 (91.9%)	3598 (90.7%)		
	Yes	190 (8.1%)	371 (9.3%)		
Systemic steroids				0.90	0
	No	2288 (98.0%)	3891 (98.0%)		
	Yes	47 (2.0%)	78 (2.0%)		

Notes: Mean(sd)/n(%). HCC= hair cortisol concentrations. [a] Excluded participants include hair samples from an unidentifiable scalp end, insufficient weight, lab errors, severe head tremor, did not provide consent, insufficient hair, ineligible for clinical screening. [b] Missingness in the analytic sample. [c] Diabetes was defined by using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006). CES-D= Center for Epidemiologic Studies Depression Scale.

3.3.2 Association between maternal separation and log-transformed HCC (pg/mg)

Table 3.3 shows that childhood factors were not significantly associated with log HCC and their effect was close to zero. Similar results were found for marital status and civil service employment grade. Overall, health behaviours and health status measures were positively associated with log HCC, but smoking was not statistically significant (p=0.06). In particular, waist-to-hip ratio and diabetes each had a relatively large effect (B=0.726, 95% CI 0.136-1.316, p=0.02; B=0.314, 95% CI 0.191-0.437, p<0.001). The association between maternal separation and log HCC is shown in Figure 3.2 and Table 3.4. Figure 3.2 displays the mean of log-HCC (pg/mg) by separation status for the basic versus fully adjusted model. In model 1, maternal separation during childhood was positively associated with log HCC (B=0.172, 95% CI 0.034-0.310, p=0.02). The effect size increased after controlling for childhood factors in model 2, which may indicate a suppressor effect. Further analysis (not shown) revealed that the increase in effect size was driven largely by the addition of parental warmth and expectations. After adjusting for adult and health measures in models 3 and 4, respectively, the effect size decreased slightly but remained statistically significant. After full adjustment, HCC was estimated to be 19.6% higher in participants who reported maternal separation during childhood, compared to those who reported no separation (B=0.179, 95% CI 0.041-0.317, p=0.01).

Table 3.3. Association between childhood, adult, and health factors and log hair cortisol concentrations (HCC) (pg/mg) (n=3969)

	B	95% CI	p-value
Age	0.002	-0.05,0.009	0.60
Male	0.270	0.151,0.389	<0.001
Ethnicity (white)	0.085	-0.088,0.257	0.34
Local steroids	0.136	-0.012,0.284	0.07
Systemic steroids	-0.636	-0.946,0.325	<0.001
ACEs	0.018	-0.058,0.094	0.63
Material disadvantage	-0.001	-0.042,0.040	0.96
Parental warmth	0.006	-0.004,0.015	0.27
Parental strictness	-0.008	-0.027,0.011	0.43
Parental expectations	0.025	-0.009,0.059	0.15
Marital status[a]			
Single	-0.009	-0.135,0.117	0.89
Divorced/separated	0.071	-0.088,0.231	0.38
Widow	-0.048	-0.196,0.99	0.52
Civil service employment grade[b]			
Professional/executive	-0.010	-0.097,0.077	0.82
Clerical/support	0.025	-0.129,0.179	0.75
Current smoker (cigarettes)	0.213	-0.010,0.435	0.06
Waist-to-hip ratio	0.726	0.136,1.316	0.02
CVD medication	0.072	-0.011,0.155	0.09
Diabetes[c]	0.314	0.191,0.437	<0.001
CESD >=16 or antidepressants	0.167	0.049,0.284	0.01

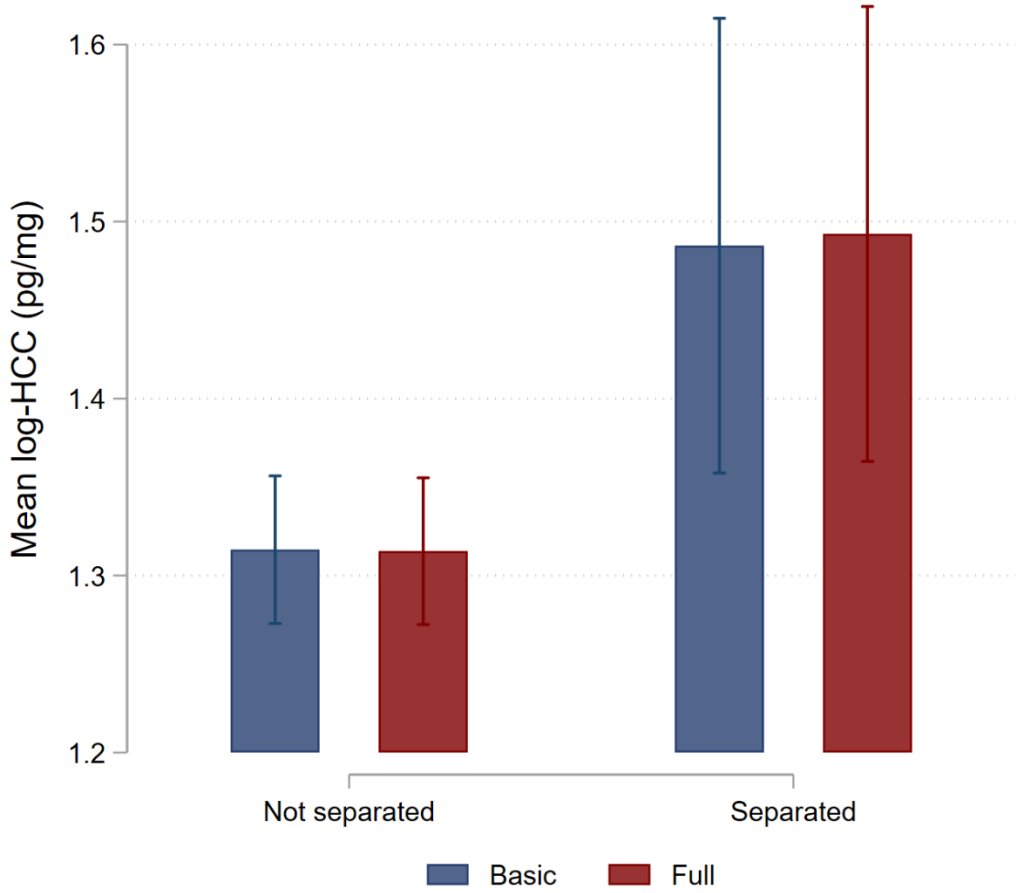
Notes: [a] Marital status reference category: married/cohabiting. [b] Employment grade reference category: administrative. [c] Diabetes was defined by using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006). CES-D= Center for Epidemiologic Studies Depression Scale.

Table 3.4. Association between maternal separation in childhood and log hair cortisol concentrations (HCC) (pg/mg) (n=3969)

	B	95% CI	p-value
Model 1	0.172	0.034,0.310	0.02
Model 2	0.185	0.047,0.324	0.01
Model 3	0.170	0.031,0.309	0.02
Model 4	0.179	0.041,0.317	0.01

Notes: Model 1: age (years), sex, ethnicity, local and systemic steroid medication. Model 2: Model 1 + adverse childhood experiences, material deprivation, and parenting dimensions. Model 3: Model 2 + marital and smoking status, civil service employment grade, and waist-to-hip ratio. Model 4: Model 3 + CVD medication, diabetes and depressive symptoms.

Figure 3.2: Predictive margins of maternal separation with 95% CI (n=3969)



Notes: Basic model: age (years), sex, ethnicity, local and systemic steroid medication. Full model: basic + adverse childhood experiences, material disadvantage, parenting dimensions, marital status, smoking status, employment grade, waist-to-hip ratio, CVD medication, diabetes, depressive symptoms. HCC = hair cortisol concentrations.

Table 3.5 shows that participants separated during middle childhood (6-11y) and adolescence (12-16y) had higher estimated log HCC (B=0.134, 95% CI 0.874-1.497, p=0.38; B=0.125, 95% CI 0.738-1.739, p=0.57), compared to participants separated during early childhood (0-5y). Compared to participants that experienced maternal separation as a result of evacuation, parental separation or divorce, adoption, and other displayed higher estimated log HCC and mother illness and death displayed lower HCC. Neither age at onset nor reason for separation reached statistical significance.

Table 3.5. Association between age and reason for maternal separation in childhood and log hair cortisol concentrations (HCC) (pg/mg) (n=397)

	B	95% CI	p-value
Age separated			
0-5 (ref)			
6-11	0.134	0.874,1.497	0.38
12-16	0.125	0.738,1.739	0.57
Reason for separation			
Evacuation (ref)			
Parents divorced/separated	0.327	-0.213,0.867	0.24
Mother died	-0.052	0.427,0.323	0.79
Mother ill	-0.040	0.499,0.419	0.87
Adoption	0.089	-0.589,0.768	0.80
Other	0.095	-0.244,0.434	0.58

Notes: Model adjusts for age (years), sex, ethnicity, local and systemic steroid medication.

3.3.3 Sensitivity analyses

The estimated effect of maternal separation on log-transformed HCC remained largely unchanged and significant across all sensitivity analyses (Appendix Table 3.C). The effect size for maternal separation on log-transformed HCC was higher when carried out on complete cases. Similarly, the effect size decreased after removing participants who reported taking local and systemic steroid medication. The inclusion of hair questionnaire variables had only a minor effect on the relationship between maternal separation and log-transformed HCC.

3.4 Discussion

We found that maternal separation during childhood for a period of up to one year or more was positively associated with HCC in late adulthood, compared to participants who reported no separation, in a large occupational cohort. This effect was independent of a wide variety of potential confounders and mediators. The association of maternal separation and HCC increased after adjusting for childhood factors. Adjusting for adulthood factors and health status explained only a small proportion of the estimated effect, suggesting that early life experiences may directly impact biological functioning into old age.

Our findings of higher cortisol accord with some studies (Kumari et al., 2013; Luecken, 2000; Luecken and Appelhans, 2006; Nicolson, 2004; Pesonen et al., 2010), but not others (Meinlschmidt and Heim, 2005; Tyrka et al., 2008; Kraft and Luecken, 2009; Hengesch et al., 2018). To our knowledge, there has been only one other study that has looked at separation and HCC in late adulthood (Iob et al., 2020), although this formed part of a dimension of loss alongside institutionalisation, parent death, and foster care. In contrast to our findings, this study found a small,

positive but non-significant association with HCC. Similarly, no association was found between HCC and other dimensions or cumulative exposure to childhood adversity.

Previous research has suggested that greater elapsed time since the onset of a stressor could lead to HPA axis hypoactivity (Fries et al., 2005; Miller et al., 2007). It is believed that this may reflect an adaptation to prolonged periods of hyperactivity. In contrast, we find evidence for elevated HCC in participants who experienced maternal separation up until age 16 in a sample where the average age of participants was 70 years. The effect of maternal separation on HCC in late adulthood may be moderated by current stressors, thereby leading to increased levels. Evidence for the interaction between early life and current stress was found in a recent study on diurnal cortisol patterns (Young et al., 2019). However, we did not have the statistical power required to examine such interaction effects.

Unlike Kraft and Luecken (2009) and Kumari et al. (2013), we did not find any substantial evidence for attenuation of an observed association by either childhood or adulthood factors. There are several potential reasons for this. First, it is possible that these factors no longer have a mediating effect in late adulthood. The aforementioned studies focus on young and middle-aged adults, respectively. Second, the lack of attenuation may be as a result of bias as the sample population in WHII became healthier and more advantaged in later waves of the study. We speculate that those at increased risk of loss to follow up or early mortality may be differentially lost to the study. Research suggests that parent-child separation is associated with adverse mental health in adulthood (Räikkönen et al., 2011; Berg et al., 2016). Mein et al.(2012) report that respondents in WHII with lower levels of physical functioning and mental health were more likely to withdraw, although a long-

standing self-reported illness decreased the risk of attrition. We found only minor differences between the distribution of early life measures at phase 5 and in our analytic sample at phase 11. However, it is possible that the participants in our analytic sample experienced maternal separation differently to those who were either not eligible for recruitment into the cohort or were lost to follow up. This may partly explain not only the lack of attenuation, but also the patterns of HPA axis hyperactivity that we observe. Third, the difference in findings may reflect differences in cortisol measurement. In two separate validation studies, hair cortisol was associated with integrated measures of salivary cortisol (AUC) over 30 days , but not the cortisol awakening response (CAR) or diurnal slope (Short et al., 2016; Sugaya et al., 2020). Moreover, Sugaya et al. (2020) found that the correlation with AUC was stronger when removing the awakening response, which suggests that hair cortisol may better reflect basal cortisol secretion than salivary cortisol.

In contrast to Pesonen et al. (2010b) we found no significant association for age of separation, but we may have had insufficient power to detect an effect. Additionally, given the chronic nature of separation in this study, the age at onset of separation may be less important than the severity experienced at different times while the separation event remained ongoing. Further research is needed to investigate this. Similarly, we found no significant association for reason for separation. Again, statistical power may have been limited. Research has suggested that the effect of early life stress on HPA axis development may be modulated by the nature of the stressor (Bruce et al., 2009), and so it's plausible that different types of separation may result in varying effects, but we did not find evidence to support this.

The strengths and limitations of this study need to be addressed. Our analysis uses data from a large cohort study, leading to a sample size larger than many of the

previous studies on parent-child separation and cortisol in adults. However, given that this study began as an occupational cohort, it is not nationally representative and issues regarding representativeness increase in later waves of the study. Participants that were excluded from the analysis reported greater material disadvantage during childhood, were more likely to smoke, more likely to be in the lowest employment grade, and measured slightly worse on a range of physical health indicators. This could explain why we do not see attenuation of effect similar to Kumari et al. (2013) in an earlier analysis of the study. Alternatively, maternal separation during childhood may be a relatively weak stressor in comparison to other stressors throughout the life-course. As a result, it is possible that we only see this magnitude of effect because of the relatively healthy and advantaged characteristics of this sample population. Räikkönen et al. (2011), for example, found that the effect of wartime evacuation during childhood on the risk of adult mental health disorders was moderated by childhood socioeconomic background. Separated individuals from upper socioeconomic backgrounds in childhood had a higher risk compared to their non-separated counterparts. Individuals from a less privileged socioeconomic background showed an increased risk, regardless of their separation status. Further research is thus needed using more representative samples to investigate whether maternal separation has a similarly strong effect.

We assess cortisol in late adulthood using hair. This has the advantage of providing an insight into chronic HPA axis activity. However, a weakness is that we only have one measure of hair cortisol at a single time-point. Research suggests that early life stress has long-term effects on HPA axis regulation, but we were unable to investigate longitudinal associations. Kumari et al. (2013) found a larger CAR and flatter diurnal slopes in those reporting maternal separation in an earlier stage of the

study when participants were aged 60y on average. Little is known about how measures of salivary and hair cortisol may relate to one another over extended periods. Previous studies have shown that early life separation is associated with a range of negative physical and mental health outcomes in later life. The findings from this study further extend the evidence base suggesting that cortisol may be an underlying biological mechanism involved in this relationship. However, we were unable to investigate the mechanisms that help to explain the relationship between separation and HPA axis dysregulation. For example, DNA methylation may play an important role in linking early life stress to HPA axis functioning (Tyrka et al., 2016; Liu and Nusslock, 2017).

In this study, we focus specifically on maternal separation during childhood. Recently, this focus on single events has been criticised for failing to account for the co-occurrence and patterning of adverse childhood experiences that are often observed (Kuhlman et al., 2017). As a result, research has increasingly moved towards the use of cumulative exposure models. However, models such as these have limited utility in investigating differences between adversity type. Although we did not compare maternal separation to other early life stressors, we were able to investigate differences between reasons for separation, but these were not significant. More recently, researchers have advocated the use of dimension-based approaches to account for patterning among adversities (McLaughlin and Sheridan, 2016). Our measure of separation in this study encompasses a range of early life events that were responsible for separation, e.g., parental separation or divorce, death of mother, adoption. Despite not using the same methods, this is consistent with dimensions of loss and disrupted caregiving described in other studies (Kuhlman et al., 2017; Iob et al., 2020). Moreover, we also adjust for a range of

adverse experiences during childhood, but these were not significantly associated with HCC.

Information on maternal separation and other childhood factors were collected using retrospective measures. Concern has been raised about the extent to which the associations between early life stress and physical and mental health outcomes in adulthood are affected by recall bias (Hardt and Rutter, 2004). Newbury et al. (2018) reported only slight to fair agreement when comparing prospective and retrospective reports of childhood maltreatment. Similarly, Sheikh (2018) found that current psychological state explained almost 25% of the association between childhood disadvantage and adult morbidity; although this could reflect mediation. Nonetheless, maternal separation may be less problematic than other retrospective measures as it does not rely heavily on subjective or emotional judgment (Hardt and Rutter, 2004). Support for this was found in a study by Reuben et al. (2016) which reported near-perfect agreement between prospective and retrospective accounts of parent-child separation at age 38. Additionally, our estimates of maternal separation were robust to adjustment for current depressive symptoms, measured using CES-D and antidepressant use. It is important to note, however, that participants that were separated during infancy or early childhood would be unlikely to have any memory of this event and would have had to have this information relayed to them by others. As such, inaccuracy of details, such as the precise age of separation, may have influenced estimates. A further weakness is that we did not have information on separation from fathers or the duration of separation. We cannot be sure of the effect this would have had. However, some cases of separation, such as evacuation, would have inevitably led to separation from both parents. Similarly, separation as a result of mother's death is of course permanent.

We adjust for a range of childhood, adulthood, and health status factors in the final model. However, we cannot rule out unobserved confounders. Given the heterogeneous nature of those separated during childhood, it is plausible that selection effects differ by reason for separation. For example, in the case of parental separation or divorce, participants may have been exposed to interparental conflict prior to separation, which has been shown to be associated with elevated cortisol in children (Harold and Sellers, 2018). Moreover, we lacked specific information regarding the post-separation environment, which we would expect to have a significant impact on the overall consequences of separation.

In conclusion, we found that maternal separation for up to one year or longer during childhood is independently associated with an increase in HCC in late adulthood, compared to participants who report no separation. This contributes towards evidence on the long-term impact of early life stress on later life biology, as evidenced by seeing an effect on hair cortisol many years later.

4 Depressive symptoms and hair cortisol in older adults: Findings from the Whitehall II study.

4.1 Background:

The hypothalamic-pituitary-adrenal (HPA) axis is thought to play an important role in the pathophysiology of depression (Pariante, 2006). Several meta-analytic studies have reported an association between depression and both basal cortisol levels (Belvederi Murri et al., 2014; Knorr et al., 2010; Stetler and Miller, 2011) and reactivity (Burke et al., 2005; Zorn et al., 2017), with hypersecretion being the most consistent finding. Despite this, considerable variation in effect size and direction has been reported, which may be a result of methodological quality, differences in the measurement of cortisol and depression, and medication use (Stetler and Miller, 2011).

Most studies on cortisol and depression have measured cortisol in saliva, urine, and serum (Belvederi Murri et al., 2014). Although these measures provide insight into acute and short-term cortisol levels, they are less suited to capturing long-term HPA axis activity and are influenced by various environmental and situational factors (Stalder et al., 2012). Hair cortisol is a more recent biomarker of the HPA axis that reflects total cortisol output over several months, depending on the length of the hair sample (Sauvé et al., 2007). The retrospective nature of hair cortisol means that it is less affected by situational confounding, and studies have reported high intra-individual stability (Short et al., 2016; Stalder et al., 2012).

Research on the association between hair cortisol concentrations (HCC) and depression is less extensive than cortisol in bodily fluids. In chapter 3, a positive association between depression, measured using antidepressant use and/or

depressive symptoms, and HCC was observed. However, studies in the literature show contradictory findings with evidence of higher (Dettenborn et al., 2012; Duncko et al., 2019; Iob et al., 2019; Song et al., 2019) and lower (Gerber et al., 2013; Pochigaeva et al., 2017) HCC, but other studies found no significant differences (Dowlati et al., 2010; Herane-Vives et al., 2018; Mayer et al., 2018; Wells et al., 2014) in HCC.

Some studies have also reported differences between a diagnosis of depression, according to DSM-IV criteria, and measures of symptom severity in their association with HCC. Wei et al. (2015) found higher HCC in depressed patients but no association with symptom severity, measured using the Hamilton Depression Rating Scale (HAMD-17). Similarly, Steudte-Schmiedgen et al. (2017) also found no association between depressive symptoms, measured using the Beck Depression Inventory (BDI), and HCC, but reported lower HCC in depressed patients versus controls. In contrast, Gerritsen et al. (2019) and Herane-Vives et al. (2020) failed to see an association with a diagnosed depressive disorder but observed a correlation between symptom severity and HCC, albeit in opposite directions. Gerritsen et al. (2019) found that Inventory for Depressive Symptomatology (IDS) scores were positively correlated with HCC, whereas Herane-Vives et al. (2020) report a negative correlation with HAMD-17.

In a recent meta-analysis, Psarraki et al. (2021) identified several factors that may partially explain the heterogeneity in reported findings. First, the recurrence of depressive episodes and symptoms could reflect an important source of variability in depressed individuals. Depression is a highly recurrent disorder characterised by periods of remission and relapse. In two separate longitudinal studies, more than 50% of depressed individuals experienced recurrent episodes following diagnosis,

and more than 15% experienced chronic episodes of at least two years duration (Kennedy et al., 2003; Verduijn et al., 2017). Few studies, however, have examined the effects of recurrent depressive episodes on HCC. Wei et al. (2015) report higher HCC among depressed patients in their first episode versus healthy controls, but no association was found for patients experiencing recurrent episodes.

Similarly, no significant differences in HCC were observed between groups before a depressive episode (Wei et al., 2015). These differences may extend beyond the first onset of depression. Duncko et al. (2019) observed higher HCC in participants with a current, but not recurrent, depressive disorder compared to depression-free participants during the study period. Similarly, Gerritsen et al. (2019) saw no differences in HCC in remitted participants versus controls. This may suggest alterations in the sensitivity of the HPA axis in recurrently depressed individuals, although further research is needed (Psarraki et al., 2021).

Second, antidepressant medication may influence the results in several ways. Antidepressants have been shown to have a regulating effect on HPA axis activity (Mason and Pariante, 2006). This may suggest lower cortisol levels in depressed individuals on medication. However, depressed patients on medication are also more likely to have experienced greater severity of symptoms, given that individuals who are worse affected are more likely to receive treatment (Subramaniam et al., 2019). To date, only a few studies have investigated antidepressant use on HCC and these have shown a positive association (Gerritsen et al., 2019; Wells et al., 2014). Moreover, Song et al., (2019) found that HCC increased in depressed patients after 4 weeks of antidepressant treatment, although this was partially determined by how well depression was controlled during this period.

Third, early life stress may also play an important role. Research has long explored the relationship between depression and both early life stress and HPA axis dysregulation (Juruena, 2014; Tofoli et al., 2011). However, existing studies on HCC have failed to observe a joint effect (Cantave et al., 2022; Hinkelmann et al., 2013; Oresta et al., 2021). Duncko et al. (2019) found higher HCC in depressed individuals without a history of childhood trauma, compared to controls. However, elevated HCC was not observed in the depressed group with a history of trauma in childhood. This may indicate a moderation effect by childhood events on the relationship between depression and cortisol.

Overall, the findings on the association between depression and HCC are mixed. The literature suggests that various factors, such as the recurrent nature of depression, the use of antidepressants, and early life stress may help to explain this variability. However, few studies have accounted for these. Research focused on older adults is also needed to see if this relationship persists across the life course. This paper aims to address these gaps by investigating the association between depressive symptoms, measured over a 10-year period, and HCC, whilst adjusting for long-term antidepressant use. In chapter 3, a positive association was observed between maternal separation in childhood and HCC in this cohort. Here, we test whether maternal separation moderates the effect of depressive symptoms on HCC in late adulthood.

4.2 Methods:

4.2.1 Data

We use data from the Whitehall II study. This is an occupational cohort of British civil servants in 20 London-based departments. Recruitment began in 1985 (phase 1) with an initial cohort of 10308 participants. A further 11 phases of data have since been collected. Hair samples were collected at phase 11 of the study (2012-2013) and depressive symptoms, measured using the 20-item version of the Center for Epidemiologic Studies Depression scale (CES-D), were assessed at phases 7 (2002-2004), 9 (2007-2009), and 11. The number of participants at phase 11 was 6308, with 5660 of these taking part in the clinical screening assessment. Hair samples were collected from 4460 participants, excluding those who did not provide consent (n=172), had insufficient hair (n=997), or a severe head tremor (n=31). Ethical approval for the Whitehall II study was obtained from the University College London Medical School committee on the ethics of human research. Informed consent was obtained from each participant.

4.2.2 Hair sample collection and analysis

Hair samples were collected by a trained nurse who carefully cut strands as close to the scalp as possible from the posterior vertex region of the head. Steroid hormone concentrations were determined from the 3cm segment closest to the scalp. Hair is estimated to grow approximately 1cm per month. The 3cm hair segment proximal to the scalp is assumed to reflect total cortisol secretion for the 3-month period preceding sample collection (Wennig, 2000). A column switching LC-APCI-MS/MS assay was used to analyse the samples. Hair samples were washed, and steroids

extracted, with minor changes performed to enable analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS). The procedure has been described in detail elsewhere (Gao et al., 2013; Stalder et al., 2012). Eleven samples were not analysed, as a result of lab error. Samples below 7.5mg in weight were removed (n=467), along with those that could not be identified as from the scalp end (n=7). The limit of quantification (LOQ) was 0.09 (pg/mg) and intra- and inter-assay coefficients of variation ranged between 7.3% and 8.8%.

4.2.3 Depressive symptoms and antidepressants

Depressive symptoms were assessed using the CES-D scale (Radloff, 1977). This self-report measure has been shown to have good predictive validity in adults aged 50 and over and has previously been validated against the interview-administered revised Clinical Interview Schedule (CIS-R) in this cohort. Sensitivity and specificity for measuring a depressive episode were 89% and 86%, respectively (Head et al., 2013). Participants were asked to record the frequency of symptoms experienced over the past week. Responses were provided on a four-point scale, ranging from 0 ‘less than once a week’ to 3 ‘5-7 days a week’. CES-D scores ranged from 0 to 60. To assess depressive symptoms over the three phases of data collection, continuous and categorical variables were created. The continuous variable is a cumulative measure of the CES-D score over phases 7, 9 and 11. This ranges from 0 to 180. The categorical variable was used to assess the recurrence of depressive symptoms and follows the categories used by Duncko et al. (2019). A cut-off score of ≥16, as previously recommended (Radloff, 1977), was used as the caseness threshold. The data were divided into four categories: no symptoms (CES-D score <16 at all three phases), past (CES-D score ≥16 at phases 7 and/or 9, but not 11),

recurrent (CES-D score ≥16 at phases 9 and 11, or all three phases), and current (CES-D score ≥16 at phase 11 only). Internal consistency was good with a Cronbach's alpha of 0.88.

At each phase of data collection, participants were asked about the medication they were currently taking. Antidepressant medication use was categorised according to the same four-level categories (none, past, recurrent, current) as depressive symptoms.

4.2.4 Maternal separation

Maternal separation was assessed at phase 5 (1997-1999), along with other retrospective childhood variables. Participants were asked: "Were you ever separated from your mother for a year or more as a child (that is, up until you were 16)?" Participants were then asked to provide details on the age they were separated, as well as the reason this occurred. Reasons for separation included: parents separated/divorced, mother died, mother ill, adoption, evacuation (referring to the Second World War), and other.

4.2.5 Covariates

Age, sex, and ethnicity were assessed by questionnaire. Participants were asked about the medication they were taking. Local and systemic corticosteroids and antipsychotic medication were recorded at phase 11. Additional childhood factors were assessed at phase 5. These refer to ages up until 16 years. Adverse childhood experiences (ACEs) were measured using a three-item scale that captured instances of abuse, family conflict, and family mental illness and alcohol abuse. Participants were asked to record whether, during childhood: "You were physically abused by

someone close to you", "Your parents often argued or fought", or "Your parent(s) were mentally ill or drank so often that it caused family problems." Material disadvantage during childhood was assessed by the following items: "Your family/household did not have an inside toilet", "Your father/mother was unemployed when they wanted to be working", "Your family/household did not own a car", and "Your family had continuing financial problems." Parent-child relationship quality was assessed using selected items from the Midlife Development in the United States (MIDUS) study (Brim et al., 2019; Shaw et al., 2004). Participants were asked: "Please show how you remember your mother (or the woman who cared for you) during the years you were growing up." The same questions were asked regarding their fathers/male carers. Three dimensions of parenting were identified: parental warmth (four items, Cronbach's alpha=0.89), parental strictness (two items, Cronbach's alpha=0.7), and parental expectations (one item, Cronbach's alpha=0.73). Responses for all childhood measures were summed. Higher scores reflect greater ACEs, material disadvantage, parental warmth, strictness, and expectations. Socioeconomic position was assessed using current or last civil service employment grade: clerical/support (lowest), professional/executive, and administrative (highest). Smoking status measured whether participants currently smoked cigarettes/pipes/cigars versus having given up/never smoked. Body mass index (BMI) was assessed using height and weight measurements collected by a trained interviewer or nurse. Physical health was assessed by current use of cardiovascular (CVD) medication and diabetes. Participants were classed as diabetic if they met one of the following criteria: self-reported diabetes, diabetic medication, fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006). All covariates were assessed at phase 11 of the study, other than childhood factors which were

assessed at phase 5, and employment grade, which uses information from all available phases.

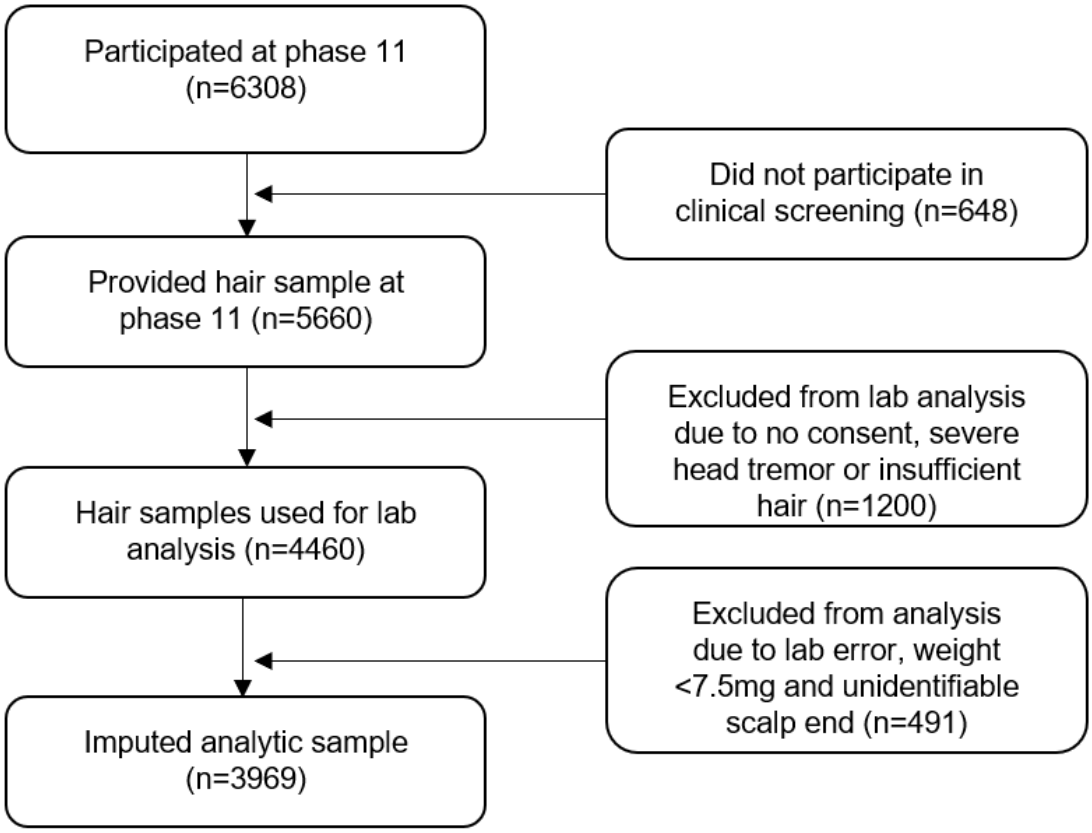
4.2.6 Statistical analysis

HCC values were skewed and so were log-transformed to have an (approximately) normal distribution. Summary statistics (shown below) present HCC in original units (pg/mg). HCC values >3 SD from the mean were winsorised to this value (n=26). Linear regression models were used to estimate the effects of depressive symptoms and antidepressant use on HCC. Depressive symptoms were first modelled using the cumulative CES-D score, followed by the categorical variable capturing the recurrence of symptoms from phases 7 to 9. Two sets of models were fitted for both. First, demographic (age, sex, ethnicity, employment grade) and medication (local and systemic corticosteroid, antipsychotics) covariates were adjusted for. Second, childhood conditions (ACEs, material disadvantage, parenting dimensions), health behaviours (smoking status), and health (BMI, CVD medication, diabetes) were added as covariates.

To assess whether maternal separation moderated the association between depressive symptoms and HCC, an interaction term was included. Nonlinearity in total CES-D score, age, and BMI was tested by including quadratic terms. None of these were statistically significant and so only linear effects are reported. Model estimates are presented throughout as unstandardised regression coefficients (B) with 95% confidence intervals. To aid the interpretation of the results, coefficients from the fully adjusted models are also displayed in the text in terms of percentage change, by exponentiating the coefficient (exp(β)*100).

Missingness in the variables ranged from 0 to 23.2%. Approximately, 61% of participants had missingness on at least one variable. To address this, missing values were imputed using multiple imputation by chained equations (MICE). Before imputation, each variable included in the substantive models was first regressed on all other variables to test convergence. The final imputation model included each variable from the substantive models described above, with further auxiliary variables added to improve the accuracy of the imputation model. These included BMI from phases 7 and 9, and variables from the accompanying hair questionnaire (hair washing frequency, hair colour, hair dye, hair treatment). The first imputation was conducted using 20 imputations. Distributions of the observed and imputed values were compared. Diagnostics of the imputation model included an investigation of Monte Carlo errors and an overview of the fraction of missing information (FMI) values for each imputed variable. A visual inspection of trace plots was carried out to evaluate the algorithm convergence. Based on the results (not shown) and following guidance from White et al. (2011) and van Buuren (2018), we increased the final number of imputations to 30 datasets based on eligible participants (hair samples of an identifiable scalp end and sufficient weight). This provided a final analytic sample of 3969 participants (Figure 4.1). Analyses were carried out using Stata, Version 16.1 (StataCorp, 2019).

Figure 4.1: Flow diagram of the analytic sample



4.2.6.1 Sensitivity analysis

Sensitivity analyses were conducted to compare the findings from the full imputed analysis to three other models. The first model used a sample restricted to complete cases. The second removed participants that had reported taking local and systemic corticosteroid and antipsychotic medication. Finally, the third model adjusted for variables from the accompanying hair questionnaire. These included: hair washing frequency, hair treatment, hair dye, and hair colour. Covariates remained the same across models, apart from the differences that are described here.

4.3 Results

4.3.1 Summary statistics

Overall, 22.54% of the sample reported depressive symptoms (CES-D ≥16) at least once between phases 7 to 11. Mean cumulative depressive symptoms was 21.27 (SD=18.26, range=0-132). Table 4.1 shows the characteristics of the participants by their depressive symptom status over phases 7 to 11. This reflects observed values, rather than imputed cases. Mean cumulative depressive symptoms were highest in the recurrent group (66.24), as expected. A value of ≥ 16 is commonly used to indicate individuals at risk for clinical depression. Participants with past symptoms had a higher cumulative CES-D score than those with only current symptoms (39.71 versus 36.36). In contrast, HCC was highest in the current group. Participants with past depressive symptoms also had higher mean HCC than those experiencing recurrent symptoms. Antidepressant use was highest in the recurrent group (17% versus 9.8% for current and past symptoms). The proportion of participants that reported maternal separation during childhood was similar between groups. Compared to participants without depressive symptoms, those in the depressed groups were more likely to be female, and reported higher ACEs, material disadvantage, parental strictness, and lower levels of parental warmth. They were also more likely to smoke, be in the lowest civil service employment grade, and have diabetes. Differences between those with and without depressive symptoms were most notable for participants in the recurrent group, other than for diabetes in which those with only current symptoms showed the highest rates. Observed versus imputed values are shown in Appendix Table 4.A.

Table 4.1: Summary statistics showing characteristics by depressive symptoms status in observed data, phases 7-11.

	No symptoms N=2361	Past N=348	Recurrent N=206	Current Only N=133	p-value	Missingness %
Total CES-D score	13.781 (9.380)	39.707 (10.282)	66.238 (17.667)	36.361 (8.574)	<0.001	23.2
HCC (pg/mg)	15.962 (71.082)	17.119 (63.292)	11.421 (28.797)	33.189 (115.824)	0.035	0
Antidepressant use					<0.001	7.8
No antidepressant use	2255 (95.8%)	297 (85.8%)	153 (74.3%)	117 (88.0%)		
Past	40 (1.7%)	15 (4.3%)	18 (8.7%)	3 (2.3%)		
Recurrent	35 (1.5%)	24 (6.9%)	27 (13.1%)	6 (4.5%)		
Current	25 (1.1%)	10 (2.9%)	8 (3.9%)	7 (5.3%)		
Maternal separation					0.62	10.2
No	1999 (89.5%)	276 (87.3%)	167 (88.4%)	105 (87.5%)		
Yes	235 (10.5%)	40 (12.7%)	22 (11.6%)	15 (12.5%)		
Age	69.848 (5.703)	69.002 (6.085)	68.933 (5.906)	70.654 (5.716)	0.003	0
Sex					<0.001	0
Female	631 (26.7%)	120 (34.5%)	89 (43.2%)	50 (37.6%)		
Male	1730 (73.3%)	228 (65.5%)	117 (56.8%)	83 (62.4%)		
Ethnicity					<0.001	0.1
Non-white	81 (3.4%)	31 (8.9%)	24 (11.7%)	6 (4.5%)		
White	2279 (96.6%)	317 (91.1%)	182 (88.3%)	127 (95.5%)		
Local steroids					0.24	0
No	2158 (91.4%)	308 (88.5%)	183 (88.8%)	121 (91.0%)		
Yes	203 (8.6%)	40 (11.5%)	23 (11.2%)	12 (9.0%)		
Systemic steroids					0.48	0
No	2318 (98.2%)	339 (97.4%)	200 (97.1%)	129 (97.0%)		
Yes	43 (1.8%)	9 (2.6%)	6 (2.9%)	4 (3.0%)		
Antipsychotic medication					<0.001	0
No	2354 (99.7%)	346 (99.4%)	205 (99.5%)	127 (95.5%)		
Yes	7 (0.3%)	2 (0.6%)	1 (0.5%)	6 (4.5%)		
ACEs	0.263 (0.556)	0.387 (0.650)	0.423 (0.715)	0.347 (0.632)	<0.001	13.1

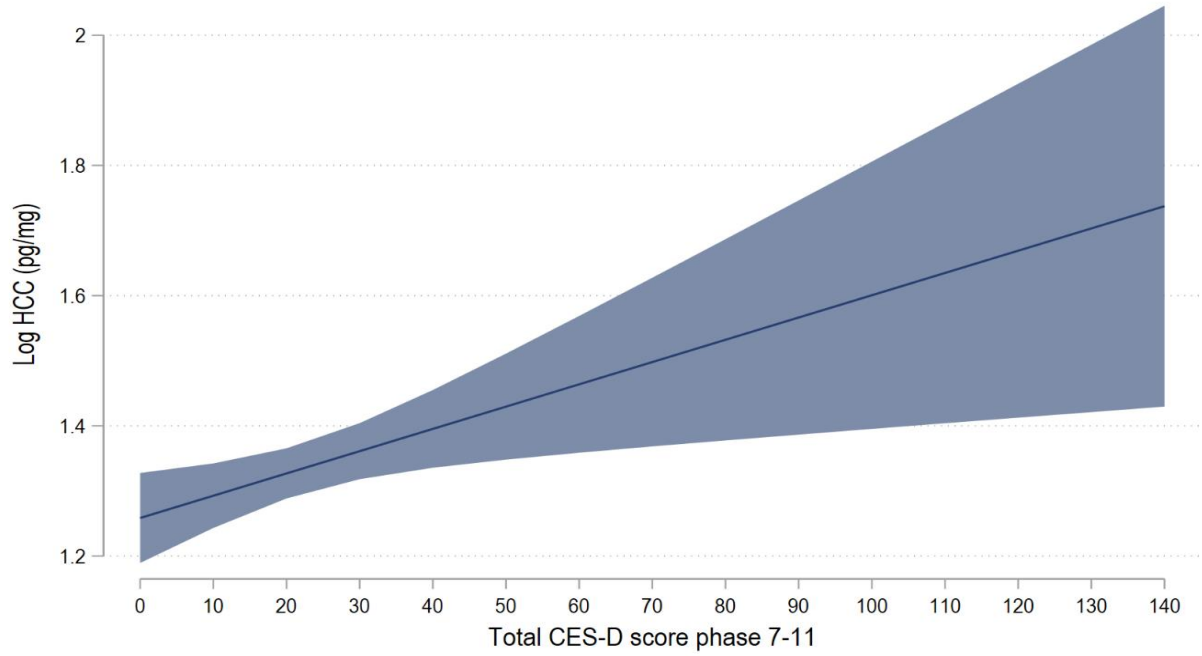
Material disadvantage	1.107 (1.033)	1.187 (1.109)	1.389 (1.116)	1.288 (1.141)	0.002	13.8
Parental warmth	22.272 (4.817)	21.334 (5.136)	20.553 (4.783)	21.062 (4.495)	<0.001	17.7
Parental strictness	9.280 (2.352)	9.559 (2.502)	9.525 (2.545)	9.696 (2.425)	0.06	17
Parental expectations	5.925 (1.441)	5.960 (1.528)	5.656 (1.529)	6.183 (1.274)	0.019	16.9
Smoking status					0.007	5.6
Never/former	2205 (96.2%)	322 (95.5%)	184 (91.1%)	121 (94.5%)		
Current	88 (3.8%)	15 (4.5%)	18 (8.9%)	7 (5.5%)		
Civil service employment grade					<0.001	0
Administrative	1204 (51.0%)	138 (39.7%)	65 (31.6%)	61 (45.9%)		
Professional/executive	997 (42.2%)	172 (49.4%)	108 (52.4%)	56 (42.1%)		
Clerical/support	160 (6.8%)	38 (10.9%)	33 (16.0%)	16 (12.0%)		
BMI	26.589 (4.425)	27.225 (4.512)	26.561 (4.617)	26.510 (4.332)	0.091	0.6
CVD medication					0.057	0
No	977 (41.4%)	133 (38.2%)	84 (40.8%)	40 (30.1%)		
Yes	1384 (58.6%)	215 (61.8%)	122 (59.2%)	93 (69.9%)		
Diabetes[a]					0.018	0
No	2112 (89.5%)	299 (85.9%)	183 (88.8%)	109 (82.0%)		
Yes	249 (10.5%)	49 (14.1%)	23 (11.2%)	24 (18.0%)		

Notes: Mean(SD)/n(%). CES-D = Center for Epidemiologic Studies Depression Scale. HCC = hair cortisol concentrations. [a] Diabetes defined using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006).

4.3.2 Association between depressive symptoms (phases 7-11) and log HCC (pg/mg) at phase 11

Cumulative CES-D scores, measured over phases 7 to 11, were positively associated with log HCC (B=0.003, 95% CI 0.001-0.006, p=0.01) and remained robust to full adjustment. Figure 4.2 shows the predictive margins for log HCC over the range of total CES-D scores across all three phases. No evidence of non-linearity was found.

Figure 4.2: Mean (95% CI) log hair cortisol concentrations (HCC) (pg/mg) by total depressive symptoms, phases 7-11 (n=3969)



Notes: 95% CI shown in the shaded error bars. Model adjusted for age, sex, ethnicity, antidepressant use, local and systemic corticosteroid medication, antipsychotic medication, maternal separation, ACEs, material disadvantage, parental warmth strictness, parental, parental expectations, smoking status, civil service employment grade, BMI, CVD medication, diabetes.

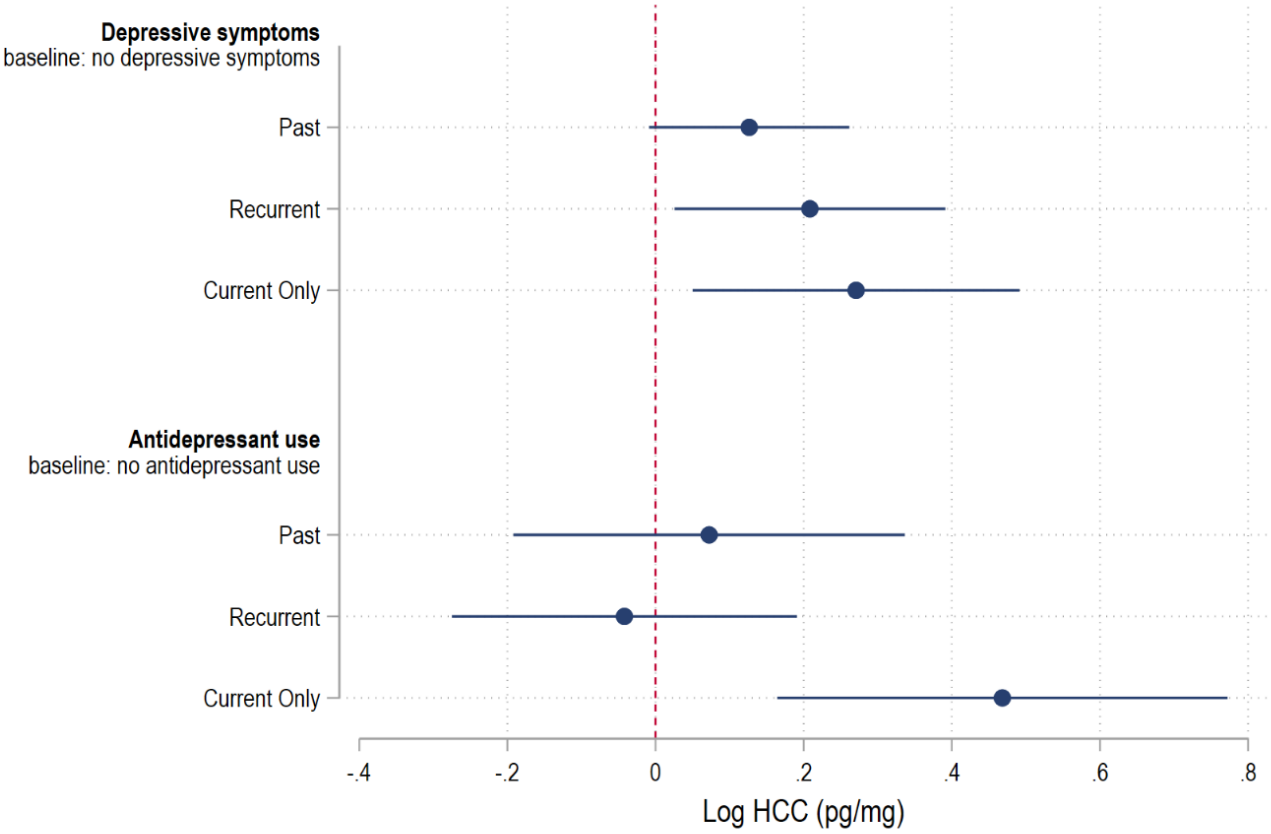
Figure 4.3 displays the associations between depressive symptoms and antidepressant use by recurrent status on log HCC. Participants experiencing past (B=0.152, 95% CI 0.015-0.289, p=0.029), recurrent (B=0.194, 95% CI 0.012-0.376, p=0.037), and current (B=0.283, 95% CI 0.065-0.502, p=0.011) depressive symptoms had higher log HCC compared to participants with symptoms below the CES-D cut-off, after adjusting for age, sex, ethnicity and medication use (Appendix Table 4.B). Estimates remained similar after full adjustment, but the effect of past depressive symptoms was fully attenuated. HCC was 23.2% and 31.1% higher for the recurrent and current only groups, respectively, when compared to participants with depressive symptoms below the CES-D threshold. However, the difference in HCC between recurrent and current symptoms were statistically non-significant. Estimates showing the association between other covariates and HCC are shown in Appendix Table 4. C.

A positive association between current antidepressant use and log HCC was observed (B=0.495, 95 CI 0.188-0.803, p=0.002). In the fully adjusted model, this reflects 59.7% higher HCC, when compared to participants that reported no antidepressant use across the study period. No significant associations were observed for recurrent and past antidepressant use. Similarly, an interaction between depressive symptoms and antidepressant use was not statistically significant.

Compared to their non-separated counterparts, participants reporting maternal separation in childhood had 18.1% higher HCC (95% CI 0.030-0.304, p=0.017). However, the interaction between maternal separation and depressive symptoms was not statistically significant. Further analysis (Appendix Table 4.D) shows that

maternal separation was also not associated with depressive symptoms, measured either continuously or by recurrent status.

Figure 4.3: Coefficient plot showing linear regression estimates (95% CI) of log hair cortisol concentrations (HCC) (pg/mg) (n=3969)

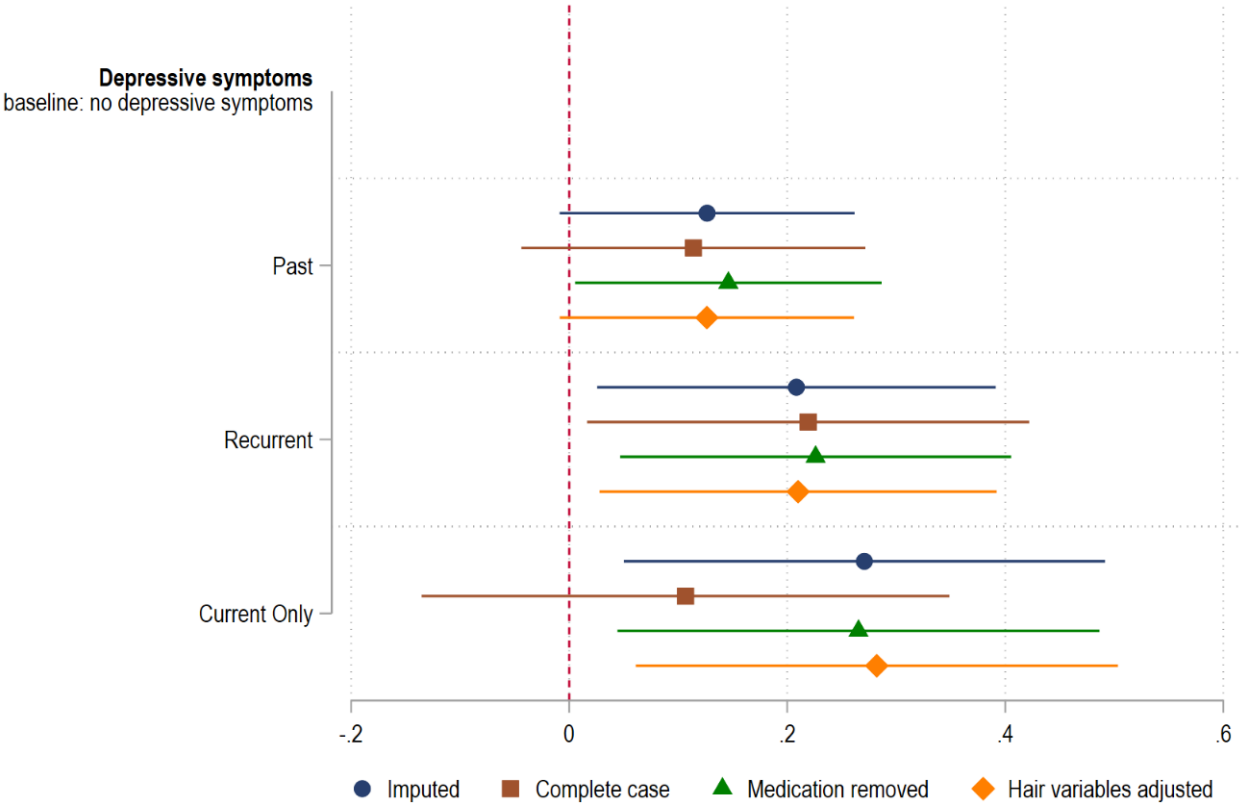


Notes: Model adjusted for age, sex, ethnicity, antidepressants, local and systemic corticosteroid medication, antipsychotic medication, maternal separation, ACEs, material disadvantage, parental warmth, parental strictness, parental expectations, smoking status, civil service employment grade, BMI, CVD medication, diabetes.

4.3.3 Sensitivity analyses

Sensitivity analyses (Figure 4.4) compared estimates from the imputed model to 1) restricting the sample to complete cases, 2) removing participants who reported taking local and systemic corticosteroid and antipsychotic medication, and 3) adjusting for variables from the accompanying hair questionnaire (hair washing frequency, hair colour, hair dyed, hair treatment). Estimates remained similar across all sensitivity analyses, other than in the complete case analysis. Here, the estimate for current versus no depressive symptoms was lower and no longer statistically significant (B=0.107, 95% CI -0.135-0.349, p=0.387). This may be explained by the relatively high levels of missingness in this variable (23.2%). Differences between complete case and imputed values are to be expected when large amounts of missingness is present. This can be a result of poor imputation model choice. However, based on the satisfactory results of the imputation diagnostics run before analysis, it is doubtful that is the case here. Instead, the differences are likely a result of improved statistical power.

Figure 4.4: Sensitivity analyses of linear regression estimates (95% CI) of log hair cortisol concentration (HCC) (pg/mg) (n=3969)



Notes: Each model adjusts for age, sex, ethnicity, antidepressants, local and systemic corticosteroid medication, antipsychotic medication, maternal separation, ACEs, material disadvantage, parental warmth, parental strictness, parental expectations, smoking status, civil service employment grade, BMI, CVD medication, diabetes. The model with medication removed refers to local and systemic corticosteroids and antipsychotic medication. Hair variables include hair washing frequency, hair colour, hair dyed, hair treatment.

4.4 Discussion:

In this study, we found that total depressive symptoms, measured over a 10-year period, were associated with higher HCC in late adulthood. This association with HCC was present for recurrent and current, but not past, depressive symptoms. The difference in HCC between the recurrent and current groups was not statistically significant. A positive association was also observed for current use of antidepressant medication, but not recurrent or past use. Lastly, we found that depressive symptoms and maternal separation appear to be independently associated with HCC in older age and found no evidence for an interaction effect between them.

Our finding of a positive association between depressive symptoms and HCC are in line with some studies (Dettenborn et al., 2012; Duncko et al., 2019; Iob et al., 2019; Song et al., 2019; Wei et al., 2015), but not others (Gerber et al., 2013; Herane-Vives et al., 2018; Pochigaeva et al., 2017; Wells et al., 2014). Few studies have examined the effect of repeat measures of depressive symptoms on HCC. This may partly explain why our findings differ from some of the literature. Furthermore, we focused our attention on depressive symptoms in late adulthood, whereas most studies included a broader range of ages.

Wei et al. (2015) and Duncko et al. (2019) each reported a positive association between first-episodic and current depression and HCC, respectively. However, neither found evidence for a difference in HCC between recurrently depressed patients and controls. In contrast, we found that participants with recurrent depressive symptoms had higher HCC, compared to participants with symptoms below the CES-D threshold at all 3 phases. The reasons for this discrepancy are

unclear but may relate to statistical power, as our study benefits from a sample size that is much larger than these two studies. This is supported by the fact that the direction of effect for recurrent depression reported by Wei et al. (2015) and Duncko et al. (2019) is the same as is reported here. Gerritsen et al. (2019) saw no differences in HCC between depressed patients in remission and healthy controls. Wei et al. (2015) similarly failed to see any differences between first-episodic, recurrent, and healthy controls before the onset of a depressive episode. These findings correspond with our failure to find an association between participants experiencing past depressive symptoms, compared to controls. This may suggest that HPA axis activity becomes hyperactive only during spells of depressive symptoms. However, further research is needed to confirm this. Studies using salivary cortisol have also been inconclusive. Differences in cortisol between remitted patients versus healthy controls have been observed in some studies (Beluche et al., 2009; Bhagwagar et al., 2003; Lok et al., 2012; Vreeburg et al., 2009), but not others (Høifødt et al., 2019; Lange et al., 2013).

Current, but not recurrent and past, use of antidepressants, were associated with higher HCC, compared with participants that reported no use of antidepressants over the study period. This finding accords with other studies on HCC (Song et al., 2019; Wells et al., 2014), but is in contrast with the general assumption that antidepressants regulate HPA axis activity (Mason and Pariante, 2006). Gerritsen et al. (2019) found higher HCC for SSRI antidepressants, but not tricyclics or other classes. We were unable to identify the types of antidepressants used by participants in this study. There is some evidence that the effects of antidepressants on HPA axis activity may differ by the class of antidepressants used (Manthey et al., 2011). However, these differences are likely restricted to acute effects. Instead, the

literature shows fairly consistent reductions in cortisol following long-term antidepressant treatment (Sarubin et al., 2014). This is thought to occur as a result of an upregulation of the mineralocorticoid (MR) and glucocorticoid (GR) receptors (Mason and Pariante, 2006), although a reduction in the severity of symptoms may also play a role (Subramaniam et al., 2019). To further assess the role of antidepressants on HCC, prospective associations are required. This study design is employed by Song et al. (2019) with a follow-up period of 1 month. Future studies would benefit from an increased follow-up period.

Early life stress has previously been proposed as playing an important role in the relationship between cortisol and depression. Rather than a consequence, it has been suggested that HPA axis hyperactivity may be a risk factor for depression, stemming from adverse events in childhood (Baumeister et al., 2016). In this study, we failed to observe such a link. Participants that experienced maternal separation during childhood had higher HCC compared to their non-separated counterparts, as was previously confirmed (Bevan and Kumari, 2021). This effect was independent of depressive symptoms measured over a 10-year period, and an interaction effect between them was statistically non-significant. Moreover, maternal separation in childhood was not associated with past or current/recurrent depressive symptoms in later life. Few studies have examined the interaction between early life and depression on HCC, but our findings of a null effect accord with the existing literature (Cantave et al., 2022; Duncko et al., 2019; Hinkelmann et al., 2013; Oresta et al., 2021).

A strength of this study is that we had repeat measures of depressive symptoms and antidepressant use, allowing us to examine the effects of depressive symptoms and medications over time. Moreover, we use data from an occupational cohort with a

relatively large sample size. As a result, we were able to show that the effect of depressive symptoms on HCC is not restricted to clinical samples. However, it is important to note that Whitehall II is a relatively advantaged and healthy cohort and therefore, is not representative of the general population. Previous research in this cohort has shown that restricting the samples to participants with valid hair samples slightly exacerbates these issues of representativeness (Abell et al., 2016b; Bevan and Kumari, 2021).

Measuring cortisol using hair has the benefit of providing insight into long-term HPA axis activity. However, our study was limited to HCC measured at a single time point. Repeat measures of HCC and depressive symptoms is required to see whether a) this association persists over time, and b) whether cortisol remains raised in participants once depressive symptoms subside. Similarly, the period captured by HCC (approximately 3 months) and depressive symptoms (within the past week) makes it difficult to tell how the two measures correspond with one another over time.

In this paper, we focus on depressive symptoms, measured using the CES-D scale. While this self-report measure has been shown to have good utility as a screening tool for depression, including in older adults (Park and Lee, 2021), it is not suitable as a diagnostic tool (Balsamo et al., 2018). Moreover, as depression commonly has an onset during adolescence or early adulthood, we lacked information on depressive history and were thus unable to create life course trajectories of depressive symptoms.

Depression is a highly heterogeneous disorder. We were able to account for some of this variability by examining symptoms experienced recurrently throughout the study

period. However, given the relatively long follow-up periods in this study (4 to 5 years), we were unable to assess the stability of depressive symptoms in between phases. This information may have provided further insight into the recurrent nature of symptoms experienced by some participants. Similarly, we lacked information on the duration of symptoms at each follow-up period. The CES-D scale measures depressive symptoms over the past week, although it is likely that some participants would have experienced symptoms for much longer than this. Depressive episodes can persist from weeks to months, and, in some cases, even years (Andrews et al., 2007). These figures serve as a useful reference, but it is important to note that a CES-D score above the cut-off does not necessarily indicate a depressive episode. Although we observed a positive association between depressive symptoms and HCC, we were unable to determine whether elevated cortisol preceded symptoms. In this cohort, hair samples and CES-D were measured within a relatively short amount of time from one another; approximately 1 to 2 weeks. The CES-D scale aims to measure current depressive symptoms (in the past week). Hair cortisol captures cortisol output over a 3-month period. It may, therefore, seem likely that cortisol was raised for at least some participants before symptoms began. However, without more detailed information, this remains speculative. The lack of association between past depressive symptoms and HCC that we found suggests that if cortisol levels are raised before symptoms begin this may occur only shortly before their onset. Future research should focus on prospective associations to provide further insight into this issue.

Other sources of potential variability include depressive subtypes, such as melancholic and atypical depression, and comorbidity with symptoms of anxiety. We were unable to adjust for these. Higher salivary and serum cortisol levels have been

reported in individuals with melancholic depression (Nandam et al., 2020), but Herane-Vives et al. (2020) failed to see a similar association in hair. The effect of comorbid depression and anxiety on HCC is also inconclusive. Steudte-Schmiedgen et al. (2017) saw no association for this sub-group. In contrast, Gerritsen et al. (2019) found higher HCC for comorbid depression and anxiety, but no associations for either disorder on their own.

The interaction effect between maternal separation and depressive symptoms was not statistically significant. However, given the low occurrence of separation in this cohort, we may have lacked the statistical power to detect an effect, even after missing values were imputed. This also applies to the interaction between antidepressant use and depressive symptoms.

We focus on the relationship between depressive symptoms and HCC in late adulthood when participants were aged 70 years on average. Depression shares a complex relationship with dementia in this age group; partly due to the long preclinical phase of dementia (Sperling et al., 2014). We were unable to identify participants with a diagnosis of dementia in this cohort. It is difficult to speculate what effect this may have had on our findings. Depression has been posited as both a risk factor and a prodromal feature of dementia (Wiels et al., 2020). Bennett and Thomas (2014) suggest that convincing evidence exists for both hypotheses, and it is likely both are valid. The difference may depend on the age at which symptoms occur. Whereas early-to-midlife depression may represent a risk factor for dementia, depression in later life, particularly the late-onset of symptoms, is more likely to be a prodrome. The HPA axis has also been implicated in dementia and cognitive impairment. Udeh-Momoh et al. (2019) found that individuals with both high cerebrospinal fluid cortisol levels and amyloid-β abnormalities (a hallmark of

Alzheimer's disease) had an increased risk of clinical progression in Alzheimer's disease (AD). Similarly, Ennis et al. (2017) found that AD risk was predicted by repeat measures of urinary cortisol. Both studies report that cortisol and depressive symptoms were independently associated with AD. It is not yet clear whether HPA axis dysregulation is a causal factor or consequence of dementia pathology (Zheng et al., 2020). However, studies suggest that the relationship exists beyond the presence of a depressive disorder (Ouanes and Popp, 2019). Therefore, we speculate that the association between depressive symptoms and HCC seen in this study is unlikely to be entirely confounded by the presence of dementia among some of the participants.

Lastly, depressive symptoms in old age may be partially confounded by the presence of physical disorders. This is particularly relevant for CES-D items focused on somatic complaints (Balsamo et al., 2018). We adjusted the analyses for health status by diabetes, CVD medication, and BMI. However, other physical disorders or conditions may also impact upon these.

In conclusion, we find that depressive symptoms measured over a 10-year period were associated with higher HCC in late adulthood. Positive associations with HCC were observed for current and recurrent, but not past, depressive symptoms, and these were not explained by the presence of maternal separation in childhood. These findings contribute towards the growing literature on HCC in depressive disorders. Future research should focus on prospective associations.

5 Discussion

The overall aim of this thesis was to examine the associations between maternal separation in childhood, cortisol, and depressive symptoms in late adulthood. The specific research aims were as follows:

- *Aim 1: Examine the association between maternal separation in childhood and cortisol, measured in saliva and hair, in late adulthood.*
- *Aim 2: Examine the association between maternal separation in childhood and depressive symptoms in late adulthood.*
- *Aim 3: Examine the association between depressive symptoms and cortisol in late adulthood.*
- *Aim 4: Investigate the interplay between maternal separation in childhood, depressive symptoms and cortisol in late adulthood.*

The findings from this thesis contribute to the literature on early life stress and cortisol and build upon existing knowledge about the relationship between depressive symptoms and HCC. The section below provides an overview of the main findings, a discussion of the strengths and limitations, and overall conclusions, policy implications, and recommendations for future research.

5.1 Main findings:

5.1.1 Maternal separation was consistently associated with cortisol in saliva and hair

The first research aim of this thesis was to examine the association between maternal separation in childhood and cortisol in late adulthood. Maternal separation in childhood was consistently associated with cortisol, using phases of data

measured ten years apart, and two types of cortisol measures: saliva and hair. In chapter 2, maternal separation was associated with a flatter diurnal cortisol slope at phase 7. Associations between separation and the CAR and AUCg were not significant. This replicates the finding by Kumari et al. (2013) in the same cohort but using a different analytic sample and extends the analysis by considering the effects of AUCg.

At phase 9, maternal separation was not associated with salivary cortisol (CAR, AUCg, diurnal slope). The diurnal slopes of the separated and non-separated groups became flatter, as is expected with age (Adam et al., 2006; Heaney et al., 2012), but the separated group showed a lower degree of change in their slopes from baseline to follow-up. To my knowledge, this is the first study to examine the effects of separation in childhood on a change in adult cortisol. It is not entirely clear why an association was observed at phase 7 but not 9. In the discussion section of chapter 2, I proposed two explanations. The first suggested that the effect of separation on adult cortisol may no longer be present as the participants become older. This is similar to the age-as-a-leveller hypothesis, which reports a narrowing of health inequalities with age (House et al., 2005). The second explanation highlighted the issue of low stability in salivary cortisol. Previous research has recommended multiple days of saliva samples to accurately measure the diurnal slope (Segerstrom et al., 2014). However, in Whitehall II, saliva samples were collected on a single day. It seems unlikely, however, that this would explain differences at the group level.

In chapter 3, a novel finding of higher HCC in participants reporting maternal separation was reported at phase 11 of the study when participants were aged 70 years, on average. This brings into question my explanation for the lack of association at phase 9. Although it remains possible that the effect of separation on

cortisol may subside and then re-emerge, this is speculative, and there are no clear explanations for why this would occur. The analyses in chapters 2 and 3 differed in terms of the analytic samples and measures of cortisol (saliva and hair). This helped to substantiate the findings as it shows the association between maternal separation and cortisol was not limited to a particular subset of participants or type of cortisol measure. However, this also makes comparisons between the findings difficult.

Previous research has suggested that the relationship between separation in childhood and adult cortisol patterns may be explained by factors occurring over the life course, such as parent-child relationships (Luecken, 2000), childhood maltreatment (Luecken and Appelhans, 2006), health behaviours, and marital status (Kumari et al., 2013). In this thesis, I adjusted for a range of similar covariates in the analyses, along with measures of health status and medication use. The effect of maternal separation on cortisol remained robust to adjustment, and the coefficients were only marginally attenuated. Other factors may similarly explain this relationship, but due to data availability, I was unable to examine them. For instance, Young et al. (2019) found that early life stress interacted with current stress to predict diurnal cortisol slopes in adulthood. Other studies have explored the role of DNA methylation in GR genes as a mechanism, but such data was unavailable (Liu and Nusslock, 2017; Tyrka et al., 2016).

Similarly, studies have suggested that the effect of separation on cortisol may differ by timing and separation type. This was tested by examining the association of age at onset and reason separation occurred in a subset of participants that had reported separation during childhood, but neither were significant. Low statistical power may have affected the likelihood of detecting an effect here.

5.1.2 Depressive symptoms and antidepressant use were associated with cortisol in hair but not saliva

The third research aim of this thesis was to examine the association between depressive symptoms and cortisol in late adulthood. Measures of depression and antidepressant use were associated with HCC at phase 11 but not in salivary cortisol at phase 7 or 9. In chapter 3, a combined measure of depressive symptoms (CES-D) and antidepressant use was cross-sectionally associated with HCC. This analysis was extended in chapter 4 by examining the effect of long-term depressive symptoms and antidepressant use on HCC, measured over phases 7 to 11. The findings showed that current and recurrent, but not past, depressive symptoms were associated with higher HCC.

Similarly, current, but not recurrent or past, antidepressant use was positively associated with HCC. Few studies have examined depression in HCC, so the findings from this chapter contribute to existing knowledge on this topic. Moreover, having repeat measures of depressive symptoms allowed me to distinguish between participants reporting current, recurrent and past symptoms.

Previous research has confirmed an association between depression and salivary cortisol (Belvederi Murri et al., 2014), but not in Whitehall II (Kumari et al., 2013, 2011). Two reasons may explain why an association between depressive symptoms and salivary cortisol was not found. First, it is possible that the association only emerges in later phases of Whitehall II. This could be because either the effect is masked at phases 7 and 9 due a suppressor effect from a third variable or low statistical power. Summary statistics show that rates of depressive symptoms were higher at phase 11 compared to phases 7 and 9. Second, the lack of association with saliva, but not hair, may be due to issues regarding the stability of salivary

cortisol. As previously discussed, studies have shown high intra-individual variability in salivary cortisol (Ross et al., 2014). Similarly, the CES-D scale is designed to measure current depressive symptoms and is sensitive to acute changes (Radloff, 1977; Smarr and Keefer, 2011). The combined variability in both measures may have decreased the chances of detecting an effect. In fact, some studies have suggested that increased variability may reflect a core feature of the effect that depression has on the HPA axis (Gex-Fabry et al., 2012; Peeters et al., 2004; Sannes et al., 2016, 2013). This provides an interesting perspective that may help explain why an association was not seen in saliva. However, it is important to note that hypercortisolism has been a common finding in many studies on depression and salivary cortisol (Stetler and Miller, 2011). Similarly, the finding of higher HCC in chapter 4 would suggest that cortisol levels were at least stable enough in this cohort to produce this result. Still, because hair and saliva were measured at distinct phases of Whitehall II, it is difficult to compare the results directly.

5.1.3 Maternal separation and depressive symptoms were independently associated with cortisol in hair

Research suggests that HPA axis dysregulation may be a risk factor for depression, which may be programmed by early life events (Pariante and Lightman, 2008). The evidence for this, however, remains mixed. Some studies have found evidence for a mediating role of cortisol in the relationship between early life stress and depression (Iob et al., 2021; Ju et al., 2020), whereas others have found support for moderation effects (Cantave et al., 2019; Goldman-Mellor et al., 2012; Harkness et al., 2011). This thesis sought to examine this potential relationship further, first by establishing whether maternal separation in childhood was associated with adult depressive symptoms (Aim 2) and then by examining the interrelationship between these variables and cortisol in late adulthood (Aim 4).

Currently, only a few studies have examined the combined effects of early life stress and depression on HCC, and they failed to find evidence for an interaction effect (Cantave et al., 2022; Duncko et al., 2019; Hinkelmann et al., 2013; Oresta et al., 2021). The findings from chapter 4 accord with these studies. Instead, maternal separation in childhood and depressive symptoms were independently associated with HCC in late adulthood. Furthermore, maternal separation was not associated with depressive symptoms at any phase. The fourth aim of this thesis was to explore the potential mediating or moderating role of cortisol in the relationship between maternal separation and depressive symptoms. However, the findings do not support this. Other studies have reported an association between separation during childhood and adult depression (SIMBI et al., 2020), including late adulthood (Pesonen et al., 2007). Further research is needed to see whether the lack of association is specific to this cohort.

The broader implications of these findings need to be considered. The positive association between maternal separation in childhood and HCC at phase 11 suggests that early life events may impact on the HPA axis decades later. Furthermore, this association was not explained by childhood conditions, socioeconomic factors, health behaviours, or health status. It is possible, therefore, that early life events may lead to chronic dysregulation of the HPA axis that persists throughout the life course. This is consistent with the critical or sensitive periods model from the life course approach. Within this framework, a distinction is made between critical and sensitive periods. A critical period refers to a specific period in which an exposure can only impact an outcome during that time. A sensitive period instead suggests the effect may just be stronger during this period (Kuh et al., 2003). However, due to the lack of association between age at onset and HCC, it is difficult to determine whether maternal separation best reflects a critical or sensitive period in this context.

Furthermore, the mechanisms linking maternal separation to HPA axis dysregulation have yet to be fully developed. It needs to be clarified whether there is something specific to the experience of separation in childhood that impacts the HPA axis, such as a loss of parental buffering (Gunnar and Hostinar, 2015), or whether it simply reflects a stressful period. The first explanation would sit in line with the definition of a critical period by Kuh et al. (2003), as it would be assumed that the effects of separation were specific to the childhood period where parents have a buffering effect on their child's stress response. However, if separation just reflects a stressful experience, then it would be best characterised as a sensitive period, given what we know about the sensitivity of the HPA axis to chronic stress during childhood (Agorastos et al., 2019).

Evidence was also found for higher HCC in depressive symptoms, but only when symptoms were ongoing. The association for past depressive symptoms was not statistically significant. Two other studies using cortisol in hair also reported a lack of association. Gerritsen et al. (2019) found no differences in HCC between remitted depressed patients versus healthy controls, and Wei et al. (2015) saw no differences in HCC between groups before the onset of a depressive episode. Mixed findings have been reported in salivary cortisol.

In contrast to Wei et al. (2015), Lok et al. (2012) found that salivary cortisol in patients with recurrent depression was consistently higher than in controls over a 2-year follow-up period and did not change during depressive episodes. Differences in salivary cortisol between remitted patients and healthy controls have also been reported in other studies (Aubry et al., 2010; Beluche et al., 2009), whilst others have found no difference (Høifødt et al., 2019; Lange et al., 2013). Despite the extensive research on depression and cortisol, it is still not clear whether HPA axis abnormalities precede depressive episodes. In a recent meta-analysis focusing on prospective biomarkers of depression, Kennis et al. (2019) report that cortisol predicted the onset, relapse, and recurrence of MDD, but this effect was attenuated after adjusting for baseline clinical diagnosis and methodological quality. This may, however, depend on the age of the participants. Focusing specifically on adolescence, Zajkowska et al. (2022) show that elevated morning cortisol is prospectively associated with the development of MDD, but the cross-sectional association in adolescents was not significant between individuals with MDD and controls. Further research using prospective data is required, particularly with cortisol in hair.

5.2 Strengths and limitations:

5.2.1 The Whitehall II sample

The data used in this thesis was exclusively from the Whitehall II study, which offered several strengths to the analyses. First, Whitehall II has a relatively large sample size and thus provided the statistical power to examine a range of questions focusing on sub-samples and interaction effects. Second, it contains extensive information on childhood, demographic, socioeconomic, and health factors. As a result, the analyses were able to adjust for a wide range of suspected confounders and mediators. Furthermore, many of these variables have been collected longitudinally. This enabled the analysis in chapter 4 to examine the effect of long-term depressive symptoms and antidepressant use on HCC rather than being limited to cross-sectional analysis. Third, Whitehall II has collected repeat biomarker measures, including cortisol in saliva (phases 7 and 9) and hair (phase 11). This helped to further validate the findings from chapters 2 and 3 by showing that the association between maternal separation in childhood and cortisol remained consistent over different phases of Whitehall II and between cortisol measured using different specimen types.

Because Whitehall II is an occupational cohort, the findings cannot be generalised to the wider population. Instead, the study reflects the demographics and characteristics of the British civil service in the mid-1980s (Marmot and Brunner, 2005). Consequentially, the sample population are relatively advantaged regarding socioeconomic background and health and display lower levels of adverse health behaviours, such as smoking. Females and participants from a non-white ethnic background are also underrepresented. The type of analyses carried out in this

thesis may have exacerbated issues around generalisability and validity in two important ways.

First, the analyses focused on the later phases of Whitehall II (phases 7-11). The data from phases 7 to 9 broadly capture the transition from employment to retirement. Research in Whitehall II has shown improvements in mental health before and shortly after retirement, but no long-term changes were observed (Fleischmann et al., 2020). Similarly, retirement was associated with steeper diurnal cortisol slopes in this cohort, but this was partially patterned by employment grade (Chandola et al., 2018). To account for this, the analysis in chapter 2 adjusted for both civil service employment grade and employment status. By phase 11, the majority of the sample was retired. Employment grade was also adjusted in chapters 3 and 4 but was not significantly associated with HCC. A focus on later phases of the study may also further impact the analyses by attrition bias. Chapter 3 showed that the proportion of participants reporting maternal separation in childhood was lower in the analytic sample at phase 11 compared to participants at phase 5 when childhood variables were first recorded. Research on attrition in Whitehall II has shown an increased risk of mortality in participants lost to attrition compared to responders (Akasaki et al., 2020). However, predictors of attrition are complex. Mein et al. (2012) found that participants with long-term health problems were more likely to remain in the Whitehall II study.

The other way validity and generalisability may have been further impacted was by restricting the analytic samples to participants who were present for the clinical screening and provided valid cortisol samples. The effect of this can be seen in chapters 2 and 3. Again, the proportion of participants reporting maternal separation in the analytic samples was lower than in the excluded groups. Furthermore,

participants in the analytic samples displayed better health (CVD medication, diabetes, depression), lower rates of smoking, and were more likely to be married and in the highest employment grade. A lack of attenuation in the effect of maternal separation on cortisol was observed after adjusting for these variables, which may partly explain why. It is plausible that participants in the analytic sample experienced maternal separation and depressive symptoms differently from those who were either lost to follow-up or were not eligible for recruitment into the cohort. However, it is difficult to estimate the overall impact this may have had on the estimates. Occupational cohorts are subject to a healthy worker effect (Chowdhury et al., 2017). The samples used in the analyses not only consist of participants that were selected into the initial cohort based on employment with the civil service but also remained in the cohort until later phases. Together, this may have biased the estimates downwards, suggesting that maternal separation and depressive symptoms have a relatively strong effect on cortisol. Alternatively, the advantaged characteristics of this cohort may have made it more likely to detect an effect. Räikkönen et al. (2011) found an increased risk of mental health disorders in adulthood for individuals evacuated during the Second World War and from an upper socioeconomic background. In contrast, individuals from lower socioeconomic backgrounds showed an increased risk, regardless of whether they were evacuated. Therefore, it is recommended that these analyses are repeated in other sample populations to see if the associations remain the same.

Analyses were also affected by missingness. To address this, MICE was employed in chapters 3 and 4. In the former, the results remained broadly similar when comparing the findings between the imputed and complete case analysis. In the latter, differences in the effect of depressive symptoms on HCC were observed. One

of the benefits of multiple imputation, aside from limiting bias, is that it increases statistical power. This may have led to the differences observed in chapter 4. However, despite imputation, statistical power was likely still too low to detect an effect in some of the analyses performed on subsets of the sample. In chapter 3, for example, no association was observed for the age and reason for separation, but the overall numbers of participants reporting separation was already low. Similarly, in chapter 4, interaction effects between depressive symptoms and maternal separation and antidepressant use were not statistically significant. Low power may have also affected these results.

5.2.2 Maternal separation and childhood conditions

Most studies examining separation in childhood have focused on a specific type of separation, such as evacuation or parent loss. Instead, Whitehall II asked participants to recall whether they were ever separated from their mother for at least one year in childhood and then asked them to provide the reason this occurred. In addition to increasing statistical power, this allowed me to analyse the effects of separation in childhood on adult cortisol more broadly. The measures of maternal separation and other childhood conditions rely on retrospective self-reports. Bias arising from such measures has been well documented in the literature (Hardt and Rutter, 2004). This needs to be considered for all the childhood measures and may help explain why I failed to see an association between ACEs, material disadvantage, and parenting dimensions with cortisol in saliva and hair. However, the risk of bias may be less severe for maternal separation, as research has shown strong agreement between prospective and retrospective reports of separation in childhood (Reuben et al., 2016). Another limitation of this measure in Whitehall II is

that information was collected only on separation from mothers. However, despite this, some types of separation would have inevitably involved separation from both parents, such as evacuation. Similarly, the measure captures reports of separation for at least one year, but the exact duration was not recorded. Again, some types of separation would have resulted in a permanent loss, such as death.

A focus on single adversities has been criticised in the literature for failing to consider the co-occurrence of adversities (Kuhlman et al., 2017; Lacey and Minnis, 2020). Cumulative approaches, however, tend to treat adversity as a homogenous construct. Summary statistics in chapters 2 and 3 showed that participants who reported separation were more likely to report higher ACEs but the latter was not associated with cortisol in either saliva or hair. I argue that this demonstrates that there is sometimes value in examining the associations between early life events and biological markers separately. Although events may co-occur, this does not imply that they are associated with biomarkers in the same way (Lacey et al., 2020). Taking this into account, a dimension-based approach to measuring adversities has recently been advocated in the literature (McLaughlin and Sheridan, 2016). These are often constructed using latent-class modelling. Although these methods were not employed in this thesis, the measure of maternal separation used here bears a strong resemblance to dimensions of loss identified in other studies (Iob et al., 2020; Lacey et al., 2020).

Despite this, separation is unlikely to impact the HPA axis in isolation and instead is shaped by circumstances that occur in the pre-and post-separation environment. Additionally, these circumstances may differ depending on the type of separation experienced (Humphreys, 2018). No significant differences were found between the reasons for separation, but as previously discussed, low statistical power may have

impacted this. Studies have suggested that the effect of separation on cortisol may depend on the levels of care that follow (Luecken, 2000; Luecken and Appelhans, 2006; Tyrka et al., 2008a). To try and account for this, I adjusted for parental warmth, strictness and expectations. These were not associated with cortisol in saliva or hair and did not attenuate the effects of separation. However, it is worth noting that participants were asked these questions relating to childhood in general; thus they do not necessarily relate to the post-separation environment.

Attention has also been placed on the role that material conditions may play. In a recent study, Lacey et al. (2022) found that poverty was associated with both individual and clusters of adversities, other than the death of a close family member. Similarly, summary statistics in chapters 2 and 3 show that material disadvantage in childhood was higher in participants who reported maternal separation. This was adjusted for in all analyses but was not associated with cortisol. While it is possible that childhood material disadvantage may not influence cortisol in late adulthood, it is important to highlight the limitations of this variable. Material disadvantage was measured using a four-item scale, consisting of self-reports of the following: family had continuing financial problems, did not have an inside toilet, father/mother were unemployed when they wanted to be working, household did not own a car. In addition to issues regarding retrospective bias, the age of the participants and the year that data was collected must considered. Childhood variables were measured at phase 5 (1997-1999) in Whitehall II when participants were aged 56 years, on average. Therefore, items such as not owning a car may not accurately reflect material disadvantage, particularly for participants growing up in urban areas. Although a range of childhood conditions was adjusted for, residual confounding cannot be ruled out.

5.2.3 Depressive symptoms

Depressive symptoms were collected at phases 7, 9 and 11. This enabled the analysis in chapter 4 to examine the association between current, recurrent and past depressive symptoms with HCC. Few studies have had the data to conduct this type of analysis. However, information on depressive history was not available. As a result, it was not possible to determine whether participants reporting depressive symptoms at phase 11 represented cases of late-onset depression. This type of depression has previously been linked with dementia (Bennett and Thomas, 2014). Research has shown that both depression and cortisol share a complex relationship with dementia (Ouanes and Popp, 2019). Due to a lack of available data, it was not possible to identify participants with a dementia diagnosis. However, studies have shown depression and dementia to be independently associated with cortisol (Ennis et al., 2017; Udeh-Momoh et al., 2019), and therefore I would speculate that it is unlikely that dementia would completely confound the associations observed here. Rates of dementia in the Whitehall II study are also relatively low – approximately 5% over a 10-year period in participants who underwent clinical screening at phase 9 - (Singh-Manoux et al., 2022), and so its effects on the association between depressive symptoms and cortisol are likely limited.

Depressive symptoms were measured using the CES-D scale. This has been shown to have good screening potential in older adults (Park and Lee, 2021) and has been validated against the CIS-R in the Whitehall II cohort (Head et al., 2013). However, CES-D is not suited for identifying cases of MDD (Balsamo et al., 2018).

5.2.4 Measures of cortisol

This thesis benefited from having repeat measures of salivary cortisol and a measure of cortisol in hair. As a result, I was able to investigate whether the effects of maternal separation and depressive symptoms persisted over time and across different measures of cortisol. However, the limitations of these measures must be considered. As previously discussed, salivary cortisol has been shown to have low stability (Ross et al., 2014). Salivary cortisol was collected on a single day at phases 7 and 9, but studies have recommended multiple days of measurement (Segerstrom et al., 2014). Having salivary cortisol measured at two phases allowed the analysis in chapter 2 to examine whether the change in diurnal cortisol slope differed by separation status. However, the repeat measures were not enough to create trajectories of diurnal cortisol patterns. Cortisol measured in hair has many benefits over saliva, serum, and urine because it is not affected by the same environmental and situational influences and provides a measure of chronic HPA axis activity (Stalder et al., 2012). However, Whitehall II currently contains hair samples measured at only one phase, and this was measured at a different time than saliva. Therefore, it was difficult to compare the results between the two measures. Some participants reported depressive symptoms at phase 11 but not phase 9. If hair had been collected during this phase, the analysis in chapter 4 could have been extended by examining the association in the reverse direction. The analyses using both saliva and hair focused on basal cortisol levels. Studies have shown that cortisol stress reactivity may also play an important role in the association between the HPA axis and depression (Burke et al., 2005; Zorn et al., 2017). However, this type of data was not available for this thesis.

5.3 Conclusions, policy implications, and future research:

The aim of this thesis was to examine the associations between maternal separation, cortisol, and depressive symptoms. The findings show maternal separation in childhood was associated with higher cortisol levels in saliva and hair. Recently, attention has focused on the impact of family separation as a result of immigration policies (Stange and Stark, 2019; Wood, 2018). Although this type of separation was not a focus of this thesis, the findings highlight the potential risks to health that separation in childhood can have. However, these findings must be approached with caution, particularly in a policy context. It is difficult to move from population-level research to policy recommendations directed at the individual, partly due to the substantial variation in outcomes (Kelly-Irving and Delpierre, 2019). Although separation may have a negative effect in some cases, it is important to highlight that there are instances where separation is essential for the overall wellbeing and safety of a child (Humphreys, 2018). The data used in this thesis are from an occupational cohort with relatively low reports of separation. Future research should aim to examine these associations in more representative cohorts. Emphasis should also be placed upon the pre- and post-separation environment to understand how they shape the experience and consequences of separation events.

The findings also contribute towards the literature on cortisol and depression. While extensive research on cortisol in saliva and serum has been conducted, few studies have examined the association in hair. Depressive symptoms were shown to be associated with higher HCC, but no association was found for past symptoms. Future research should aim to examine prospective associations, particularly in cortisol measured in hair, to build a better picture of HPA axis activity in between depressive spells. Additionally, depressive symptoms were operationalised as a

unidimensional construct. Further research may provide greater insight by examining symptoms either as clusters or individually (Fried and Nesse, 2015).

Finally, maternal separation in childhood and depressive symptoms were found to be independently associated with cortisol, and the evidence did not support mediation or moderation. Analyses were carried out using cortisol measured in both saliva and hair, but comparisons were difficult due to measurements existing at different phases of data collection. Future research should aim to collect measures of saliva and hair at the same time to understand if and how these exposures relate to different aspects of the HPA axis. This thesis focused on cortisol in late adulthood. However, future research should examine the role of early life stress in the relationship between depression and cortisol in different age groups to see if alternative patterns emerge.

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Appendices to Chapter 1

Appendix 1.A: Retrospective childhood measures collected at phase 5 (1997-1999) of Whitehall II study

This section is about influences in your early life and the whole of your childhood up to when your were aged 16.

5.17 a. Were you ever separated from your mother for a year or more as a child (that is, up until you were 16)? TMATSEP

Yes No

b. What age were you when you were first separated from your mother for at least a year? TMATSEPA

years old

c. Why did the separation happen? TMATSEPR

Parents separated/ divorced | Mother died | Mother ill | Adoption | Evacuation | Other reason

d. Did any of the following things happen during your childhood (that is, up until you were 16)?

TCHHOS4W	You spent 4 or more weeks in hospital	Yes	No
TCHDIVOR	Your parents were divorced	Yes	No
TCHUNEMP	Your father/mother were unemployed when they wanted to be working	Yes	No
TCHMIALC	Your parent(s) were mentally ill or drank so often that it caused family problems	Yes	No
TCHABUSE	You were physically abused by someone close to you	Yes	No
TCHARGUE	Your parents very often argued or fought	Yes	No
TCHORPHG	You were in an orphanage/childrens' home	Yes	No

e. Did you experience any of the following circumstances during your childhood (that is, up until you were 16)?

TCHFINPR	Your family had continuing financial problems	Yes	No
TCHOSLOO	Your family/household did not have an inside toilet	Yes	No
TCHCAR	Your family/household owned a car	Yes	No

Appendix 1.B: Retrospective childhood measures collected at phase 5 (1997-1999) of Whitehall II study

5.18 Please show **how you remember your mother** (or the woman who cared for you) during the years you were growing up.

(Please mark one answer on each line)

	A great deal	Quite a lot	A little	Not at all
a. How much did she understand your problems and worries? TMOTUNDE				
b. How much could you confide in her about things that were bothering you? TMOTCONF				
c. How much love and affection did she give you? TMOTLOVE				
d. How much time and attention did she give you when you needed it? TMOTTIME				
e. How strict was she with her rules for you? TMOTSTRI				
f. How harsh was she when she punished you? TMOTHARS				
g. How much did she expect you to do your best in everything you did? TMOTEXBE				

5.19 Please show **how you remember your father** (or the man who cared for you), during the years you were growing up,

If you were brought up in a home without a male parent please go to Question 5.20.

(Please mark one answer on each line.)

	A great deal	Quite a lot	A little	Not at all
a. How much did he understand your problems and worries? TFATUNDE				
b. How much could you confide in him about things that were bothering you? TFATCONF				
c. How much love and affection did he give you? TFATLOVE				
d. How much time and attention did he give you when you needed it? TFATTIME				
e. How strict was he with his rules for you? TFATSTRI				
f. How harsh was he when he punished you? TFATHARS				
g. How much did he expect you to do your best in everything you did? TFATEXBE				

Appendices to Chapter 2

Appendix 2.A: Sensitivity analyses: estimates (95% CI) from linear regression models of cortisol awakening response (CAR) and area under the curve with respect to the ground (AUCg) at baseline and follow-up

	Baseline		Follow-up	
	CAR (n=1980)	AUCg (n=1980)	CAR (n=1736)	AUCg (n=1746)
Model 1[a]	0.424	-0.330	-0.505	-2.602
	(-1.17,2.02)	(-6.78,6.12)	(-2.06,1.05)	(-8.98,3.77)
Model 2[b]	0.558	-1.627	0.568	0.500
	(-1.19,2.30)	(-8.56,5.30)	(-1.15,2.28)	(-6.32,7.32)

Notes: [a] Fully adjusted model using analytic sample. [b] Fully adjusted model but participants on steroid medication and more than 15 minutes late taking cortisol sample 1 were removed.

Appendix 2.B: Sensitivity analyses: estimates (95% CI) from multilevel models of log diurnal cortisol slope (nmol/l) at baseline and follow-up

	Baseline		Follow-up	
	Model 1[a] (n=1980)	Model 2[b] (n=1736)	Model 1[a] (n=1980)	Model 2[b] (n=1746)
Hours since awakening	-0.136***	-0.135***	-0.118***	-0.117***
	(-0.14, -0.13)	(-0.14, -0.13)	(-0.12, -0.12)	(-0.12, -0.11)
Maternal separation[c]	-0.062	-0.070	-0.01	-0.01
	(-0.13,0.01)	(-0.14,0.00)	(-0.09,0.07)	(-0.08,0.08)
Maternal separation x	0.014**	0.014**	-0.001	-0.000
hours since awakening[d]	(0.01,0.02)	(0.01,0.02)	(-0.01,0.01)	(-0.01,0.01)
Random effects				
var(hours since awakening)	0.001***	0.001***	0.001***	0.001***
	(0.00,0.00)	(0.00,0.00)	(0.00,0.00)	(0.00,0.00)
var(intercept)	0.014***	0.002***	0.057***	0.052***
	(0.00,0.08)	(0.00,0.01)	(0.03,0.10)	(0.03,0.10)

Notes: * p<0.05, ** p<0.01, *** p<0.001. [a] Fully adjusted model using analytic sample. [b] Fully adjusted model but participants on steroid medication and more than 15 minutes late taking cortisol sample 1 were removed. [c] Maternal separation ref: not separated. [d] Interaction term between maternal separation and hours since awakening.

Appendix 2.C: Sensitivity analysis: estimates (95% CI) from linear regression model of log diurnal cortisol slope (nmol/l) difference score

	Model 1[a] (n=1980)	Model 2[b] (n=1736)
Maternal separation[c]	-0.005**	-0.004*
	(-0.01,-0.00)	(-0.01,-0.00)

Notes: * p<0.05, ** p<0.01, *** p<0.001. [a] Fully adjusted model using analytic sample. [b] Fully adjusted model but participants on steroid medication and more than 15 minutes late taking cortisol sample 1 were removed. [c] Maternal separation ref: not separated.

Appendices to Chapter 3

Appendix 3.A: Summary statistics showing characteristics by reason for separation status

		Parents divorced/ separated (n=28)	Mother died (n=82)	Mother ill (n=34)	Adoption (n=17)	Evacuation (n=118)	Other (n=101)	p-value
HCC (pg/mg)		33.56 (130.65)	15.48 (82.74)	18.81 (69.20)	8.35 (13.25)	35.46 (120.25)	29.68 (112.39)	0.75
Maternal separation age								<0.001
	0-5y	10 (37.0%)	32 (39.5%)	15 (44.1%)	15 (100.0%)	44 (38.3%)	32 (32.3%)	
	6-11y	12 (44.4%)	28 (34.6%)	16 (47.1%)	0 (0.0%)	70 (60.9%)	48 (48.5%)	
	12-16y	5 (18.5%)	21 (25.9%)	3 (8.8%)	0 (0.0%)	1 (0.9%)	19 (19.2%)	
Age (years)		72.32 (5.51)	70.57 (6.32)	68.61 (5.36)	69.84 (6.34)	77.82 (2.67)	70.86 (5.42)	<0.001
Sex								0.01
	Female	11 (39.3%)	22 (26.8%)	7 (20.6%)	8 (47.1%)	58 (49.2%)	40 (39.6%)	
	Male	17 (60.7%)	60 (73.2%)	27 (79.4%)	9 (52.9%)	60 (50.8%)	61 (60.4%)	
Ethnicity								<0.001
	Non-white	2 (7.1%)	15 (18.3%)	0 (0.0%)	1 (5.9%)	1 (0.8%)	23 (22.8%)	
	White	26 (92.9%)	67 (81.7%)	34 (100.0%)	16 (94.1%)	117 (99.2%)	78 (77.2%)	
ACEs		0.73 (0.78)	0.33 (0.62)	0.82 (0.88)	0.38 (0.65)	0.31 (0.61)	0.38 (0.63)	<0.001
Material disadvantage		1.65 (1.06)	1.25 (1.09)	1.16 (1.14)	1.50 (1.16)	1.66 (1.03)	1.43 (1.16)	0.08
Parental warmth		18.29 (4.60)	21.12 (5.44)	19.83 (5.38)	16.94 (4.80)	20.90 (5.64)	20.11 (5.25)	0.03
Parental strictness		9.50 (2.74)	8.74 (2.46)	9.59 (2.16)	9.53 (2.90)	8.88 (2.70)	10.13 (2.31)	0.02
Parental expectations		5.59 (1.33)	5.86 (1.20)	5.69 (1.39)	5.47 (1.84)	5.26 (1.59)	6.07 (1.59)	0.02
Marital status								0.81
	Married/cohabiting	18 (64.3%)	57 (70.4%)	25 (73.5%)	11 (64.7%)	78 (66.1%)	73 (75.3%)	
	Single	3 (10.7%)	11 (13.6%)	2 (5.9%)	2 (11.8%)	11 (9.3%)	8 (8.2%)	

	Divorce/separated	3 (10.7%)	4 (4.9%)	1 (2.9%)	1 (5.9%)	10 (8.5%)	9 (9.3%)	
	Widow	4 (14.3%)	9 (11.1%)	6 (17.6%)	3 (17.6%)	19 (16.1%)	7 (7.2%)	
Civil service employment grade								0.01
	Administrative	7 (25.0%)	31 (37.8%)	14 (41.2%)	10 (58.8%)	39 (33.1%)	30 (29.7%)	
	Professional/executive	18 (64.3%)	39 (47.6%)	17 (50.0%)	3 (17.6%)	44 (37.3%)	55 (54.5%)	
	Clerical/support	3 (10.7%)	12 (14.6%)	3 (8.8%)	4 (23.5%)	35 (29.7%)	16 (15.8%)	
Current smoker (cigarettes)								0.23
	No	23 (88.5%)	77 (98.7%)	31 (93.9%)	15 (93.8%)	110 (97.3%)	90 (93.8%)	
	Yes	3 (11.5%)	1 (1.3%)	2 (6.1%)	1 (6.3%)	3 (2.7%)	6 (6.3%)	
Waist-to-hip ratio		0.93 (0.08)	0.92 (0.08)	0.93 (0.07)	0.91 (0.07)	0.90 (0.09)	0.92 (0.10)	0.17
CVD medication								0.11
	No	11 (39.3%)	34 (41.5%)	17 (50.0%)	11 (64.7%)	39 (33.1%)	36 (35.6%)	
	Yes	17 (60.7%)	48 (58.5%)	17 (50.0%)	6 (35.3%)	79 (66.9%)	65 (64.4%)	
Diabetes[a]								0.54
	No	25 (89.3%)	68 (82.9%)	32 (94.1%)	16 (94.1%)	100 (84.7%)	85 (84.2%)	
	Yes	3 (10.7%)	14 (17.1%)	2 (5.9%)	1 (5.9%)	18 (15.3%)	16 (15.8%)	
CES-D >=16 or antidepressants								0.94
	No	23 (85.2%)	66 (85.7%)	30 (88.2%)	13 (81.3%)	97 (86.6%)	79 (82.3%)	
	Yes	4 (14.8%)	11 (14.3%)	4 (11.8%)	3 (18.8%)	15 (13.4%)	17 (17.7%)	
Local steroids								0.21
	No	24 (85.7%)	75 (91.5%)	31 (91.2%)	12 (70.6%)	100 (84.7%)	90 (89.1%)	
	Yes	4 (14.3%)	7 (8.5%)	3 (8.8%)	5 (29.4%)	18 (15.3%)	11 (10.9%)	
Systemic steroids								0.46
	No	27 (96.4%)	78 (95.1%)	34 (100.0%)	15 (88.2%)	112 (94.9%)	98 (97.0%)	
	Yes	1 (3.6%)	4 (4.9%)	0 (0.0%)	2 (11.8%)	6 (5.1%)	3 (3.0%)	

Notes: Mean(sd)/n(%). HCC= hair cortisol concentrations. [a] Diabetes was defined using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006). CES-D= Center for Epidemiologic Studies Depression Scale.

Appendix 3.B: Comparison between phase 5 and analytic sample in phase 11

		Phase 5 (n=7870)	Analytic sample (n=3969)
Maternal separation	No	6,092 (86.5%)	3,169 (88.9%)
	Yes	947 (13.5%)	397 (11.1%)
Maternal separation age			
	0-5y	345 (37.8%)	154 (40.4%)
	6-11y	442 (48.4%)	177 (46.5%)
	12-16y	126 (13.8%)	50 (13.1%)
Age (years)		55.97 (6.04)	69.83 (5.85)
Sex			
	Female	2,397 (30.5%)	1,284 (32.4%)
	Male	5,473 (69.5%)	2,685 (67.6%)
Ethnicity			
	Non-white	675 (8.6%)	237 (6.0%)
	White	7,186 (91.4%)	3,729 (94.0%)
ACEs		0.28 (0.57)	0.30 (0.59)
Material disadvantage		1.212 (1.071)	1.172 (1.063)
Parental warmth		22.009 (4.988)	22.002 (4.938)
Parental strictness		9.338 (2.403)	9.388 (2.385)
Parental expectation		5.887 (1.462)	5.912 (1.454)

Notes: Mean(sd)/n(%)

Appendix 3.C. Sensitivity analyses: association between maternal separation in childhood and log hair cortisol concentrations (HCC) (pg/mg)

	B	95% CI	p-value
Model 1 (n=3969)	0.179	0.041,0.317	0.01
Model 2 (n=2793)	0.247	0.086,0.408	<0.01
Model 3 (n=3598)	0.159	0.018,0.3	0.03
Model 4 (n=3969)	0.186	0.048,0.324	0.01

Notes: Model 1: Full model. Adjusts for age (years), sex, ethnicity, local and systemic steroid medication, adverse childhood experiences, material disadvantage, parenting dimensions, marital status, civil service employment grade, smoking status, waist-to-hip ratio, CVD medication, diabetes, depressive symptoms. Model 2: Model 1 with complete cases. Model 3: Model 1 + removed participants reported taking local and systemic steroid medication. Model 4: Model 1 + hair colour, hair dye, hair treatment, hair washing frequency.

Appendicies to Chapter 4

Appendix 4.A: Comparison of observed and imputed values

	Observed	Imputed	Missing %
HCC (pg/mg)	16.38	16.38	0
Depressive symptoms			23.2
No depressive symptoms	0.78	0.75	
Past	0.11	0.12	
Recurrent	0.07	0.08	
Current	0.04	0.05	
Total CES-D score	21.27	22.24	23.2
Antidepressant use			7.8
No antidepressant use	0.93	0.92	
Past	0.03	0.03	
Recurrent	0.03	0.03	
Current	0.02	0.02	
Maternal separation			10.2
No	0.89	0.89	
Yes	0.11	0.11	
Age	69.86	69.86	0
Sex			0
Female	0.32	0.32	
Male	0.68	0.68	
Ethnicity			0.1
Non-white	0.04	0.06	
White	0.96	0.94	
Local steroids			0
No	0.91	0.91	
Yes	0.09	0.09	
Systemic steroids			0
No	0.98	0.98	
Yes	0.02	0.02	
Antipsychotic medication			0
No	0.995	0.99	
Yes	0.005	0.01	
ACEs	0.300	0.303	13.1
Material disadvantage	1.172	1.175	13.8
Parental warmth	22.002	21.969	17.7
Parental strictness	9.388	9.396	17
Parental expectations	5.912	5.898	16.9
Smoking status			5.6
Never/former	0.96	0.95	
Current	0.04	0.05	
Civil service employment grade			0
Administrative	0.45	0.45	

Professional/executive	0.44	0.44	
Clerical/support	0.11	0.11	
BMI	26.745	26.748	0.6
CVD medication			0
No	0.41	0.41	
Yes	0.59	0.59	
Diabetes[a]			0
No	0.87	0.87	
Yes	0.13	0.13	

Notes: Mean/proportions. HCC= hair cortisol concentrations. CES-D = Center for Epidemiologic Studies Depression Scale. [a] Diabetes defined using self-reported diabetes, or diabetic medication, or fasting glucose ≥7 (WHO, 2006), or HbA1c ≥6.5% (WHO, 2006).

Table 4.B: Linear regression estimates (95% CI) of log hair cortisol concentration (HCC) (pg/mg) (n=3969)

	Model 1[a]			**Model 2**[b]		
	B	95% CI	p-value	B	95% CI	p-value
Depressive symptoms[c]						
Past	0.152	0.015,0.289	0.029	0.127	-0.009,0.262	0.067
Recurrent	0.194	0.012,0.376	0.037	0.208	0.026,0.391	0.026
Current	0.283	0.065,0.502	0.011	0.271	0.050,0.491	0.016
Antidepressant use[d]						
Past	0.083	-0.178,0.344	0.531	0.072	-0.192,0.337	0.591
Recurrent	-0.030	-0.263,0.203	0.800	-0.042	-0.275,0.191	0.724
Current	0.495	0.188,0.803	0.002	0.468	0.164,0.772	0.003
Maternal separation				0.167	0.030,0.304	0.017

Notes: [a] Model 1 adjusts for age, sex, ethnicity, antidepressants, local and systemic corticosteroid medication, antipsychotic medication. [b] Model 2 adjusts for model 1 covariates + maternal separation, ACEs, material disadvantage, parental warmth, parental strictness, parental expectations, smoking status, civil service employment grade, BMI, CVD medication, diabetes. [c] Depressive symptoms reference category: no depressive symptoms. [d] Antidepressant use reference category: no antidepressant use.

Appendix 4.C: Linear regression estimates (95% CI) of log hair cortisol concentration (HCC) (pg/mg) (n=3969)

	B	95% CI	p-value
Age	0.004	-0.004,0.011	0.333
Male	0.378	0.286,0.470	0.000
Ethnicity[a]	0.056	-0.118,0.229	0.530
Local steroids	0.145	-0.002,0.292	0.053
Systemic steroids	-0.605	-0.912,-0.297	0.000
Antipsychotic medication	0.254	-0.228,0.736	0.302
ACEs	0.011	-0.067,0.088	0.786
Material disadvantage	-0.009	-0.049,0.031	0.655
Parental warmth	0.005	-0.005,0.014	0.344
Parental strictness	-0.010	-0.030,0.010	0.333
Parental expectations	0.019	-0.013,0.052	0.250
Smoking status[b]	0.187	0.002,0.371	0.047
Civil service employment grade[c]			
Professional/executive	-0.030	-0.116,0.056	0.492
Clerical/support	-0.026	-0.178,0.127	0.741
BMI	0.032	0.023,0.041	0.000
CVD medication	0.034	-0.050,0.117	0.428
Diabetes	0.261	0.139,0.383	0.000

Notes: [a] Ethnicity reference category: non-white. [b] Smoking status reference category: former/never. [c] Civil service employment grade reference category: administrative.

Appendix 4.D: Linear regression and multinomial logistic regression estimates (95% CI) showing depressive symptoms (continuous and categorical) regressed on maternal separation (n=3969)

	B	95% CI	p-value
Linear regression[a]	1.531	-0.710,3.772	0.180
Multinomial logit[b]			
Past	0.205	-0.159,0.569	0.269
Recurrent	0.194	-0.246,0.634	0.387
Current	0.003	-0.570,0.575	0.993

Notes: [a] The beta-coefficient is the effect of total depressive symptoms, measured continuously, regressed on maternal separation. [b] Baseline reference: no depressive symptoms. Interpretation is the same as for the linear regression model.

Publications

Bevan, K., Kumari, M., 2021. Maternal separation in childhood and hair cortisol concentrations in late adulthood. Psychoneuroendocrinology 130. https://doi.org/10.1016/J.PSYNEUEN.2021.105253

Conference abstract:

Bevan, K. 2021. Maternal separation in childhood and hair cortisol concentrations in late adulthood. Poster session presented at: Longitudinal Studies 2021, Wellcome Genome Campus; Virtual Conference.