Adverse childhood experiences and diurnal cortisol pattern and heart rate variability in adults

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Abstract

Dysregulation of hypothalamic–pituitary–adrenal axis (HPA axis) and of the autonomic nervous system may link stress throughout the life course with poorer health. This study aims to investigate whether multiple adverse childhood experiences have a long-term impact on markers of these systems - cortisol secretion and heart rate variability - in adulthood. Data were from the Whitehall II cohort study. Fourteen adversities, collected retrospectively in midlife, were considered. Outcomes were total amount of cortisol

secretion during the day (area under the curve [AUC]), cortisol awakening response (CAR), and diurnal slope, estimated from six saliva samples taken on a weekday; and resting heart rate (rHR) and heart rate variability (HRV) measured for five minutes at three time points over 10 years with the last measures taken at the same time as the salivary measurement. Regression models were used to examine the association of adversities with AUC, CAR, rHR and HRV and multilevel modelling was applied to analyses of cortisol diurnal slope and the 10-year follow-up of rHR and HRV. At least one early life adversity was reported by 68% of participants. There was little evidence that increasing number of adversities was associated with any measures of cortisol, rHR or HRV or 10-year change in rHR or HRV. Of the individual adversities, only parental death was associated with increased AUC and CAR. In conclusion, although the HPA axis and autonomic nervous system have been hypothesized as mechanisms relating to adverse childhood experiences with health, our study finds no evidence to support this.

Key words: Adverse childhood experience, salivary cortisol, heart rate variability, circadian rhythm, ageing

Introduction

Adverse childhood experiences, which include experiences such as parental separation and abuse, have been extensively studied and shown to have potentially harmful effects on lifelong health.[1] These traumatic and stressful events in childhood and adolescence appear to be associated with adverse brain development and function, resulting in potential negative behavioural and physiological changes, and disturbed stress reactivity over the life course, which impact a range of health outcomes.[2-4]

One of the possible underlying biological pathways linking adverse childhood experiences and health is via the hypothalamic–pituitary–adrenal axis (HPA axis), a central component of the stress response system.[2] The final effectors in the HPA axis are glucocorticoids, primarily cortisol, which play important roles in metabolism, immune function, and inflammatory responses.[2] Short-term fluctuations of cortisol are essential to respond to the environment to maintain allostasis. However, prolonged deviations, due to sustained or repeated exposures to stressors, may gradually dysregulate system functioning.[2] Cortisol secretion has a circadian rhythm, with a peak approximately 30-45 minutes after awakening, called the cortisol awakening response (CAR), followed by a decline towards evening. The CAR appears to involve extra-pituitary pathways along with the HPA axis. Furthermore, the volume of cortisol secretion is an important indicator of health, as highlighted by individuals with Cushing syndrome (hypercortisolism) and Addison's disease (hypocortisolism). Cortisol secretion has been shown to be associated with mental health outcomes,[5] and a flatter diurnal slope was found to be associated with mortality and with adverse physiological outcomes including obesity.[6]

Previous findings on the association between adverse childhood experiences and cortisol secretion are heterogeneous.[7] It is challenging to compare results across existing studies, partly due to variations in the measurement of cortisol and of adverse childhood experiences.[6] Systematic reviews and meta-analyses concluded that there is little evidence that adverse childhood experiences are associated with the CAR,[7,8] cortisol level at awakening,[7,9] or a blunted diurnal slope.[9] The few studies examining the total volume of cortisol secretion during the day in general population samples have reported no

association with adverse childhood experiences.[10] Most of the existing studies, however, examine only specific types of adverse childhood experience, while studies considering multiple childhood adversities have applied a simple sum of the number of adversities. This approach, which has been commonly used for a range of health outcomes, aims to capture accumulation of adverse experiences. However, it has been criticised[11] as it makes the unlikely assumption that each adversity has exactly the same impact on the outcome, and that each combination of adversities has the same impact. Therefore, it does not take account of the potential differential impact of each adversity, which have been highlighted in some studies in relation to health outcomes.[12,13] Moreover, many previous studies had small sample sizes, so associations have not been estimated with adequate precision.

Another possible biological pathway linking adverse childhood experiences and health is through disturbances of the autonomic nervous system, composed of sympathetic and parasympathetic nervous systems. The sympathetic nervous system triggers the fight-or-flight response, accelerating heartbeat and inhibiting peristalsis, while the parasympathetic nervous system functions conversely by controlling the rest-and-digest response to calm the body down. Autonomic activity, such as an increase in blood pressure triggered by adrenaline, is an instantaneous response to stressors, while the activation of the HPA axis happens, more slowly, in minutes.[14] Autonomic dysfunction has been found to predict postinfarction mortality,[15,16] as well as to have an association with a wide range of cardiovascular conditions and risk factors in people without prior cardiovascular history.[17-19]

Heart rate variability (HRV), which reflects short-term variations in the interval between consecutive heart beats, has been used as a measurement of autonomic nervous function.[20]

This measurement is particularly valuable due to its non-invasive derivation from electrocardiogram (ECG) recordings.[20] Using HRV or other existing procedures for measurement, disturbed autonomic nervous function, either heightened sympathetic or depressed parasympathetic activity, has been linked to fatal arrythmia.[20] A systematic review with meta-analysis reported no overall association between childhood adversity and vagal activity, mostly measured in childhood and adolescence.[21] However, there was significant heterogeneity in estimates between studies, possibly reflecting the variation in study design, and most only considered specific types of severe adversity, most commonly maltreatment and interparental agression.[21] Therefore, the impact of accumulation of multiple adverse childhood experiences has been little examined. Further, as HRV decreases with increasing age, it may be that associations between childhood adversity and HRV could vary by age and could become stronger in later life. Therefore adversity may be related to the rate of change in HRV across later life. The autonomic nervous system is also known to function differently in men and women,[22] and the type of childhood adversities experienced differ between men and women,[23] with stronger associations being observed between childhood adversity and health outcomes, such as cardiovascular disease, in women compared with men.[24] It is therefore possible that adversities have a potentially stronger effect on the biological pathways in women compared with men.

Our objective is to investigate whether, when allowing for the potential differential impact of each adversity, an increasing number of adverse childhood experiences is related to greater disruption of diurnal cortisol secretion and autonomic activity in midlife to older adulthood. We also assessed whether associations were stronger in women than men. Finally, using repeated measures of the outcome, we investigated whether a greater

number of adverse childhood experiences is related to more rapid changes in autonomic activity over a 10-year period.

Materials and Methods

Study sample

The study sample are participants in the Whitehall II study, a cohort study that was established in 1985 with 10 308 participants (men 6895; women 3413, aged 33-55), recruited from non-industrial civil servants from 20 Civil Service Departments in London.[25] There have been 13 waves of data collection at an average three-year interval. Self-administered questionnaires were conducted at all waves, and medical examinations were performed at even-numbered waves. This current study uses data up to the 9th wave, where the response rate remained over 70%.

Measurements

Adverse childhood experiences

Adverse childhood experiences before the age of 18 were assessed retrospectively using a questionnaire at waves 1 (1985-1988) and 5 (1997-1999), when the mean age (standard deviation (SD)) was 44.4 (5.9) and 55.4 (6.0) years, respectively. Among 20 items, five items were adopted from the European Prospective Investigation into Cancer and Nutrition study (EPIC);[26] two from the Childhood Experience of Care and Abuse (CECA) interview;[27] 10 items from the Midlife Development in the United States (MIDUS) study;[28] and the rest were designed in the Whitehall II study. Cronbach α indicated internal consistency in two groups of four items on parenting style from MIDUS, from which we created two summary score variables to take severity of parental lack of attachment and harsh punishment into

account. Consequently, 14 variables were included in analysis (details provided in table S1) and were either *binary (coded as 0=no, 1=yes)* - "maternal separation ≥1 year", "hospitalisation ≥4 weeks", "parental divorce", "parental unemployment", "mental illness and alcohol problems", "physical abuse", "parental argument", "orphanage", "financial problems", "parental death before the participant was aged 16" - or *continuous (range)* - "lack of attachment to mother" (scored from 4 to 16), "lack of attachment to father" (4 to 16), "mother's harsh punishment" (1 to 4), "father's harsh punishment" (1 to 4).

Salivary cortisol

Salivary cortisol was collected at wave 9 (2007-2009), when the mean age (SD) was 65.9 (6.0) years. Participants were asked to provide six saliva samples in salivettes on a weekday at waking (T_1), and at +30 min (T_2), +2.5 hours (T_3), + 8 hours (T_4), +12 hours (T_5) since awakening, and at bedtime (T_6). The participants were instructed not to brush teeth, drink nor eat anything in the 15 minutes before each saliva sample collection, to record time of sample collection in a logbook, and to send back the six salivettes and the logbook to the study team in a Freepost envelope. Salivette devices were centrifugated at 3000 rpm for 5 minutes, resulting in a clear supernatant of low viscosity. Cortisol levels were assessed using a commercial immunoassay kit with chemiluminescence detection (CLIA, IBL-Hamburg, Hamburg Germany). The lower concentration limit of this assay was 0.44 nmol/l, with intraassay and interassay precision of <8%.

Resting heart rate and Heart rate variability

Resting heart rate (rHR) and Heart rate variability (HRV) were recorded at three waves (waves 5 [1997-1999], 7 [2002-2004] and 9 [2007-2009]), when the mean age (SD) was 55.8

(6.0), 61.1 (6.0), and 65.9 (6.0) respectively. Five-minute supine 12-lead electrocardiograms were performed at rest using SEER MC recorders (GE Medical Systems, Milwaukee, Wisconsin). The recorders were set to capture 10-second electrocardiograms every 10 seconds. Therefore the signal collected from the recorders was continuous because there are no gaps between adjacent 10 second elementary electrocardiogram recordings.[29] Five minutes of beat-to-beat data were re-sampled at a frequency of 500 Hz to obtain digitised sequences of R waves. Electrocardiographic abnormalities, such as ectopic beats, right bundle-branch block, and respiratory arrhythmia, were detected using an automatic algorithm, and normal QRS complexes adequate for HRV analyses were identified.

HRV was analysed in two domains: time and frequency (Table 1). The time domain measures were the standard deviation of normal-to-normal RR intervals (SDNN; milliseconds), and the square root of successive differences of normal-to-normal RR intervals (RMSSD; milliseconds). Frequency-domain measures were the low-frequency (LF: 0.04 to 0.15 Hz) and high-frequency (HF: 0.15 to 0.4Hz) spectral power (milliseconds squared), which were computed using a Blackman-Tukey algorithm. In resting conditions, higher HF and larger RMSSD indicate greater parasympathetic control, while lower HF and shorter RMSSD reflect predominance of sympathetic activation.

Covariates

Potential confounders of the association of adverse childhood experiences with salivary cortisol, with HRV, and predictors of the outcome were identified based on existing literatures and by a directed acyclic graphs (DAGs) (figure S1 and S2).

All analysis included sex, ethnicity (white vs non-white), childhood socioeconomic position, and adult socioeconomic position. Fathers' occupational social class was used as a marker of childhood socioeconomic position, classified according to the Registrar General's Social Class Scheme, with categorisations of 'professional', 'managerial/technical', 'skilled-non-manual', 'skilled-manual', 'partly skilled', and 'unskilled'. Employment grade at saliva sampling, as a marker of adult socioeconomic position, was categorised as 'administrative' (high grade), 'professional/executive' (intermediate grade), and 'clerical/support' (low grade). For cortisol, age at saliva collection in years, cigarette smoking (yes vs no) and waking time on the day of sample collection were also included. For rHR and HRV, medication intake at the time of electrocardiographic samples collection was also included as potential a predictor of rHR and HRV. Types of medication were beta-blocker, angiotensin-converting enzyme inhibitors, calcium channel blocker, and diuretics.

Statistical analysis

We first described the outcomes at every time point and covariates by the count of adverse childhood experiences.

Adverse childhood experiences and Salivary cortisol

Area under the curve (AUC) was used as an index of the amount of cortisol secretion during a day, and cortisol awakening response (CAR) and diurnal slope as the indices of the diurnal cortisol pattern (Table 1).

AUC was calculated for each individual based on the six observed values using the Trapezoidal rule;[30]

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

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

CAR was computed by subtracting cortisol measured at awakening time (T_1) from cortisol measured at 30 minutes after awakening (T_2). The samples at T_1 were restricted to those taken within 10 minutes after awakening, and samples at T_2 to those taken within 60 minutes after awakening, irrespective of time of the day at awakening, to minimise measurement error.

Participants who were taking steroids, including hormone replacement therapy at the time when salivary cortisol was measured were excluded from analysis. Our analytic sample was 3419 participants with complete data on outcome, exposure, and covariates (Figure 1).
We first described cortisol levels at every time point, and covariates by the count of adverse childhood experiences. As the distribution of salivary cortisol values and the AUC were right skewed, we used the median and inter-quartile range. We applied linear regression to examine the association of adverse childhood experiences with AUC and CAR. The natural logarithm of the AUC measures was taken, to reduce skewness, and then multiplied by 100 so that the resulting regression coefficients are interpreted as percent change in AUC per unit change in an exposure.[31] In preliminary analyses, we included interaction terms for sex by individual adversities and performed likelihood ratio tests to assess whether sex modified the association of adverse childhood experiences with each index of cortisol. With no

evidence for interactions, we present estimates from models including men and women together, with adjustment for sex, as the primary analyses, while showing sex-stratified estimates as a supplementary analysis. Model 1 included all 14 adverse childhood experiences, sex, and age, and model 2 additionally adjusted for ethnicity, childhood and adult socioeconomic position, and smoking and waking time on the day of sample collection.

To investigate the association between adverse childhood experiences and the diurnal slope, we used a multilevel regression model in which measurement occasions were considered as level 1, nested within individuals as level 2. The outcomes were measures of log cortisol (x 100) at T_1, T_3, T_4, T_5, and T_6. Cortisol at T_2 was not included because the awakening response appears to have a different biological system from diurnal slope.[32] A random quadratic term and interaction terms of adversities with time were not included because their inclusion did not improve the model fit. Model 1 included all 14 adverse childhood experiences. Model 2 adjusted awakening time, sex, age in years, ethnicity, childhood and adult socioeconomic positions, smoking on the day of sample collection, and their interaction terms with time since awakening.

Adverse childhood experiences and rHR and HRV

We excluded participants with a history of cardiac events by the time when rHR and HRV were measured. Log-transformations (multiplied by 100) were used for SDNN, RMSSD, LF, and HF as their distributions were right skewed.

We carried out two types of analyses for these outcomes. First, we applied multiple linear regression to measurements only at wave 9 (2007-2009: which is the same wave at which

the cortisol is collected). Second, we examined the association between adverse childhood experiences and the longitudinal profiles using multilevel models. Preliminary analyses provided no evidence of sex differences in the relationships between adverse experiences and outcomes, so the primary analyses include men and women with adjustment for sex.

The analytical sample for the analysis with the outcomes at wave 9 (2007-2009) was 2878 with complete information on outcome, adversity exposures and covariates. We examined the association of adverse childhood experiences with rHR and each indicator of HRV, using models including all 14 adverse childhood experiences, sex, and age (model 1). Model 2 additionally adjusted for ethnicity, childhood socioeconomic position, adult socioeconomic position, and medication intake.

The longitudinal analysis included 3827 with at least one measure of the outcome, and complete exposure and covariate information (Figure 1). A multilevel linear regression was applied in which measurement occasions was level 1, nested within individuals as level 2. We estimated random effects for intercept and for the linear term of age, which was centred at age 60. The inclusion of a random quadratic term, and interaction terms of adverse childhood experiences and other covariates with age did not improve the model fit and were thus not included. The fixed part of the model included the linear and quadratic terms for centred age in years, and age at baseline (i.e., age at the 1st measurement); model 1 included all 14 ACEs and adjusted for sex; and model 2 included ethnicity, childhood socioeconomic position, adult socioeconomic position (time-varying), and medication intake (time-varying).

For both salivary cortisol and HR and HRV analyses, we predicted a value of outcome in each individual based on the final model when all the covariates were set to their baseline values (coded as zero in the model). We then calculated the average of the predicted values in individual level per count of adverse childhood experiences. To obtain the observed count of ACEs for each individual, the worst quartile or worst score on the Likert scale for ordinal ACEs was counted as 1 (yes) with all other responses coded as 0 (no). However, to account for severity of these adversities when estimating the marginal effects, we subtracted the highest score in the third quartile from the score in the worst quartile. *We* used the StataMP 18 for all the analyses.

Results

Table 2 summarises the reports of adverse childhood experiences. Among the participants included in the analyses, 68% reported at least one adversity. The highest prevalence was observed for “financial problems”, followed by “arguments between parents”. Table S2a and S2b present the distribution of covariates and outcomes by count of adverse childhood experiences. Non-white participants were more likely to report a larger number of adverse childhood experiences than white participants. Participants who had fathers in manual occupations, and those in lower employment grades in adulthood tended to have a larger count of adversities than those from more advantaged socioeconomic positions.

Adverse childhood experiences and Salivary cortisol

There is little evidence of any consistent association between adverse childhood experiences and AUC or CAR in either sex and age adjusted models or fully adjusted models (Table S3). The largest estimate was for parental death in childhood, where those who had

experienced this adversity showed a 7% increase in AUC (95%CI 0.25 to 13.83, p = 0.04), and a 2.801 nmol/l (0.398 to 5.204, p = 0.02) greater CAR than those who did not.

For both AUC and CAR, the predicted values from the model showed no pattern across the count of adverse childhood experiences (Figure 2 and Table S3). None of the adversities were associated with either the awakening value (i.e., the intercept) or with the slope. No clear trend was observed in the awakening value (intercept) by the count of adverse childhood experiences (Figure 2).

Adverse childhood experiences and rHR and HRV

The mean value of all indicators of HRV exhibited a downward trend from the baseline to 10 years follow-up time (Tables S4-S8). Consistent with this, there are also age-related decreases in all HRV measures within each wave.

There was no association between adverse childhood experiences and rHR or HRV and their changes over time (Table 3). Figure 3 shows rHR and HRV in wave 9 (2007-2009) by the count of adverse childhood experiences.

As preliminary analysis found no evidence of adverse childhood experiences by age interactions, the associations of adverse childhood experiences with rHR and HRV is consistent across all three timepoints. There is little evidence of any consistent effect of individual adversities on rHR or on HRV.

Discussion

We found little evidence to support our hypothesis that there would be an association of an increasing number of adversities with disrupted cortisol, or autonomic activity and its rate of change. This extends previous research on these outcomes which has predominantly investigated specific types of adversity rather than the accumulation of multiple adversities. We found no evidence of modification by sex. When investigating individual adversities, only parental death showed an association with increased AUC and CAR.

To date, there are a limited number of studies examining the amount of cortisol secretion during the day in relation to childhood adversity.[10] Our findings of no relationship between adversities and AUC are consistent with evidence from a previous study with cortisol measured from four samples over the day.[10] A systematic review reported no association between adversities and CAR.[8] However, most of the studies included in that review used data from people with underlying health conditions, such as psychosis, and the sample sizes were small.[8] Our study, which has a relatively large sample size in a working population, also provided little evidence to support an association between increasing number of adversities and CAR.

In contrast to previous studies, we also found no difference in cortisol levels on waking by adversities in childhood. However, these previous studies were conducted in specific risk groups such as people with fibromyalgia,[33] depression,[34] and international adoptees.[35] Of the previous studies based on samples from the general population, they are limited by small sample size (61 healthy adults),[36] a small number of cortisol samples per day (three samples),[37] or examination of only one form of adversity (maternal separation).[38]

Although our focus was on multiple adversities, we observed an association between the death of a parent when the participant was less than 16 years old and increased total cortisol secretion during the day and with elevated CAR, consistent with existing studies.[39] There may therefore be types of adversity that are more harmful, depending on the severity and timing of the experience, and its longer-term consequences. Parental death is a particularly severe adversity, probably because it involves the permanent loss of a crucial relationship and attachment figure, and it may happen suddenly without warning.[40] A study of youths aged 10 to 29 years has reported that parental death dysregulated the HPA axis during five years of follow-up,[41] and our findings suggest that this association might continue into later life.

Although the autonomic nervous system responds vigorously to acute stress, it remains unclear whether chronic stress across the life course leads to disruption of autonomic activity. Two systematic reviews have reported low vagal activity, characterised as low RMSSD and HF, in relation to self-reported chronic stress[42] and posttraumatic stress disorder (PTSD).[43] Our null findings suggest that early life stress is not associated with HRV in later life, but we do not account for chronic or current stress and subsequent life course events after childhood. Some individuals who experienced adversities in childhood have difficulties in coping with stress in adulthood,[44] or may have PTSD arising from these earlier adversities.[45] If a key determinant of disrupted autonomic activity is the presence of current stress[46] this may modify the association of adverse childhood experiences with later life HRV. Our study used rHR and HRV measured at three times unlike existing studies which used measurements collected at a single time point.[47,48] Given that analysis using a single measurement is susceptible to regression dilution bias,[49] our estimation may be less biased

than many existing studies. The repeated measures also allowed us to investigate whether the association of adversities changes with age and we found no evidence that this was the case.

Most previous studies of adverse childhood experiences have applied a cumulative score, which is a simple sum of adversities, possibly due to its convenience of use. However, this approach has been criticised for not taking account of different effect sizes for each adversity.[11,50] This assumption is, however, unlikely and each adversity has different effect size and direction of the association, as demonstrated by our results as well as in existing literature. To address this, we fitted models which included all types of adversities as separate variables, and then averaged effect sizes according to the count of adversities, similar to some previous research.[51-53]

The current study has some limitations. We used saliva samples collected only during the daytime from a single day and it was not possible to assess the total circadian rhythm of cortisol secretion (i.e., including nighttime). Additionally, only the self-reported time of sample collection was available, although these measures have been shown in previous analyses to predict mortality outcomes.[54] Measurements of rHR and HRV were recorded for five minutes in resting conditions that may not reflect everyday levels of autonomic tone. We used retrospectively collected adverse childhood experiences, which are prone to reporting bias. Reuben et al documented that participants' dispositions, such as agreeableness or neuroticism, may influence reporting bias, potentially leading to under- or over-estimation of the effect of adversities on the outcomes.[55] Our statistical methodology of simultaneously adjusting for all 14 adverse childhood experiences assumes that

adversities are independent from each other, and do not lie on a causal pathway between any of adversities and the outcomes. The participants initially recruited into the Whitehall II cohort study were all civil servants at the time of recruitment, so may have been healthier than a general population sample. It has been also reported that some of the characteristics of participants remaining in the study at later waves differ from those at baseline.[56] For example, men and those who were white, married, and in higher employment grade had higher response rates than other groups.

In conclusion, our study shows that multiple adverse childhood experiences contribute little to dysregulation of the HPA axis and of the autonomic nervous system in later life. Given that association of adverse childhood experiences with poor health outcomes have been repeatedly reported, it would be interesting to investigate other potential pathways further to better understand the link in order to provide practical implications for those who experienced adversities in childhood.

Credit authorship contribution statement

Mifuyu Akasaki: conceptualisation, methodology, formal analysis, investigation, data curation, writing – original draft, writing – review&editing, visualisation, project administration. **Andrew Steptoe:** conceptualisation, methodology, investigation, writing – review&editing, supervision. **Rebecca Hardy:** conceptualisation, methodology, formal analysis, investigation, writing – review&editing, supervision.

Declaration of competing interest

Nothing to declare.

Data availability

Data of the Whitehall II study are available to the scientific community. Data sharing policy is available at https://www.ucl.ac.uk/epidemiology-health-care/research/epidemiology-and-public-health/research/whitehall-ii/data-sharing.

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Table 1. Summary of the study outcomes

Study	Outcome	Index
1. Adverse childhood experiences and salivary cortisol	Area under the curve (AUC)	Amount of cortisol secretion during the day
	Cortisol awakening response (CAR)	A peak of cortisol secretion after awakening
	Diurnal slope	Direction of change in cortisol secretion over the day
	Resting heart rate (rHR)	General indicator of heart function

2. Adverse childhood experiences and heart rate variability	Standard deviation of normal-to-normal RR intervals (SDNN)	Changes in heart rate due to cycles longer than five minutes of measurement time
	Square root of successive differences of normal-to-normal RR intervals (RMSSD)	Short-term changes in heart rate
	High frequency (HF)	Parasympathetic activity at normal breathing rate
	Low frequency (LF)	Sympathetic activity at normal breathing rate

Table 2. Prevalence of adverse childhood experiences

	Sample 1 (n=3419)	Sample 2 (n=3932)
Adverse childhood experiences		
Maternal separation 1yr+, n (%)	330 (9.7)	370 (9.4)
Parental death, n (%)	241 (7.1)	266 (6.8)
Hospitalisation 4wks+, n (%)	408 (11.9)	455 (11.6)
Divorce, n (%)	58 (1.7)	72 (1.8)
Mental illness and alcohol problems, n (%)	196 (5.7)	208 (5.3)
Arguments between parents, n (%)	651 (19.0)	747 (19)
Unemployment, n (%)	334 (9.8)	372 (9.5)
Financial problems, n (%)	848 (24.8)	992 (25.2)
Physical abuse, n (%)	69 (2.0)	82 (2.1)
Orphanage, n (%)	15 (0.4)	18 (0.5)
Lack of attachment to mothers, median (IQR)	8 (6 to 10)	8 (6 to 10)
Lack of attachment to fathers, median (IQR)	10 (8 to 12)	10 (8 to 12)
Mother's harsh punishment, median (IQR)	2 (1 to 2)	2 (1 to 2)
Father's harsh punishment, median (IQR)	2 (1 to 3)	2 (1 to 3)
No adverse childhood experiences, n (%)	1101 (32.2)	1261 (32.1)

Sample 1: analysis of adverse childhood experiences and salivary cortisol

Sample 2: analysis of adverse childhood experiences and resting heart rate and heart rate variability

Table 3. Estimated resting heart rate and heart rate variability with 95% confidence intervals (CIs) in association with adverse childhood experiences

	b (95% CI)									
	rHR		SDNN		RMSSD		LF		HF	
Age centred at 60 (year)	-0.158	(-0.197, -0.119)	-1.153	(-1.369, -0.936)	-0.925	(-1.234, -0.615)	-2.897	(-3.361, -2.433)	-3.145	(-3.681, -2.609)
Age squared (year2)	0.004	(0.001, 0.007)	0.007	(-0.009, 0.023)	0.038	(0.015, 0.061)	0.027	(-0.008, 0.062)	0.064	(0.023, 0.104)

Age at baseline (year)	-0.239	(0.174, 0.304)	-0.262	(-0.561, 0.036)	-0.456	(-0.883, -0.028)	-0.933	(-1.579, -0.287)	-0.498	(-1.246, 0.249)
Adverse childhood experiences; binary (ref. no experience)										
Maternal separation 1yr+	-0.004	(-1.185, 1.177)	0.963	(-3.909, 5.836)	1.081	(-5.920, 8.082)	1.341	(-9.305, 11.987)	3.608	(-8.722, 15.937)
Parental death	-0.49	(-1.810, 0.831)	0.463	(-4.935, 5.855)	3.649	(-4.082, 11.379)	-0.935	(-12.731, 10.861)	6.281	(-7.392, 19.953)
Hospitalisation 4wks+	0.723	(-0.275, 1.721)	-1.644	(-5.704, 2.415)	-1.543	(-7.355, 4.269)	-2.488	(-11.364, 6.388)	-0.106	(-10.397, 10.185)
Divorce	-0.429	(-2.818, 1.960)	-0.016	(-9.572, 9.540)	7.158	(-20.779, 6.463)	-2.454	(-23.377, 18.469)	-15.226	(-39.516, 9.065)
Mental illness and alcohol problems	-0.462	(-1.953, 1.028)	-0.017	(-6.022, 5.988)	4.973	(-3.598, 13.544)	-0.074	(-13.213, 13.064)	7.015	(-8.232, 22.261)
Arguments between parents	-0.027	(-0.868, 0.922)	-0.087	(-3.702, 3.527)	-2.715	(-7.882, 2.452)	-3.021	(-4.887, 10.930)	-0.229	(-9.403, 8.944)
Unemployment	0.060	(-1.087, 1.207)	-1.574	(-6.198, 3.051)	-0.494	(-7.102, 6.114)	-4.129	(-14.248, 5.990)	-1.884	(-13.623, 9.855)
Financial problems	-0.137	(-0.667, 0.940)	-0.933	(-4.169, 2.302)	-0.164	(-4.458, 4.786)	-2.405	(-9.486, 4.676)	-2.023	(-10.238, 6.192)
Physical abuse	-0.297	(-1.947, 2.542)	-3.227	(-12.155, 5.701)	-2.866	(-15.596, 9.865)	-9.051	(-28.612, 10.510)	-9.983	(-32.691, 12.726)
Orphanage	-4.797	(-9.404, -0.190)	18.865	(0.542, 37.189)	15.231	(-11.039, 41.502)	40.196	(0.066, 80.325)	19.735	(-26.791, 66.261)
Adverse childhood experiences; ordinal										
Lack of attachment to mothers	0.003	(-0.140, 0.146)	-0.198	(-0.398, 0.758)	-0.199	(-0.626, 1.025)	-0.397	(-0.867, 1.661)	-0.033	(-1.433, 1.499)
Lack of attachment to fathers	-0.002	(-0.138, 0.142)	-0.194	(-0.761, 0.372)	-0.302	(-1.111, 0.508)	-0.495	(-1.735, 0.745)	-0.303	(-1.742, 1.135)
Mother's harsh punishment	-0.277	(-0.742, 0.187)	1.347	(-0.526, 3.220)	3.038	(0.361, 5.716)	-3.591	(-0.508, 7.690)	6.069	(1.314, 10.825)
Father's harsh punishment	-0.200	(-0.605, 0.206)	-0.380	(-2.016, 1.257)	-0.057	(-2.282, 2.395)	-1.599	(-5.179, 1.981)	-0.890	(-5.043, 3.263)

[a] rHR is presented in an original scale (bpm). The markers of HRV are presented in %.

[b] Models adjusted for sex, age centred at 60, age squared, age at baseline, all adverse childhood experiences, ethnicity, childhood socioeconomic position, adult socioeconomic position (time-varying), and medication intake (beta-blocker, ACE inhibitor, calcium channel blocker, diuretics: time-varying)

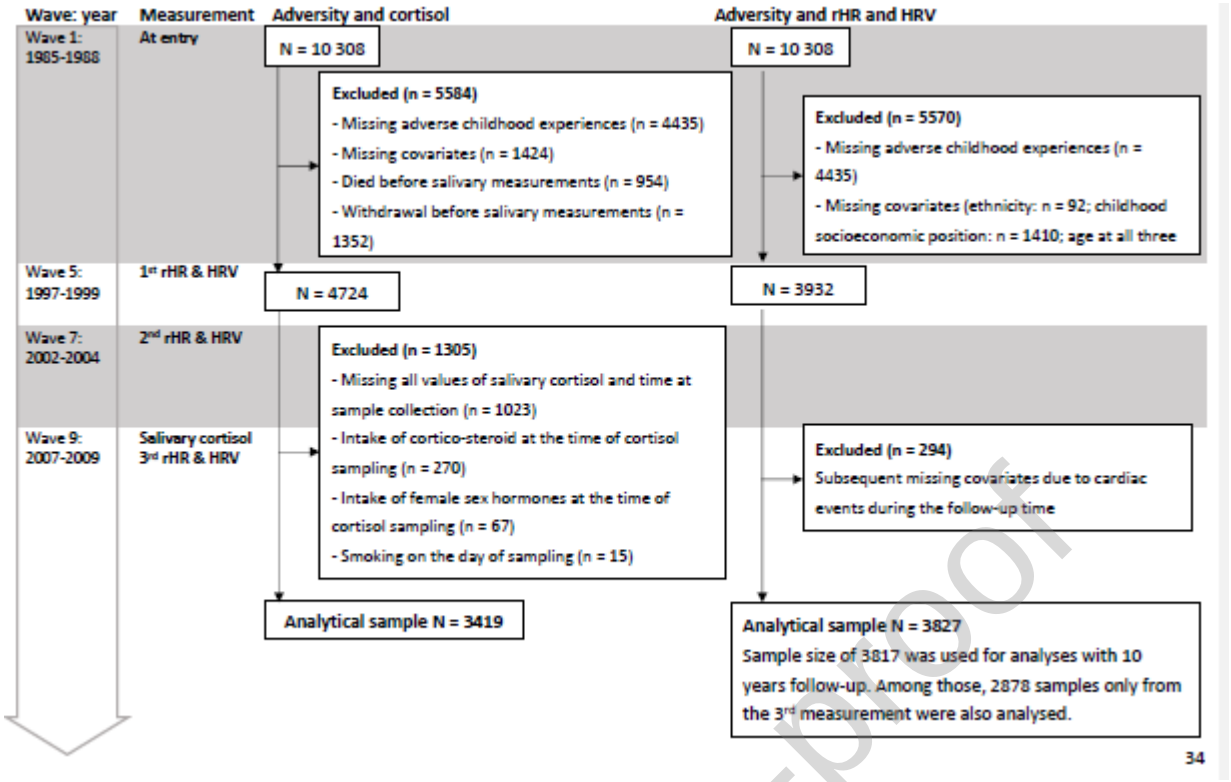
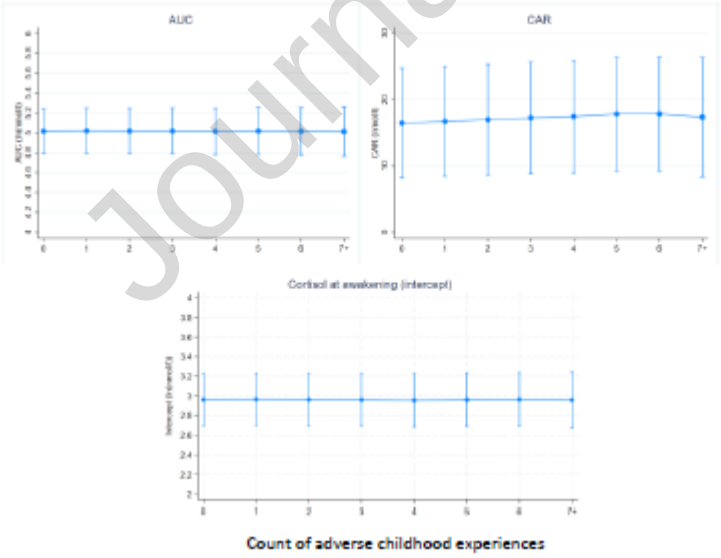


Figure 1. Flow chart of follow-up

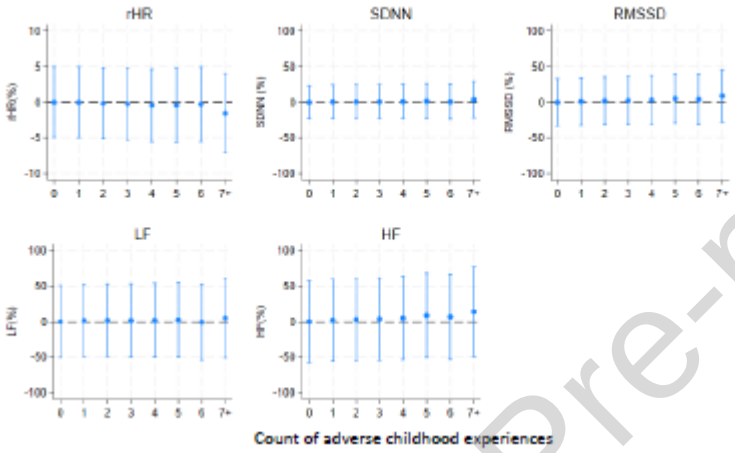
Figure 2. Estimated area under the curve (AUC), cortisol awakening response (CAR), and intercept of diurnal pattern with 95% CIs by the count of adverse childhood experiences



[a] AUC and CAR: model adjusted for all 14 adverse childhood experiences, and adjusted for sex, age, ethnicity, childhood and adult socioeconomic position, and smoking and awakening time on the day of sample collection

[b] Intercept of diurnal pattern: model adjusted for all 14 adverse childhood experiences age in years, sex, ethnicity, childhood socioeconomic position, adult socioeconomic position, and awakening time and smoking on the day of saliva sampling.

Figure 3. Relative changes in resting heart rate and heart rate variability by the count of adverse childhood experiences



[a] rHR: resting heart rate, SDNN: standard deviation of normal-to-normal RR intervals, RMSSD: square root of successive differences of normal-to-normal RR intervals, LF: low frequency, HF: high frequency
[b] Derived from models adjusted for sex, age in years, ethnicity, childhood socioeconomic position, adult socioeconomic position, and medication intake

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- No evidence for the association of multiple adverse childhood experiences with any of salivary cortisol measurements during the day, resting heart rate, heart rate variability, or their changes over 10 years in adults.
- Of the individual adversities, only parental death showed associations with increased total amount of cortisol secretion during the day, and cortisol awakening response in adults.
- This study applied a methodology allowing for the potential differential impact of each adversity on the outcomes.