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How childhood trauma and recent adverse events are related to hair cortisol levels in a large adult cohort

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ABSTRACT

Background: Exposure to adversity is a risk factor for many mental and somatic health problems. Hypothalamic-pituitary-adrenal (HPA) axis dysregulation is a potential mechanism linking adversity exposure and negative health outcomes. However, associations between adversity exposure and HPA-axis activity have been inconsistent. To understand the impact of adversity on the HPA-axis, we examined associations between early-life and recent adversity with hair cortisol concentration, an indicator of long-term systemic cortisol levels.
Methods: We included 1166 adult participants of the Netherlands Study of Depression and Anxiety (NESDA). Hair cortisol was measured in 3 cm of proximal hair, representing cortisol exposure during the previous 3 months. Childhood maltreatment, childhood negative life events, and recent negative life events were retrospectively assessed using interview and self-report questionnaires. Linear regression analyses were performed to assess the associations between childhood maltreatment, childhood life events and recent life events with hair cortisol. Associations with cumulative adversity exposure and with subtypes of childhood maltreatment, childhood and recent negative life events were also investigated, as were interaction effects between adversity and sex, age and psychopathology.
Results: Childhood maltreatment ($\beta = 0.034$, $p = 0.243$), childhood life events ($\beta = -0.017$, $p = 0.544$), and recent life events ($\beta = -0.021$, $p = 0.456$) were not significantly associated with hair cortisol levels. Subtypes of childhood maltreatment and specific childhood and recent life events were not significantly associated with hair cortisol ($p_{FDR} > 0.05$). There were no significant interaction effects between adversity exposure and sex, age or depression/anxiety diagnostic status on hair cortisol.
Conclusions: There were no significant associations between childhood and recent adversity with systemic cortisol levels in adults. Effects of early-life and adult adversity are complex and may not directly impact on long-term systemic cortisol levels as measured in hair.

1. Introduction

Acute and chronic exposure to psychosocial stressors have been widely associated to impaired physical and mental health; in particular, people experiencing adversities, both in childhood and in adulthood, are at increased risk for mental disorders, such as depression and anxiety, and for somatic diseases, such as metabolic syndrome and cardiovascular disease (Carr et al., 2013; Hughes et al., 2017; Steptoe and Kivimäki, 2013; Wegman and Stetler, 2009). Furthermore, adversity exposure is associated with less favourable psychiatric disease

trajectories and reduced treatment response (e.g. Lippard and Nemeroff, 2020), showing the need to improve our knowledge of the biological sequelae of adversity exposure. The hypothalamic-pituitary-adrenal (HPA) axis, one of the main stress-response systems, has been widely studied as a possible explanation for these associations (Heim et al., 2008; Russell and Lightman, 2019).

The hormone cortisol, the main glucocorticoid in humans, is the most well-studied regulator of the HPA-axis (Saxbe, 2008). Acute stress exposure induces a rapid increase in cortisol levels (Dickerson and Kemeny, 2004). Cortisol binds with glucocorticoids and

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mineralocorticoid receptors that, after being activated, produce a feedback inhibition signal that leads to the decrease of HPA axis activity and, consequently, to the end of the arousal state produced by the stressor (Holsboer and Ising, 2010; Pariante and Lightman, 2008). However, situations of chronic or extreme stress could lead to long-term disrupted functioning of the HPA axis, hypothesized to play a role in development of psychiatric disorders (Pariante and Lightman, 2008).

Most research on the role of cortisol in adversity and psychopathology has assessed cortisol in saliva (Staufenbiel et al., 2013). Assessing cortisol in saliva samples has been recognized as a validated measure of the retrospective release of the hormone in blood, giving an indication of acutely circulating cortisol concentrations (Kirschbaum and Hellhammer, 1994). Thus, salivary cortisol assessment as well as plasma cortisol, reflect a relatively short timeframe (minutes to hours if collected repeatedly), best fitted to assess transient and direct HPA-activity, such as daily fluctuations or responsivity to acute stress. However, acute cortisol levels are highly variable and prone to intra- and interindividual differences due to cortisol's circadian rhythm and reactivity to factors such as physical activity and food intake (Kudielka et al., 2003; Lightman et al., 2008). In more recent years, it has been possible to assess cortisol in scalp hair, a validated measure and stable retrospective marker of *long-term* systemic cortisol levels over periods of weeks to several months (one cm of scalp hair reflecting one month in time), reflecting more chronic cortisol exposure (Manenschijn et al., 2011; Noppe et al., 2015; Stalder and Kirschbaum, 2012). Thus, cortisol measurement in hair samples captures a different aspect of HPA-axis activation compared to more transient cortisol measures such as from salivary, blood or urinary samples, and can provide new avenues to understand the relation between cortisol regulation and both chronic stress and mental illness (Staufenbiel et al., 2013).

A recent meta-analysis assessed the strength and direction of the relation between traumatic experiences and hair cortisol concentrations in 28 studies in a total of 3397 participants (Khoury et al., 2019). Overall, results showed a significant positive association between adversity exposure and hair cortisol concentrations ($d = 0.213$), although significant heterogeneity between studies was observed (see further discussion below). We previously conducted a systematic review (Staufenbiel et al., 2013) and investigated the association between hair cortisol and stress exposure in adulthood, also suggesting increased hair cortisol concentrations after stress exposure. Similarly, a meta-analysis by Stalder et al. (2017) showed 22% higher median hair cortisol concentrations in relation to chronic stress in a variety of contexts such as stressful working conditions and unemployment as well as in relation to serious life events such as death of close relatives or serious illness. Interestingly, hypercortisolism seems to depend on the period of stress exposure as increased hair cortisol were observed particularly when stress exposure was ongoing at the time of study (+43%) whereas no significant association was found for exposure to stressors in the past (−9%) (Stalder et al., 2017). Fitting with these observations, a recent study by (Iob et al., 2019) observed no significant associations between childhood adversity and hair cortisol concentrations in a sample of over 3000 older adults. Indeed, cortisol levels may vary in response over time, with levels rising during acute stress exposure, and returning to baseline after stress exposure has ceased. Chronic stress exposure, particularly in childhood, has been hypothesized to lead to more long-term changes in HPA axis functioning and potentially even down-regulation, such as reduced salivary cortisol awakening levels (Bernard et al., 2017), though findings are inconsistent (Fogelman and Canli, 2018).

Overall, previous studies seem to suggest that adversity exposure may lead to (potentially transient) hyperactivation of the HPA axis as indicated by increased hair cortisol levels. However, findings are inconclusive, with previous work suggesting that increased levels of hair cortisol may be present only - or most strongly - during and in the direct aftermath of adversity, and whether increased hair cortisol levels are observed in adults exposed to childhood adversity remains to be

established. Meta-analyses described significant heterogeneity between studies in terms of samples, types of adversity exposure, and methodological differences, as well as in between-study variance in direction and magnitude of effects. They provided pooled estimates of effect-sizes across heterogeneous single-study results (d = 0.213, Khoury et al., 2019), and have indicated relevant potential moderators of associations between adversity exposure and hair cortisol levels, such as type, timing and severity of adversity exposure, and clinical status (Khoury et al., 2019; Stalder et al., 2017). However, meta-analyses do not always allow for consistent control for potential confounding variables, and may be sensitive to noise introduced by heterogeneity of sample populations and methods. Using a large-scale single study allows us to address different types and timings of adversity - alone and in combination-, assess potential moderating variables, and rigorous control for potential confounders, within a single sample using consistent methodological assessment across individuals.

Therefore, the present research aimed to investigate, in the largest sample to date, the association between psychosocial adversity exposure both in childhood and in adulthood, with hair cortisol concentrations measured in adulthood, seen as a stable measure of long-term cortisol regulation. To establish potential effects of type and timing of adversity exposure on hair cortisol levels, distinct adversity measures were included. As primary research questions, we aimed to establish if measures of childhood maltreatment, childhood life events and recent adult life events were associated with hair cortisol levels in adulthood. Secondly, in exploratory analyses we further investigated specific characteristics of adversity exposure. As it has been suggested that HPA functioning in adulthood may be affected particularly by accumulation of early- and later-life adversity (e.g. Daskalakis et al., 2013), we investigated a cumulative adversity index combining childhood and adult adversity exposure in relation to adult hair cortisol levels. Also, HPA functioning may be differentially affected by specific types of adversity exposure, such as neglect relative to abuse. For example, in children, neglect has been associated with reduced salivary cortisol and hair cortisol levels, relative to *increased* cortisol levels associated with abuse, though findings have not been consistent (Bruce et al., 2009; Schalinski et al., 2019; Fogelman and Canli, 2018; Bernard et al., 2017). In the meta-analysis of hair cortisol by Khoury et al. (2019), moderation by adversity type could not be assessed reliably due to a paucity of studies. Therefore, we explored associations between hair cortisol and specific subtypes of childhood maltreatment, such as emotional neglect, psychological, physical and sexual abuse, and with specific life events, such as death of a parent or loved one, end of a relationship or unemployment. Then, as HPA axis dysregulation has previously been observed particularly in participants with severe forms of childhood adversity (Kuzminskaite et al., 2020; Bernard et al., 2017), exploratory extremes-comparison analyses investigated this as well. Furthermore, demographic and health characteristics that have previously been associated with both adversity exposure and/or hair cortisol levels, i.e. sex, age, presence of depression and anxiety disorders, depression severity, and metabolic factors (e.g. Staufenbiel et al., 2015; Gerritsen et al., 2019) were explored as potential moderating or explanatory variables. Lastly, to assess robustness of any observed associations between childhood maltreatment and hair cortisol levels, we included sensitivity analyses using a different measure of childhood maltreatment that was available in the current sample. Thus, a deeper investigation could shed light on the consequences of adversities during life on biological mechanisms and may explain the cascade of processes that could link adversity exposure to psychopathology and impaired health.

2. Methods

2.1. Study sample

Analyses were conducted on data previously collected within the Netherlands Study of Depression and Anxiety study (NESDA), an

ongoing naturalistic longitudinal cohort study exploring predictors, course and consequences of depression and anxiety on a sample of 2981 participants including healthy controls and people with remitted or current diagnosis of depressive and/or anxiety disorder. The Ethical Review Board of the VU University Medical Centre and the review boards of all participating centres approved the study protocol. For further information about the NESDA cohort, see Penninx et al. (2008).

The study sample consisted of a subgroup of 2256 subjects that participated at the 6-year follow-up wave of NESDA, when hair samples collection was included. Individuals were selected if they gave permission, had a sufficient number of hairs on the posterior vertex position of the scalp and the hair sample had a minimum weight of 5 mg. Considering these inclusion criteria, 1677 out of 2256 participants (74.3%) contributed hair.

Following methods previously used in this sample (Gerritsen et al., 2019; Staufenbiel et al., 2015), participants were excluded in case of a diagnosis of bipolar disorder in the last year (n = 22), use of lithium (n = 11), use of systemic or local corticosteroids in the past three months (n = 320), or in case of measurement errors in hair cortisol assessments due to contamination of the hair sample by other hormones, such as steroids or metabolites (n = 98). Thus, the final study sample included 1166 subjects with available cortisol data. The participants included in the final sample were more often female ($\chi^2(1) = 65.514$, $p < 0.001$), younger (t(2254) = − 3.372, p = 0.001) and nominally more educated (t(2254) = 1.935, p = 0.053) compared to the participants from this measurement wave that were not included in the current hair cortisol analyses (n = 1090) (see Supplementary Table S1).

2.2. Hair cortisol measurement

Long-term cortisol variation was assessed at the NESDA 6-year follow-up measurement, by analysing its secretion and incorporation in hair samples, a well-validated measure shown to correlate with cortisol assessed in saliva, plasma and urine (for overview, see Stalder and Kirschbaum, 2012). Approximately 100 hairs were cut in strands as adjacent as possible from the posterior vertex position of the scalp. Considering that scalp hairs have a mean growth rate of one cm per month (Wennig, 2000), analyses were computed on the most proximal three cm from the scalp assuming that this sample would represent the secretion of cortisol in the previous three months for each subject. To assess cortisol levels, hair samples were meticulously cut with surgical scissors and their weight was calculated; afterwards, the procedure for cortisol extraction consisted in overnight incubation with 1.4 mL LC-MS grade methanol and in presence of 100 μL internal standard (cortisol-d4) for 18 h at 25 °C while gently shaking (Staufenbiel et al., 2015). More information about this hair analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS) including matrix interferences can be found elsewhere (Noppe et al., 2015). At time of measurement of hair cortisol assessment (i.e. 6-year follow-up), participants were on average 46.90 years old (SD = 13.29, range 23–72).

2.3. Adversity exposure

2.3.1. Childhood maltreatment - Childhood Trauma Interview

Childhood maltreatment and childhood negative life events were assessed at NESDA baseline interview with the Childhood Trauma Interview (CTI, Hovens et al., 2010) as used in the Netherlands Mental Health Survey and Incidence Study (NEMESIS, e.g. de Graaf et al., 2002) which explores traumatic events prior to the age of sixteen years. Childhood maltreatment was measured as exposure to (a) emotional neglect, defined as lack of parental attention or support, or ignorance of one's problems and experiences; (b) psychological abuse, defined as unjustified punishment, verbal abuse, subordination or blackmailing; (c) physical abuse, defined as being kicked or hit with hands or objects, or physical abuse in any other way; and (d) sexual abuse, defined as being sexually approached against ones will, being touched or being obliged to

touch someone in a sexual way (Hovens et al., 2010). In a face-to-face interview, after each form of maltreatment was specifically defined, participants were asked whether they had experienced any of these kinds of abuse before the age of 16, and in case of affirmative answer, they were asked to report the frequency of these events on a 5-point scale (1 = "Once"; 2 = "Sometimes"; 3 = "Regularly"; 4 = "Often"; 5 = "Very often"). Frequency of each type of maltreatment was recoded to a 3-point scale (0 = "Never"; 1 = "Once, sometimes"; 2 = "Regularly, often, very often") following previous work (e.g. Hovens et al., 2010), as these were considered qualitatively comparable categories, and as depending on the maltreatment type either the low or high scores (i.e. "Once", or "Often"/"Very often") were reported very infrequently (< 1%). Based on these, a total childhood maltreatment score was computed from the presence and frequency of the four different types of childhood maltreatment variables before the age of 16 (minimum/maximum score 0–8) (Hovens et al., 2010), with higher scores indicating more types and a higher frequency of childhood maltreatment. Next to the total childhood maltreatment score, the four maltreatment types (emotional neglect, psychological abuse, physical abuse, sexual abuse) were also used as separate predictors (range 0–2).

2.3.2. Childhood negative life events - Childhood Trauma Interview

As part of the Childhood Trauma Interview, three indicators of childhood negative life events – before the age of 16 - were included: (a) separation, such as being placed in a juvenile prison or being raised in a foster family, (b) divorce of parents and (c) parental loss. Each category was recorded in a different binary variable with a score of either 0 (not happened) or 1 (happened). Based on this, an overall number of childhood life events score was computed as the sum of the experienced events ranging from 0 to 3. Next to the total childhood life events score, the three indicators (separation, divorce, parental loss) were also used as separate predictors (range 0–1). At time of measurement of the Childhood Trauma Interview (i.e. baseline), participants were on average 40.86 years old (SD=13.30, range 18–65).

2.3.3. Recent negative life events - Brugha List of Threatening Events Questionnaire

Recent exposure to negative life events was assessed using the Brugha List of Threatening Events Questionnaire (Brugha et al., 1985). This questionnaire investigates experience of negative life events with a list of 12 experiences that have been associated with marked or moderate long-term stress (Brugha et al., 1985) (a) Being seriously ill, wounded or victim of violence; b) Close relative being seriously ill, wounded or victim of violence; c) Parent, child, sibling or partner died; d) Good friend or close relative died; e) Separation from partner; f) Ended a longstanding relationship with a friend or relative; g) Serious problem with a close friend, relative or neighbour; h) Became unemployed or looked for a job without result; i) Being fired from job; j) Facing serious financial problems; k) Contact with the police or court by misdemeanour; l) Money or something valuable was stolen or lost). For each event, participants indicated if they had experienced this event (yes/no) since the previous NESDA measurement. For each endorsed event, the specific timing (date) was assessed, allowing for calculation of the events experienced in the past 12 months. An overall number of recent life events scale was computed as the sum of the negative life events endorsed in the past 12 months (min/max score 0–12). Also, total number of recent life events for the past three months was computed, to coincide with the period reflecting hair cortisol assessment. Next to the total 12- and 3-month recent life events scores, individual endorsements of the 12 indicators of specific life events in the past 12- or 3-months were also used as separate predictors (range 0–1).

2.3.4. Cumulative adversity index

To assess potentially cumulative effects of childhood maltreatment, childhood life events and recent life events, a cumulative adversity index was calculated by transforming childhood maltreatment, childhood life

events and recent life events scores to z-scores, and subsequently adding the three z-scores (Vinkers et al., 2014).

2.3.5. Childhood maltreatment - Childhood Trauma Questionnaire

Within the NESDA sample, an alternative measure of childhood maltreatment was available; the Childhood Trauma Questionnaire Short-Form (CTQ-SF; Bernstein et al., 1994), assessed at 4-year follow-up. Interviews have been shown to be more reliable retrospective investigations of childhood trauma (Baldwin et al., 2019), and the Childhood Trauma Interview (see 2.3.1) was therefore chosen as our primary predictor. However, the Childhood Trauma Questionnaire was used to run additional sensitivity analyses. This allowed us to assess robustness of any observed associations between childhood maltreatment and hair cortisol levels across different types and timepoints of assessment. In the current sample, scores from the Childhood Trauma Questionnaire showed a moderate-to-strong correlation with scores on the Childhood Trauma Interview assessed 4 years apart (Spearman's $\rho = 0.681$, $p < 0.001$) (see Spinhoven et al., 2014 for an in-depth comparison). The Childhood Trauma Questionnaire and Childhood Trauma Interview assess largely overlapping constructs but are not completely synonymous; The Childhood Trauma Questionnaire assesses a broader age range (i.e. including teenage years vs. before age of 16), and was slightly more sensitive in detecting emotional abuse and emotional neglect compared to the Childhood Trauma Interview in the current sample (Spinhoven et al., 2014). Also, as people differ in likelihood to disclose adverse experiences in either self-report assessment or face-to-face interviews, these modes of assessment can be complimentary (Spinhoven et al., 2014). Thus, including the Childhood Trauma Questionnaire as a secondary measure next to the Childhood Trauma Interview will 1). provide an opportunity to validate the primary findings, and 2). provide the most complete and sensitive overview of childhood adversity, thereby reducing chances of adversity being missed in use of only one measure of adversity.

The Childhood Trauma Questionnaire includes 25 retrospective self-report questions concerning experiences of emotional neglect and abuse, physical neglect and abuse, and sexual abuse while growing up as a child and teenager, and 3 control items. Each item is scored on a Likert-scale ranging from 1 ("Never true") to 5 ("Very often true"). The alternative childhood maltreatment score was computed as the total score of the 25 abuse and neglect items of the CTQ-SF (minimum/maximum score 25–125). Next to the total Childhood Trauma Questionnaire score, scores on the five maltreatment type subscales (emotional neglect, emotional abuse, physical neglect, physical abuse, sexual abuse) were used as separate predictors (ranges 5–25). The subscales "emotional neglect", "physical abuse" and "sexual abuse" of the Childhood Trauma Questionnaire aim to measure the same constructs as the Childhood Trauma Interview subscales with the same names; the "Emotional abuse" subscale is synonymous to "Psychological abuse" subscale of the Childhood Trauma Interview (Spinhoven et al., 2014). The "Physical neglect" subscale is unique to the Childhood Trauma Questionnaire and not included in the Childhood Trauma Interview.

The Childhood trauma questionnaire was administered at 4-year follow-up, 2 years prior to hair cortisol measurement and 4 years after NESDA baseline interview at which the Childhood Trauma Interview was administered. At time of measurement of the Childhood trauma questionnaire (i.e. 4-year follow-up), participants were on average 45.00 years old (SD = 13.34, range 21–70).

2.4. Covariates

Previous observations of determinants of hair cortisol concentrations in the current sample described significant effects of age, sex, hair colour, hair washing frequency, season of hair assessment, use of oral contraceptives, diabetes and waist circumference (Staufenbiel et al., 2015), as well as depression symptom severity and anti-depressant use, specifically selective serotonin reuptake inhibitors (SSRI's) (Gerritsen et al., 2019). Following these results, all analyses were controlled for the following variables as potential confounders (Model 1); age in years, sex (male, female), hair washing frequency (0–2 times/week, 3 or more times/week), dummy-coded variables for hair colour (brown, red, grey, black, reference = blond), season of hair assessment (spring, summer, fall, reference = winter), oral contraceptive use (no, yes).

As the psychopathology and metabolic factors associated with hair cortisol in the current sample (Gerritsen et al., 2019; Staufenbiel et al., 2015) are often increased in associations with adversity exposure, these were seen as potential explanatory variables. As a second step, analyses were therefore additionally controlled for psychopathology severity and metabolic factors, i.e. severity of depressive symptoms, antidepressant use, diabetes mellitus and waist circumference (Model 2a). Antidepressant use (no, yes) was defined as current frequent use (i.e. > 50% of days) of SSRI's, TCA's, or other types of antidepressants (ACT-code N06A). Severity of depressive symptoms was assessed using the sum-score of the 30-item Inventory of Depressive Symptomatology Self-Report (IDS-SR; Rush et al., 1996), assessing severity of a broad range of depressive symptoms over the past 7 days. Each question scored on a 4-item scale from none to severe symptoms (min/max score 0–84). Diabetes was assessed as self-report of being under treatment of a physician for diabetes mellitus (both type 1 and type 2) and waist circumference (cm) was assessed by a trained research assistant at the central point between the lowest front rib and the highest front point of the pelvis. As depression severity measured with the IDS was most strongly associated with hair cortisol concentrations relative to psychiatric status and anxiety symptoms in a previous report of the current sample (Gerritsen et al., 2019), and depression severity and anxiety severity symptoms (Beck Anxiety Index (BAI) score) were strongly correlated ($r = 0.722$), we included only depression severity scores in Model 2a.

However, to test if associations between adversity and hair cortisol concentrations were also independent of remitted and current diagnosis of depressive and/or anxiety disorders we additionally performed Model 2b; which included lifetime psychiatric diagnostic status as a covariate instead of severity of depressive symptoms (2 dummy-coded variables: remitted depressive and/or anxiety disorder (no, yes); current depressive and/or anxiety disorder (no, yes); reference group: healthy controls without lifetime or current depressive or anxiety disorders). Depressive and anxiety diagnoses were assessed with CIDI clinical interview using DSM-IV criteria (Robins et al., 1988). Education, smoking and alcohol intake were not included in the model as covariates because these were not significantly associated with hair cortisol concentrations in the current sample (Staufenbiel et al., 2015).

2.5. Statistical analysis

2.5.1. Descriptive analyses

Hair cortisol levels was not normally distributed; therefore, descriptive analyses are reported as median and interquartile range (IQR). For further analyses using hair cortisol concentrations, a logarithmic transformation was computed to obtain normal distribution. For other variables, descriptive characteristics were investigated and reported as mean (M) and standard deviation (SD) for continuous variables, whereas categorical variables were described in size (n) and percentage (%). For descriptive purposes, correlation analyses were conducted across all study variables, using Spearman's rank order correlations (for this purpose, categorical variables with > 2 non-ordinal levels were transformed to dummy-coded dichotomous variables).

2.5.2. Associations between adversity exposure and hair cortisol concentrations

Primary analyses were performed to explore the association between hair cortisol and psychosocial stressors. Three separate multivariate linear regression analyses were assessed with hair cortisol concentrations as dependent variable and adversity exposure scales (childhood

maltreatment, childhood negative life events, recent negative life events) as predictors. Furthermore, we assessed if there was a cumulative effect of childhood and recent adversity exposure on hair cortisol by assessing associations with the cumulative adversity index (see 2.3, Adversity exposure). All regression analyses were run initially with sociodemographic and hair characteristics as covariates, and repeated with potential explanatory variables, i.e. additional psychopathology and metabolic factors known to be associated with adversity exposure and hair cortisol concentrations (Gerritsen et al., 2019; Staufenbiel et al., 2015). In model 1, we corrected for age, sex, hair colour, hair washing frequency, season of hair assessment and use of oral contraceptives. Correction for multiple comparisons was done using false discovery rate (FDR) (Benjamini and Hochberg, 1995). Results were considered to be significant with an FDR-corrected p-value lower than 0.05. All analyses were assessed using SPSS 24.0 for Windows (IBM, Chicago, Illinois).

Several follow-up analyses were conducted; the main analyses stated above (Model 1) were repeated while additionally correcting for psychopathology severity and metabolic factors as potential explanatory variables (i.e. severity of depressive symptoms, use of antidepressant medication, diabetes and waist circumference) (Model 2a). A variant of model 2a was also conducted, correcting for lifetime psychopathology diagnostic status instead of severity of depressive symptoms (i.e. adding 2 dummy-coded variables; current depressive and/or anxiety disorders, remitted depressive and/or anxiety disorders) (Model 2b). Also, recent life events analyses were repeated looking at total number of life events in the past 3 months, to coincide with the period reflected in hair cortisol assessment. We were also interested to test associations between childhood and recent adversity, while controlling for life events during the period reflected in hair cortisol assessment, in case current events may obscure associations between hair cortisol and prior adversity. To do so, Model 1 was performed as described above, but additionally controlling for number of recent life events in the past 3 months (Model 3).

2.5.3. Exploratory analyses investigating subtypes of adversity exposure

Furthermore, in exploratory analyses, individual subtypes of childhood maltreatment, childhood life events and recent life events adversity exposure were investigated in relation to hair cortisol concentrations (see 2.3, Adversity Exposure) by including each of these subtypes of childhood maltreatment, and childhood or recent life events separately as a predictor in Model 1 instead of the total scores for Childhood maltreatment, Childhood life events, or Recent life events.

2.5.4. Interactions with age, sex and psychopathology

Next, analyses were run to explore potential modifiers of adversity effects, by including the following interaction terms to the original models: age by adversity, sex by adversity, depression severity by adversity, and diagnostic status by adversity. Hereby we aimed to check if associations between adversity exposure and hair cortisol concentrations were potentially dependent on age or depression severity, or different between men and women, or different between healthy controls, people with remitted depression/anxiety diagnosis and people with current depression/anxiety diagnosis.

2.5.5. Sensitivity analyses for childhood maltreatment

Additionally, since a secondary measure of childhood maltreatment was available (i.e. Childhood Trauma Questionnaire, CTQ-SF; Bernstein et al., 1994), assessed with a different instrument and at a different time point, we repeated the childhood maltreatment analyses with childhood maltreatment scores based on the Childhood Trauma Questionnaire-Short Form instead of the Childhood Trauma Interview (de Graaf et al., 2002) to assess robustness of any observed associations between childhood maltreatment and hair cortisol levels. If two different types and timepoints of childhood maltreatment assessment would show similar associations with the primary outcome measure, that would signify a robust association and underline our confidence in

the observed associations. The Childhood Trauma Questionnaire-Short Form was available for 1106 participants of the hair cortisol sample. Childhood maltreatment analyses were repeated as above, while replacing childhood maltreatment scores based on the Childhood Trauma Interview with total Childhood Trauma Questionnaire-Short Form scores.

2.5.6. Exploratory extremes-comparison analyses

To check if associations between adverse events and hair cortisol concentrations might be obscured by psychiatric status in participants not exposed to adversity (Gerritsen et al., 2019), or if associations were only present in more extreme exposure to adversity (Kuzminskaite et al., 2020), additional exploratory analyses were performed comparing healthy controls without adversity exposure *and* without lifetime diagnosis of depressive/anxiety disorders (i.e. reference group), to participants with mild adversity exposure and to participants with severe adversity exposure (following procedures of Kuzminskaite et al., 2020), to allow for a more extreme, clear-cut contrast. Three separate analyses were run, for childhood maltreatment, for childhood life events and for recent life events. Healthy controls without adversity exposure and without psychopathology were the reference group, and dummy variables for mild and for severe adversity exposure were used to compare mild and severe exposure to the reference group. In each analysis an additional dummy was included for participants without that particular type of adversity exposure but with lifetime or current depressive/anxiety disorders to control for psychopathology in participants without adversity exposure. Cut-offs were implemented as followed: Healthy controls with no lifetime depressive or anxiety diagnosis and childhood maltreatment score = 0 (n = 197), childhood life events score = 0 (n = 206), recent life events score = 0 (n = 130); Controls with lifetime depressive or anxiety diagnosis and childhood maltreatment score = 0 (n = 427), childhood life events score = 0 (n = 731), recent life events score = 0 (n = 424); Mild adversity exposure: childhood maltreatment score = 1–3 (n = 313), childhood life events score = 1 (n = 177), recent life events score = 1 (n = 360); Severe exposure: childhood maltreatment score ≥ 4 + (n = 217), childhood life events score ≥ 2 (n = 35), recent life events score ≥ 2 (n = 243).

3. Results

3.1. Sample characteristics

The characteristics of the study sample (n = 1166) are reported in Table 1. 74.1% of the study sample was female and participants were, on average, 47 years old (range 23–72 years). A large portion of the sample had a current or remitted diagnosis of depressive and/or anxiety disorder (current 28.6%, remitted 50.6%) and 19.4% of participants currently used antidepressants. Around one-fifth of the sample had experienced one or more childhood negative life events; 4.5% were separated from their home during childhood, 11.5% experienced a divorce of parents and 5.4% parental death during childhood. In terms of childhood maltreatment, 35.1% reported emotional neglect, 21.5% psychological abuse, 11.2% physical abuse and 18.4% sexual abuse before the age of 16 years. Half of the sample (51.7%) reported to have experienced at least one type of negative life event in the past year; 0.88 (SD = 0.99) negative life events were experienced on average in the sample. Childhood maltreatment scores were positively associated with age ($\rho = 0.137$, $p < 0.001$) and higher in females (t(608.164) = − 4.278, $p < 0.001$). Number of childhood life events was negatively associated with age ($\rho = − 0.074$, $p = 0.011$) and did not differ between males and females (t(1164) = − 0.838, $p = 0.402$). Number of recent life events in the past 12 months was not associated with age ($\rho = − 0.048$, $p = 1.00$) or sex (t(1163) = − 0.506, $p = 0.613$). A correlation matrix describing correlations between all study variables can be found in Supplementary Table S2.

Table 1
Sample characteristics (n = 1166).

Demographic	
Sex (male/female), n (%)	302/864 (28.9%/74.1%)
Age, M (SD), [range]	46.90 (13.29), [23–72]
Education, years, M (SD), [range]	13.00 (3.34), [5–18]
Endocrine measures	
Hair cortisol concentration (pg/mg), median (IQR), [range]	3.26 (2.20–5.47), [0.34–212.05]
Hair characteristics	
Hair washing frequency, n (%)	
0–2 times per week	346 (29.7%)
3 + times per week	820 (70.3%)
Hair colour, n (%)	
Brown	389 (33.4%)
Red	27 (2.3%)
Grey	211 (18.1%)
Black	46 (3.9%)
Blond	493 (42.3%)
Season of hair assessment, n (%)	
Winter	291 (25.0%)
Spring	278 (23.8%)
Summer	290 (24.9%)
Autumn	307 (26.3%)
Psychopathology	
Diagnostic status	
No lifetime depression or anxiety	242 (20.8%)
Current depression and/or anxiety	334 (28.6%)
Remitted depression and/or anxiety	590 (50.6%)
Depressive symptom severity score (IDS), M (SD), [range]	14.37 (11.38), [0–65]
Antidepressant use, n (%)	226 (19.4%)
Health	
Waist circumference (cm), M (SD), [range]	90.38 (13.62), [50–162]
Current diabetes under treatment, n (%)	43 (3.7%)
Use of hormonal contraceptives, n (%)	120 (10.3%)
Adversity exposure	
Any childhood maltreatment (none/>=1), n%	633 / 530 (54.4% / 45.6%)
Total childhood maltreatment score, M (SD), [range]	1.44 (2.01), [0–8]
Emotional neglect	
Sometimes, n (%)	57 (4.9%)
Regularly, n (%)	353 (30.3%)
Psychological abuse	
Sometimes, n (%)	49 (4.2%)
Regularly, n (%)	202 (17.4%)
Physical abuse	
Sometimes, n (%)	66 (5.7%)
Regularly, n (%)	65 (5.6%)
Sexual abuse	
Sometimes, n (%)	170 (14.6%)
Regularly, n (%)	45 (3.9%)
Any childhood negative life events (none/> = 1), n%	954 / 212 (81.8% / 18.2%)
Total childhood negative life events, M (SD), [range]	0.21 (0.48), [0–2]
Divorce of parents, n (%)	134 (11.5%)
Death of a parent, n (%)	63 (5.4%)
Separation from home, n (%)	53 (4.5%)

	past 12 months	past 3 months
Any recent negative life event (none/ >=1), n%	562/603 (48.2%/ 51.8%)	931/234 (79.9%/ 20.1%)
Total recent negative life events, M (SD), [range]	0.81 (0.99), [0–7]	0.25 (0.55), [0–4]
Being seriously ill, wounded or victim of violence, n (%)	46 (3.9%)	7 (0.6%)
Close relative was seriously ill, wounded or victim of violence, n (%)	196 (16.8%)	56 (4.8%)
	63 (5.4%)	17 (1.5%)

Table 1 (*continued*)

	past 12 months	past 3 months
Parent, child, sibling or partner died, n (%)		
Good friend or close relative died, n (%)	240 (20.6%)	83 (7.1%)
Separation from partner, n (%)	60 (5.1%)	20 (1.7%)
Ended a longstanding relationship with a friend or relative, n (%)	81 (6.9%)	22 (1.9%)
Serious problem with a close friend, relative or neighbour, n (%)	57 (4.9%)	15 (1.3%)
Became unemployed or looked for a job without result, n (%)	64 (5.5%)	21 (1.8%)
Being fired from job, n (%)	27 (2.3%)	11 (0.9%)
Facing serious financial problems, n (%)	38 (3.3%)	8 (0.7%)
Contact with the police or court by misdemeanour, n (%)	15 (1.3%)	6 (0.5%)
Money or something valuable was stolen or lost, n (%)	60 (5.1%)	24 (2.1%)
Cumulative adversity index, M (SD), [range]	0.00 (1.96), [− 1.98–9.74]	
Childhood trauma questionnaire, M (SD), [range]	39.53 (13.21), [25–119]	
Emotional abuse, M (SD), [range]	8.44 (4.24), [5–25]	
Physical abuse, M (SD), [range]	5.80 (2.33), [5–25]	
Sexual abuse, M (SD), [range]	5.99 (2.78), [5–25]	
Emotional neglect, M (SD), [range]	12.01 (5.05), [5–25]	
Physical neglect, M (SD), [range]	7.30 (2.70), [5–22]	

Note: Status of depressive and anxiety disorder diagnoses were based on CIDI clinical interviews, current = depression and/or anxiety diagnosis in past 6 months, remitted = lifetime depression and/or anxiety diagnosis but no diagnosis in past 6 months. Exposure to childhood maltreatment and childhood life events based on the childhood trauma interview (CTI); exposure to recent life events based on the Brugha List of Threatening Events Questionnaire interview; Cumulative adversity index based on summed z-scores for childhood maltreatment, childhood life events and recent life events scores. Range denotes minimum and maximum score on variable in current sample. Abbreviations: M = mean, SD = standard deviation, n = number of participants, IQR = interquartile range; IDS = Inventory of depressive symptomatology.

3.2. Hair cortisol concentrations

The median hair cortisol concentration was 3.26 pg/mg (IQR 2.20–5.47) (see Table 1). As described in Staufenbiel et al. (2015), hair cortisol concentration was positively associated with age (Spearman's $\rho = 0.204$, p < 0.001, Supplementary Table S2) and was higher in men compared to women (t(1, 1164) = 2.103; p = 0.036).

3.3. Associations between adversity exposure and hair cortisol concentrations

Multivariate linear regression analyses were performed to examine the association between psychosocial stressors and hair cortisol concentration, measured in severity scales, while adjusting for sociodemographic and hair characteristics. While adjusting for sociodemographic and hair characteristics (model 1), neither childhood maltreatment score ($\beta = 0.034$, p = 0.243), number of childhood negative life events ($\beta = - 0.017$, p = 0.544) nor number of recent negative life events experienced in the past year ($\beta = - 0.021$, p = 0.456) were significantly associated with hair cortisol concentration (Table 2, Fig. 1). A cumulative adversity index of childhood maltreatment, childhood life events and recent life events also did not show any significant associations with hair cortisol levels (Table 2; model 1, $\beta = - 0.010$, p = 0.732).

Also with additional adjustment for psychopathology severity and metabolic characteristics (model 2a, additional covariates; depressive symptom severity, antidepressant use, waist circumference, diabetes),

Table 2
Associations between hair cortisol and psychosocial stressors, based on multivariate linear regression analyses (n = 1166).

	Model 1, basic adjustment				Model 2a, additional adjustment - Psychopathology severity & metabolic factors				Model 2b, additional adjustment - Psychopathology diagnostic status & metabolic factors			
	B	SE	β	p	B	SE	β	p	B	SE	β	p
Childhood maltreatment score	0.005	0.004	0.034	0.243	0.001	0.005	0.008	0.799	0.002	0.005	0.014	0.638
Childhood life events (number)	− 0.011	0.019	− 0.017	0.544	− 0.014	0.019	− 0.021	0.472	− 0.014	0.019	− 0.021	0.468
Recent life events (number)	− 0.007	0.009	− 0.021	0.456	− 0.008	0.009	− 0.026	0.364	− 0.006	0.009	− 0.020	0.488
Cumulative adversity index	− 0.002	0.005	− 0.010	0.732	− 0.004	0.005	− 0.028	0.351	− 0.003	0.005	− 0.022	0.450

Note: Model 1: adjusted for sex, age, hair colour, hair washing frequency, season of hair assessment and oral contraceptive use; Model 2a: includes covariates of model 1, plus additional adjustment for depressive symptom severity, use of antidepressants, diabetes and waist circumference; Model 2b: includes covariates of model 2a, except instead of depression symptom severity includes current depression and/or anxiety disorders and remitted depression and/or anxiety disorder. Childhood maltreatment and Childhood Life event were based on the Childhood Trauma Interview; Recent life events were measured across the past 12 months and based on the Brugha Questionnaire; Cumulative adversity index based on summed z-scores for childhood maltreatment, childhood life events and recent life events scores. Abbreviations: n = number of participants, B = unstandardized beta, SE = standard error, β = standardized beta.
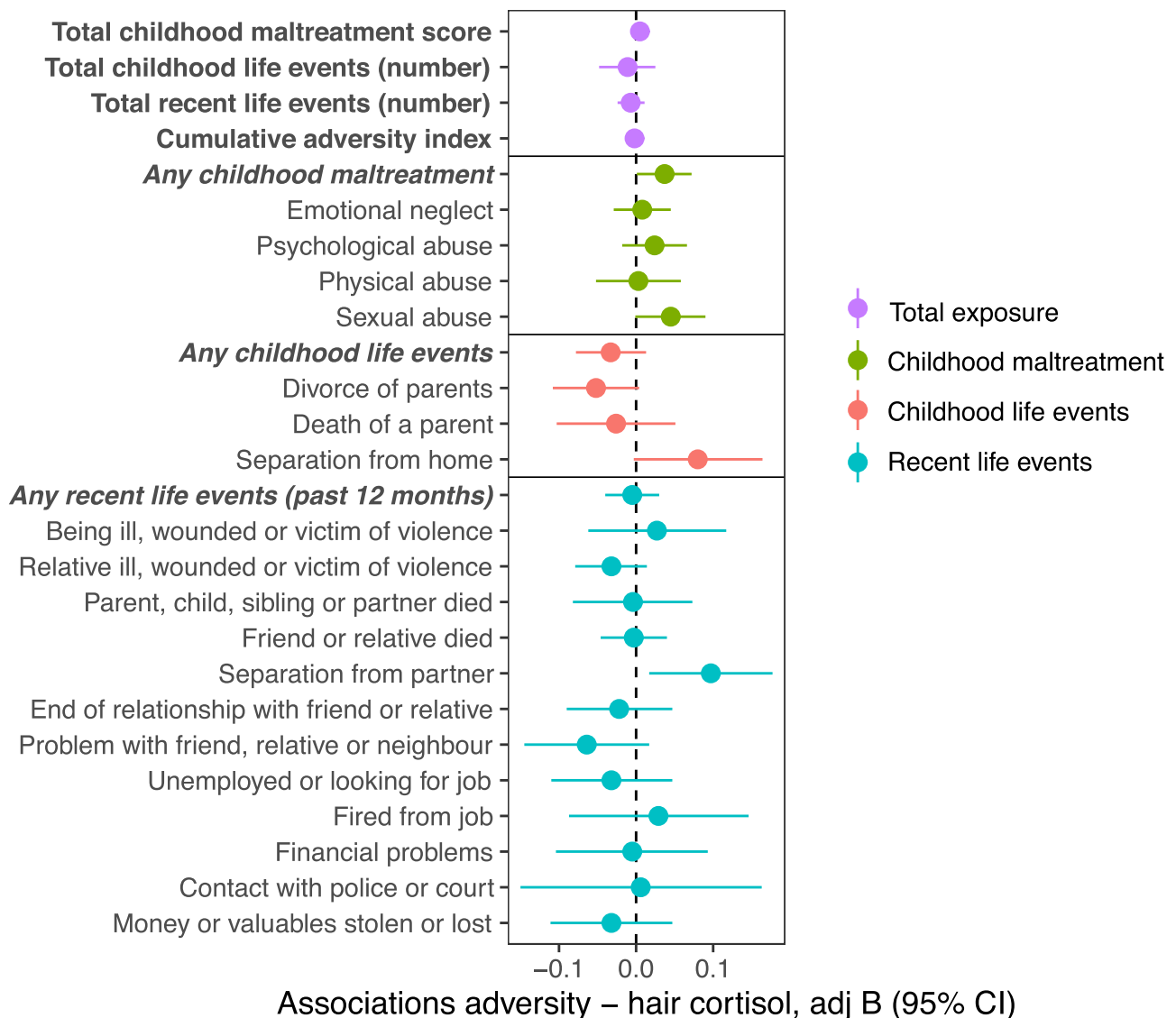

Fig. 1. Forest plot of adjusted regression estimates of associations between different types of adverse events and hair cortisol concentrations. Note: Top panel: Total exposure scores per adverse event type in the top panel; Bottom three panels: Endorsement (yes [score ≥ 1], no [score = 0]) of exposure to (subtypes of) childhood maltreatment, childhood life events and recent life events. Dots represent the adjusted regression estimates (adjusted for basic socio-demographic and hair variables [sex, age, hair colour, hair washing frequency, season and oral contraceptive use] according to model 1), horizontal lines represent 95% confidence intervals.

childhood maltreatment ($\beta = 0.008$, $p = 0.799$), childhood life events ($\beta = -0.021$, $p = 0.472$), recent life events ($\beta = -0.026$, $p = 0.364$) and the cumulative adversity index ($\beta = -0.028$, $p = 0.351$) were not significantly associated with hair cortisol (Table 2, Fig. S1).

Similarly, when adjusting for remitted and current psychiatric diagnostic status instead of current severity of depressive symptoms (model 2b), no significant associations were observed between hair cortisol and childhood maltreatment score ($\beta = 0.014$, $p = 0.638$), childhood negative life events ($\beta = -0.021$, $p = 0.468$), recent negative life events ($\beta = -0.020$, $p = 0.488$), or the cumulative adversity index ($\beta = -0.022$, $p = 0.450$) (Table 2, Fig. S1). Parameter estimates for all model covariates in model 1, 2a and 2b are provided in Supplementary Table S3.

Hair cortisol was also not significantly associated with total number of life events in the past 3 months (instead of 12 months, to coincide with the period covered by the hair assessment) (model 1, $\beta = -0.041$, $p = 0.150$), though frequency of negative life events was relatively low during this time-period, with n = 234 (20.1%) endorsing ≥ 1 events. Last, controlling for recent life events in the past 3 months did not change the main findings described above, as childhood maltreatment, childhood life events, recent life events in the past 12 months and the cumulative adversity index were not significantly associated with hair cortisol levels after controlling for recent life events in the past 3 months ($p > 0.190$, see Supplementary Table S4).

3.3.1. Exploratory analyses investigating subtypes of adversity exposure

Further exploration aimed to check whether there were any associations between exposure to specific subtypes of childhood maltreatment, childhood negative life events, or recent negative life events and hair cortisol concentrations (Fig. 1, Supplementary Table S5). There were no significant associations with any of the early or recent event or maltreatment subtypes ($p > 0.05$) with hair cortisol, with one exception; partner separation in the past 12 months being associated with increased hair cortisol ($\beta = 0.069$; $p = 0.017$), though this was no longer significant after FDR-correction for multiple comparisons (Supplementary Table S5). Also, exposure to any form of childhood maltreatment (childhood maltreatment score ≥ 1 vs 0) was associated with increased hair cortisol ($\beta = 0.059$; $p = 0.042$), though also no longer significant after FDR-correction.

3.3.2. Interactions with age, sex and psychopathology

Though there were significant main effects of age, sex and depressive symptoms on hair cortisol concentrations (previously described in Gerritsen et al., 2019; Staufenbiel et al., 2015) we did not observe any significant interaction effects of age, sex, depressive symptoms or depression and/or anxiety diagnosis with childhood maltreatment score, number of childhood life events or number of recent life events on hair cortisol (Model 1, all $p > 0.063$, see Supplementary Table S6). This suggests the (lack of) associations between adversity measures and hair cortisol were the same across age groups, within men and women, and comparable within healthy controls, people with remitted depression/anxiety disorders and people with current depression/anxiety disorders.

3.3.3. Sensitivity analyses for childhood maltreatment

Sensitivity analysis using CTQ scores as alternative measure for childhood maltreatment similarly yielded no significant results; CTQ total score was not significantly associated with hair cortisol (model 1, n = 1.108, $\beta = 0.024$, $p = 0.420$), and none of the CTQ subscales were associated with hair cortisol (all $p > 0.263$, see Supplementary Table S7).

3.3.4. Exploratory extremes-comparison analyses

Severe childhood maltreatment (childhood maltreatment score ≥ 4, n = 217) relative to healthy controls with no adversity exposure and no lifetime diagnosis of depression or anxiety (n = 198) was not associated

with different levels of hair cortisol ($\beta = 0.021$; $p = 0.578$); Severe childhood life events (childhood life events score ≥ 2, n = 35) relative to healthy controls (n = 208) was not associated with hair cortisol ($\beta = 0.050$; $p = 0.099$); and likewise severe exposure to recent life events (recent life events score ≥ 3, n = 243) relative to healthy controls (n = 132) was not associated with hair cortisol ($\beta = 0.029$; $p = 0.499$) (Table 3, Supplementary Fig. S2). Mild exposure to childhood maltreatment, childhood life events or recent life events, and lifetime depression or anxiety without adversity exposure were also not significantly associated with hair cortisol concentrations relative to healthy controls with no adversity exposure and no lifetime diagnosis of depression or anxiety ($p > 0.135$, see Table 3).

4. Discussion

The aim of this study was to explore the association between early-life and recent exposure to psychosocial adversity and long-term systemic cortisol levels, as measured in scalp hair, in a large adult cohort. We did not observe any significant associations between cumulative scores of childhood maltreatment, childhood negative life events, or recent negative life events and hair cortisol concentrations. Follow-up analyses investigating specific types of childhood or recent adverse events (such as physical abuse, loss of a parent, job loss) also did not reveal any significant associations with hair cortisol concentrations. Only partner separation in the past 12 months seemed to be associated with marginally higher hair cortisol; however, this association did not survive FDR-correction for multiple comparisons and disappeared after correction for metabolic factors. Furthermore, we did not observe any significant associations in more extreme levels of exposure, additive effects of different types of adversity, nor were there indications of interactions between early- or recent adversity and sex, age or psychopathology.

Table 3

Associations between mild and severe adversity exposure and hair cortisol levels, based on multivariate linear regression analyses.

	Model 1, basic adjustment			
	B	SE	β	p
Childhood Maltreatment (CM)				
Healthy, no CM (n = 198)	ref.			
Depr/anx, no CM (n = 435)	− 0.019	0.026	− 0.030	0.459
Mild CM (n = 313)	0.028	0.028	0.040	0.316
Severe CM (n = 217)	0.016	0.030	0.021	0.587
Childhood Life Events (CLE)				
Healthy, no CLE (n = 208)	ref.			
Depr/anx, no CLE (n = 746)	0.029	0.024	0.045	0.221
Mild CLE (n = 177)	− 0.030	0.031	− 0.035	0.334
Severe CLE (n = 35)	0.091	0.055	0.050	0.099
Recent Life Events (RLE)				
Healthy, no RLE (n = 132)	ref.			
Depr/anx, no RLE (n = 430)	0.054	0.030	0.084	0.076
Mild RLE (n = 360)	0.047	0.031	0.069	0.135
Severe RLE (n = 243)	0.022	0.033	0.029	0.499

Note: Multiple regression results on associations between mild and severe adversity exposure, relative to healthy controls without adversity, and hair cortisol levels. Model 1: adjusted for sex, age, hair colour, hair washing frequency, season of hair assessment, and oral contraceptive use. Reference group is Healthy, no adversity = score 0 on relevant adversity measure and no current or remitted depressive or anxiety disorder; Depr/anx, no adversity = score 0 on relevant adversity measure and current or remitted depressive or anxiety disorder; Mild childhood maltreatment = score 1–3, severe childhood maltreatment = score > = 4; Mild childhood life events = score 1, severe childhood life events = score > = 2; Mild recent life events = score 1, Severe recent life events = score > = 2. Childhood maltreatment and Childhood Life event were based on the Childhood Trauma Interview; Recent life events were measured across the past 12 months and based on the Brugha Questionnaire. Abbreviations: B = unstandardized beta, SE = standard error, β = standardized beta, ref. = reference group.

The observed standardized beta coefficients were small (β < [− 0.021, 0.035]), and at first sight, our results do not seem to corroborate previous meta-analytic observations of increased hair cortisol in relation to adversity exposure (Khoury et al., 2019; Stalder et al., 2017). However, within subgroup analyses, these meta-analytic reviews observed increased hair cortisol concentrations only in samples with adult adversity exposure and not in samples with childhood adversity (Khoury et al., 2019), and only in samples with ongoing stress exposure but not when stress exposure had ceased (Stalder et al., 2017). A recent study in the largest sample to date (n = 3357) also showed no association between childhood adversity and hair cortisol in adulthood (Iob et al., 2019), and small regression coefficients comparable to ours. Together, these findings fit with our observation of a lack of associations between childhood adversity exposure and adult hair cortisol. Possibly, childhood adversity may have been associated with altered hair cortisol levels when measured more proximal to the time of exposure, but if any changes occurred, these were transient in nature and no longer detectable in adulthood. We did not observe significant associations between recent adversity exposure in adulthood and hair cortisol, which were reported by Stalder et al. (2017) and Khoury et al. (2019). A possible explanation is that long-term hypercortisolism induced by chronic psychosocial stress diminishes after the stress exposure has ceased (Stalder et al., 2017). It is likely that the recent life events, assessed over the past 12 months in the current study, were not always still ongoing at the time of cortisol measurement, and effects may no longer have been present or measurable in hair. However, we also did not observe any association between adversity exposure in the past 3 months, coinciding with the timeframe of hair assessment. On the other hand, downregulation of HPA axis activity, leading to (salivary) *hypo*-cortisolism has also been reported in response to severe and/or chronic stress (e.g. institutionalized children, Bernard et al., 2017), which may dilute group differences. Though we could not establish chronicity of exposure with the instruments used in the current sample, subgroup analyses did not indicate hypo- (or hyper-) cortisolism in response to severe exposure. Future studies of adversity exposure may therefore want to inform whether or not adversity exposure is still ongoing and address severity, intensity and chronicity of adversity exposure. Another important point to address is that even if there is no association between prior adversity exposure and current hair cortisol levels, temporary stress-induced changes in HPA-functioning in the immediate aftermath of adversity may still have long-term effects on health via other biological pathways, such as changes in gene expression, glucocorticoid receptor density, neurotransmitter functioning, or brain structure (e.g. De Bellis and Zisk, 2014; McCrory et al., 2010).

As our analyses were run in a large single sample, with consistent assessment across participants, investigating multiple types and timings of adversity and moderating variables, our data and similar large-scale studies such as Iob et al. (2019) provide an additional perspective to meta-analytic observations, allowing to check the associations suggested by many small studies within a single study. Of relevance, associations between adversity and hair cortisol observed by Iob et al. (2019) and the current study were weak, and markedly smaller relative to the earlier reported meta-analytic observations (Khoury et al., 2019; Stalder et al., 2017). Together, these complimentary approaches provide a more nuanced view on the association between adversity and hair cortisol levels.

Interestingly, previous meta-analyses in *salivary* cortisol have also suggested a lack of associations between adversity exposure and cortisol levels (Bernard et al., 2017; Fogelman and Canli, 2018). Subgroup analyses by Bernard et al. (2017) suggested cortisol dysregulation may be present only in more extreme or specific cases, as they observed stronger dysregulation in the form of blunted cortisol awakening responses in agency-referred children (e.g. child welfare, institutionalized children) compared to studies relying on self-reported maltreatment (possibly due to more reliable reporting and/or more extreme maltreatment). In adults included in the current NESDA study, Kuzminskaite et al. (2020) observed associations in the opposite direction, with higher levels of salivary cortisol markers only in adult participants with the highest levels of childhood maltreatment compared to healthy controls without childhood adversity or psychopathology, though this was partially explained by differences in smoking, BMI and chronic diseases. However, our subgroup analyses, similar in design to those by Kuzminskaite et al. (2020), severe adversity exposure groups did not yield significantly different hair cortisol levels relative to non-exposed healthy controls. This may indicate that it is more difficult to disentangle associations with hair cortisol compared to salivary cortisol measures, and different measures of cortisol may yield different results. Possibly, effects of adversity exposure may be present only in short-term salivary cortisol responsivity, or perhaps only in cortisol responses to acute stressors, as was suggested by a meta-analysis reporting blunted salivary cortisol responses to social stress but not in baseline cortisol levels in participants exposed to early life adversity (Bunea et al., 2017). Another explanation may be that subjective stress experience is more important than number/types of events as assessed in the current study.

It is important to consider some intrinsic features of our sample. Our sample was characterized by depressive and anxiety disorders with 29% of participants having a current diagnosis of depression and/or anxiety, and 51% a remitted depression and/or anxiety disorder. A previous study in the current sample reported increased hair cortisol concentrations in association with depressive symptom severity (Gerritsen et al., 2019). Also, Khoury et al. (2019) reported the clinical status of the sample was a significant moderator in the association between hair cortisol and adversity exposure, with hair cortisol and adversity only significantly associated in non-clinical samples. However, in the current study, psychiatric status was not a significant moderating variable, as no significant interaction effects were observed between adversity exposure and depressive symptoms nor diagnostic status on hair cortisol concentrations. This suggests that even though depressive symptoms were associated with increased hair cortisol, the lack of associations between adversity and hair cortisol were similar within healthy controls, within participants with a remitted depression or anxiety disorder, and within participants with a current depression or anxiety disorder. In addition, our analyses were corrected for possible confounding effects of depressive symptoms severity, depressive/anxiety disorder diagnostic status, and antidepressant use, suggesting that presence and treatment of depressive/anxiety disorders did not influence our results.

Both timing of adversity exposure and timing of hair cortisol measurement are relevant issues to address. Specific age-ranges of timing of adversity exposure, particularly in early childhood around 3–5 years of age, have previously been related to adult hair cortisol (Schalinski et al., 2019), which we might not have picked up by grouping all childhood exposure. Also, considering the wide age-range of the participants (i.e. 23–72), the time between childhood adversity exposure and hair cortisol measurement was highly variable in the current sample. Though Baldwin et al. (2019) did not observe any age-effects on agreement between prospective and retrospective measures of childhood maltreatment in their meta-analysis, a larger time-lag might increase recall-bias. We did observe a positive main effect of age on hair cortisol, and on childhood maltreatment, and a negative effect of age on childhood life events, yet we did not detect evidence for any age by adversity interactions. This suggests associations between adversity exposure and hair cortisol did not differ as a function of age at time of hair cortisol measurement. The recent large hair cortisol study in older adults by Iob et al. (2019) (n = 3357) observed a small but significant age by childhood adversity interaction, with increasing hair cortisol concentrations in participants above 70 years of age with high childhood adversity exposure. With an age range between 23 and 72 years, this may not have been observed in the current NESDA sample. Though meta-analytic observations suggest that hair cortisol is mostly associated with recent and not past adversity (Stalder et al., 2017; Khoury et al., 2019), early life adversity might advance biological aging later in life via HPA axis alterations (Iob et al., 2019). Yet, a paucity of adversity-related research in elderly participants

impedes drawing conclusions, and signifies the need for more research in this generally underrepresented group.

Interestingly, significant effects of waist circumference and diabetes on hair cortisol concentrations were observed (see also (Stalder et al., 2017; Staufenbiel et al., 2015)). Increased prevalence of obesity, diabetes and other cardio-metabolic risk markers are steadily observed in adults exposed to (childhood) adversity (Felitti et al., 2019; Jakubowski et al., 2018), and are associated with changes in HPA-activity (Incollingo Rodriguez et al., 2015). Obesity and cardiometabolic risk markers have also been associated with increased risk of developing a broad range of somatic and mental health problems (Avila et al., 2015). Thus, these factors may be possible explanatory variables that play a role in both altered HPA-responses and in adverse health outcomes observed in people with exposure to (childhood) adversity.

4.1. Strengths and limitations

The present study was characterized by several strengths, such as the large sample; participants with different demographic features and from different clinical settings; combination of several different measures of early and recent adversity exposure; in-depth adversity and psychopathology measurements through (clinical) interviews; and extensive correction for potential confounding and explanatory factors. However, our observations could have been influenced by biases related to sample features or measurement. First of all, adversity exposure was measured with retrospective interviews in adulthood. Though measurements based on retrospective interviews were in higher agreement with prospective measures of adversity than retrospective questionnaire data (Baldwin et al., 2019), retrospective reports are likely subject to recall-bias, particularly in participants with depression and/or anxiety, who tend to report more adversity during depressive or anxious episodes (Baldwin et al., 2019). Still, retrospective recall of childhood adversity was previously shown to be stable within the current sample, with moderate overlap (Spearman's correlation $\rho = 0.57$–0.61) observed between interview and questionnaire-based measures of childhood maltreatment measured 4 years apart, and this association was not affected by presence of depressive or anxiety disorders (Spinhoven et al., 2014). Yet, recall-bias is an important issue to bear in mind in retrospective reporting of childhood adversity, that may further have been dependent on age at time of hair cortisol measurement. However, as age was included as a covariate, and no age-by-adversity interactions were observed, we conclude age is unlikely to have affected our main outcomes. Secondly, the clinical features of the sample might limit the generalisability of the current findings, considering the large proportion of our sample with lifetime or current depressive and anxiety disorders. However, our observations suggest that the association between adversity exposure and hair cortisol was similar within healthy controls and participants with remitted or current psychopathology. Also, it must be noted that in samples exposed to adversity, rates of depression and anxiety disorders are expected to be high (Spinhoven et al., 2011). Of clinical relevance, treatment of trauma-related issues, such as trauma-focussed psychotherapy, or other ways of effective coping with adversity, may mitigate the long-term psychological and biological sequelae of adversity exposure, including HPA axis functioning. Within the current study we did not have information on treatment of trauma-related issues though. A fourth issue, also concerning generalisability, is the fact that our study sample was primarily composed of women. The NESDA cohort includes an overrepresentation of females reflecting the female preponderance in depressive and anxiety disorders (Penninx et al., 2008), and hair cortisol assessment further excluded a small portion of male subjects due to insufficient hair/hair length. However, associations between adversity and hair cortisol concentrations were not affected by sex, similar to previous observations (Iob et al., 2019). Last, frequencies of exposure to recent negative live events were relatively low, especially when considering the past 3 months, which may have compromised power in these analyses. Considering the fact that particularly ongoing stressful life events may be associated with hair cortisol (Stalder et al., 2017), hair cortisol measurement *during* chronic stress exposure would be an interesting avenue to explore. For future studies, it would further be relevant to investigate other aspects of adversity that we did not take into account, for example specific age-periods of exposure, duration of the exposure, and levels of perceived stress.

4.2. Conclusions

This study was the largest study to date to explore the relation between psychosocial adversity experienced both in childhood and in adulthood, and hair cortisol concentrations. Our results suggest there is no association between adversity exposure in either childhood or adulthood and hair cortisol in adults. The (lack of) associations between adversity and hair cortisol were not affected by psychopathology in the current sample. Together with previous observations, this suggests adversity may not lead to long-term alterations of systemic cortisol levels as measured in hair. Thus, it remains unclear if and how the HPA-axis is involved in health and disease after adversity exposure. Nevertheless, we know that adversity exposure has major somatic and mental health implications, while at the same time, adversity is associated with reduced response to currently available treatment options. Therefore, future studies are needed to reveal the biological mechanisms related to adversity exposure, which are crucially important to improve treatment and prevention strategies, and to ultimately advance health outcomes after adversity.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105150.

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