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Running head: Determinants of hair cortisol

Article title: Sociodemographic, lifestyle, and psychosocial determinants of hair cortisol in a South London community sample

For submission to Psychoneuroendocrinology

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Abstract

Objective

Hypothalamic-pituitary-adrenal (HPA) measures are crucial for research into stress and stress-related disorders. Most HPA measures fluctuate depending on diurnal rhythms and state confounders. Hair cortisol concentrations (HCC) are less susceptible to such fluctuations, but less is known about trait-like confounders. Using a community sample, we tested the relationship between HCC and a range of variables including demographic variables, hair treatment, and medication, as well as psychosocial variables, namely childhood trauma, critical life events, and depressive symptoms.

Methods

Hair samples were collected from 144 individuals from the South East London Community Health (SELCoH) study. Childhood trauma, life events, and depressive symptoms were measured, together with age, sex, ethnicity, relationship status, educational attainment, employment status, occupational social class, hair washing frequency, hair treatments, season reflected in the hair sample, hazardous drinking, smoking, medication intake, and body mass index. Hair samples reflecting the past 3 months were collected and analysed using immunoassays. First, correlations (continuous variables) and simple linear regressions (dichotomous variables) were conducted to identify sociodemographic, hair-related, and lifestyle determinants of HCC. Next, multiple linear regressions were conducted to test the relationship between psychosocial variables and HCC when controlling for the identified confounders.

Results

Age ($r=-0.17$, $p=.050$), White British ethnicity ($\beta=-0.19$, $p=.023$), heat-based treatments ($\beta=-0.22$, $p=.010$), and winter season ($\beta=-0.18$, $p=.024$) were associated with lower HCC, whereas summer season ($\beta=0.24$, $p=.024$), painkillers ($\beta=0.25$, $p=.003$),

Susanne Fischer

anxiolytics/antidepressants ($\beta=0.21$, $p=.014$), and hormonal contraceptives ($\beta=0.27$, $p=.006$) were associated with higher HCC. Controlling for these confounders, physical neglect during childhood ($\beta=-0.17$, $p=.057$), war-related experiences ($\beta=0.20$, $p=.027$), separation ($\beta=0.18$, $p=.054$), and being the victim of a serious crime ($\beta=-0.17$, $p=.062$) were linked with altered HCC.

Conclusion

Our findings suggest that variation in HCC occurs according to sociodemographic, hair-related, and lifestyle variables, and that certain associations between stress and altered HCC can only be revealed when accounting for these confounders.

Keywords: childhood trauma; hair cortisol; life events; lifestyle; sociodemographic; stress

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is the most relevant endocrine system in stress-related research. It follows a distinct basal circadian rhythm, and is highly responsive to stimulation. On the one hand, this allows for an adaptation of the body to mild, short-term stress. On the other hand, it renders the HPA axis vulnerable to the detrimental effects of severe and long-term stress. There is ever-increasing evidence for the long-lasting ramifications of early life adversity and chronic psychosocial stress via epigenetic modification of genes involved in HPA axis regulation (Lupien et al., 2009). It is therefore not surprising that the presence of psychosocial stressors, such as childhood trauma and critical life events, is frequently associated with dysregulations of the HPA axis, which in turn may predispose an individual to develop stress-related disorders, such as depression or somatoform disorders (Heim and Nemeroff, 2001; Nater et al., 2011). Hypothalamic-pituitary-adrenal measures have therefore become crucial parameters for research looking into stress and the pathophysiology of stress-related disorders.

Indexing of HPA activity and reactivity has traditionally relied on peripheral markers, such as total and free cortisol measured in blood, 24 hour urine, and saliva (Kirschbaum and Hellhammer, 1989). The determination of hair cortisol concentrations (HCC) was introduced as a novel method to measure HPA activity. It makes use of the fact that endogenous cortisol is incorporated into hair follicles, mainly via passive diffusion from the blood stream (Stalder and Kirschbaum, 2012). As hair is known to grow at an average rate of about 1cm per month (Wennig, 2000), a scalp-near collected hair segment of 3cm would therefore provide a window into cortisol secretion over the past three months. The advantages offered by the measurement of HCC are manifold: it is non-invasive, economical in that repeated sampling can be avoided, and for the first time provides an assessment of cumulative cortisol concentrations over an extended period of time akin to the glycosylated haemoglobin as a long term measure of blood glucose concentration. This seems to render the HCC methodology an ideal candidate for stress-related research, where invasive procedures are

often not feasible due to limited financial and time resources, and repeated sampling schedules often present with adherence problems. Most importantly, compared to conventional specimens, HCC could prove a more reliable indicator of HPA abnormalities which is less susceptible to the influence of state variables (e.g., day of the week, time of day; Kudielka and Wüst, 2010). However, less is known about potential trait-like confounders of HCC, such as sex, educational attainment, or body mass index (BMI).

In a systematic review on confounders of HCC, the relationship between age and HCC was deemed “complex” and most likely “non-linear”, and it was concluded that more studies with a broad age range are needed (Wosu et al., 2013). Similarly, evidence on sex differences was inconsistent, while very few studies were identified that allowed investigation of potential differences across ethnicities, or by relationship and socioeconomic status. The literature on lifestyle variables suggested that alcohol intake and vigorous physical activity are positively correlated with HCC, while smoking status and intake of medication including oral contraceptives showed no association, and findings regarding BMI were inconsistent. Research using the HCC methodology was still at an early stage when this review was conducted, and information on potential confounders mainly relied on small studies using highly selected populations (e.g., mothers of new-borns and patients with endocrine disorders). Some of the more recent studies using larger and more diverse samples suggest that in adults, HCC increases with age (Feller et al., 2014; Stalder et al., 2013; Staufenbiel et al., 2015), is higher in men (Abell et al., 2016; Dettenborn et al., 2012; Feller et al., 2014; Manenschijn et al., 2013; Staufenbiel et al., 2015), in black people (Abell et al., 2016; Wosu et al., 2015), and the unemployed (Feller et al., 2014); is positively associated with alcohol consumption (Feller et al., 2014; Manenschijn et al., 2013); is higher in smokers (Feller et al., 2014; Wosu et al., 2015); higher in women on hormonal contraceptives (Staufenbiel et al., 2015); and positively linked with BMI (Abell et al., 2016; Stalder et al., 2013). However, evidence overall is still scarce and inconclusive. It is therefore uncertain which

sociodemographic and lifestyle confounders should be considered in the design and interpretation of future studies investigating HCC in relation to psychosocial stress.

The present study was conducted in order to study a population of community residents regarding a potential relationship between childhood trauma, critical life events, psychological distress (i.e. depressive symptoms) and HCC. This seemed especially important in light of the fact that so far, evidence for a link between psychosocial stress and HCC is sparse and findings regarding an association between HCC and psychological distress are inconsistent, highlighting the need for further research (Herane Vives et al., 2015). In a first step, however, potential sociodemographic and lifestyle confounders of HCC, and potential hair-related confounders (e.g., washing frequency) were investigated. Finally, to eliminate any potential confounding influence, the initial analyses looking at psychosocial stressors, depressive symptoms, and HCC were repeated, this time controlling for the identified confounders. The current study complements the above-mentioned research by, for the first time, reporting on the influence of a wide range of commonly used medications on HCC. Based on the literature, it was expected that older people, men, people of Black African and Black Caribbean ethnicity, and people outside of regular employment would have higher HCC. It was further hypothesised that people reporting a high number of life events would exhibit comparably higher levels of HCC.

2. Methods

2.1 Participants

Hair samples were collected from a total number of 144 individuals who took part in phase 3 of the South East London Community Health (SELCoH) study. SELCoH was a longitudinal study that aimed to identify antecedents and consequences of various health outcomes in a local community sample. Details of the initial recruitment procedures can be found elsewhere (Hatch et al., 2011). Briefly, in phases 1 and 2, households located in the two

adjacent South London boroughs Lambeth and Southwark were randomly selected and repeatedly visited by researchers trained in conducting clinical interviews. All residents aged 16 and over were eligible. Phase 3 used a purposive subsample of 500 of these people and included biomedical data collections. Ethnicity was assessed at a previous phase. The study was approved by the King's College London Psychiatry, Nursing and Midwifery Research Ethics Committee, reference PNM/12/13-152. All procedures were in accordance with the Declaration of Helsinki and written informed consent was obtained from all participants.

2.2 Sociodemographic measures

Age and sex were recorded. In addition to being included as a continuous variable, age was divided into bands: 18-29, 30-59, and 60+. For ethnicity, data came from phase 2 and was recorded based on UK Census categories: White British, Black African, Black Caribbean, Asian, and Other. Relationship status was categorised as “not in a relationship” vs. “in a relationship”. The former category included single, separated, divorced, and widowed people, whereas the latter included people being in a long-term relationship or marriage. Indicators of socioeconomic status included educational attainment, employment status, and occupational social class. Educational attainment was divided into no school qualifications or up to GCSE levels, A levels or vocational, and higher qualifications (three categories). Employment status was indicated as “not in regular employment” vs. in regular employment”. Importantly, the vast majority of people that were classified as “not in regular employment” were retired rather than currently seeking a job. Occupational social class was categorised according to the Registrar General's classification (Office of Population Censuses and Surveys, 1980) and as “non-manual” vs. “manual” based on participants' description of their jobs.

2.3 Hair treatment measures

Hair washing frequency per week and the use of hair treatments including hair dye, bleach, hair chemicals (e.g., relaxers), and heat (e.g., hair straighteners) were recorded (“no” vs. “yes”). The season that was primarily represented in the hair sample was determined based on the collection date.

2.4 Lifestyle measures

Hazardous drinking during the past year was assessed using the ten-item Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2001). This instrument measures alcohol consumption, dependence, and problematic behaviours related to alcohol use by means of a five-point scale (0-4). The maximum attainable score is 40, with a cut-off of eight or more indicating hazardous drinking. Smoking status and intake of medication during the past three months was recorded, with medication being categorised as topical steroids, painkillers, antacids, cold medication, allergy medication, antibiotics, inhalers, diabetes medication, anxiolytics and antidepressants, cardiovascular medication, thyroid medication, and hormonal contraceptives by study personnel after participants had written down the names of their medication at home and brought this information to the study appointment. The BMI was calculated for all participants and divided into bands labelled “underweight” (<18.5), “normal” (18.5-24.99), “overweight” (≥ 25), and “obese” (≥ 30).

2.5 Psychosocial stress

The Childhood Trauma Questionnaire (Bernstein et al., 2003) was used to ask about past experiences of abuse and neglect. The questionnaire distinguishes between five domains of trauma, each measured by five items: emotional neglect, physical neglect, emotional abuse, physical abuse, and sexual abuse. Answers are indicated on a five-point Likert scale (score range from 25-125). Both continuous and dichotomous (trauma absent vs. present) scores

can be calculated for each subscale, with the latter being based on validated cut-off values for moderate to severe trauma (Bernstein and Fink, 1998).

Critical life events were assessed by a list of 22 items that were based on Turner and Lloyd's 20 item list (Turner and Lloyd, 1995), the Life Events Questionnaire (Meyer et al., 2006), and the List of Threatening Events (Brugha et al., 1985). Participants were given a questionnaire containing this combined list of events to fill out but were able to contact research personnel in case they had difficulties in doing so. The list contained both traumatic and other life events. Example items are being hit or forced to have sex (traumatic life event) or loss or theft of a valued possession (other life event). Participants indicated whether they had ever experienced the life event in question (no vs. yes) and whether this had been the case in the past twelve months (no vs. yes). The prevalence of each event was recorded and a lifetime and twelve months count of traumatic and other life events was calculated for each person.

2.6 Depressive symptoms

Depressive symptoms were measured using the Revised Clinical Interview Schedule (CIS-R; Lewis et al., 1992). The CIS-R is a structured clinical interview developed and validated for the assessment of common mental disorders in community settings. The interview asks about 14 clinical domains, including depression. It uses skips to allow asymptomatic individuals to answer a minimum of 28 questions. The severity of depressive symptoms is rated on a scale ranging from 0 to 4.

2.7 Hair sampling and extraction of cortisol

Hair strands were taken as close to the scalp as possible and from the posterior vertex of all consenting participants with a hair length of at least 3cm. The root end was marked and all

samples stored in aluminium foil at room temperature. After visual inspection, three centimetres representing the past three months were cut off each sample and washed twice for 2 minutes in glass vials using 3mL of isopropanol. Samples were dried for 24 hours at room temperature and then pulverised by means of a Retsch ball mill. Ten to 15mg of hair powder were taken from each sample and incubated for one hour with 1.5mL of methanol. After centrifugation, 1.3mL of the methanol supernatant was evaporated at 60°C under a gentle stream of nitrogen. The samples were then reconstituted in 1mL phosphate buffer and stored at -30°C until the immunoassay. The Immulite DCP's Immunoassay analyser (www.diagnostics.siemens.com) was used to conduct immunoassays, as previously described (Mondelli et al., 2010). The inter- and intra-assay variance of this assay is below 10%.

2.8 Data preparation and statistical analysis

All data were explored and HCC outliers of more than three standard deviations (SD) excluded (n=1). Similarly, all individuals using systemic corticosteroids (n=4) were excluded from the analysis, which resulted in a final sample size of 139 participants. Due to the fact that HCC were not normally distributed as indicated by the Kolmogorov-Smirnov test, all values were log-transformed for statistical analyses. Untransformed HCC were, however, reported for descriptive purposes. Descriptives were given as means and standard deviations (normally distributed variables), median and interquartile range (non-normally distributed variables), or absolute and relative frequencies (categorical variables), respectively. Bivariate Pearson or Spearman's rank order correlation coefficients were calculated, as well as simple linear regressions and univariate ANOVAs with a post-hoc Scheffé test where appropriate. This was to identify all potentially significant determinants of HCC (unadjusted analyses). For the psychosocial variables childhood trauma, critical life events and depressive symptoms linear regressions were calculated; first unadjusted and

then controlling for the previously identified determinants/confounders. Finally, a multiple linear regression was run, with all relevant confounders (defined as $p < .10$) of HCC entered simultaneously. This allowed for mutual adjustment and as such enabled us to identify the most relevant confounders of HCC. The statistical significance level was set at $\alpha = .05$. All analyses were conducted in SPSS 22.

3. Results

3.1 Sample characteristics

Of the original 500 individuals that were part of the SELCoH phase 3 sample, 211 (42%) were *unable* to give hair due to shortness or baldness, and 145 (29%) *refused* for various reasons. Younger people were equally unable and unwilling to give hair (83% loss in total). It was not possible to collect hair in 74% of middle-aged people and in 59% of older people, which in both cases was mainly due to people's inability to give hair (rather than their unwillingness). It was for the same reason that we were unable to harvest hair from the majority of men in the sample (76%). Nearly half of the women, on the other hand, did not consent to give hair (47%). There was a large proportion of people of Black African and Black Caribbean ethnicity who could not provide a hair sample (71%).

As is evident from the descriptive data given in Table 1, the 139 individuals who gave hair and were therefore eligible for the present study still mainly consisted of middle-aged and older adults. The age range was 20-79 years. There was a female preponderance in the sample and most people were of White British ethnicity. The majority of people were not in a relationship, had a higher education, and were regularly employed in non-manual professions.

On average, participants washed their hair every second day. Hair dye was the most frequently used hair treatment, followed by heat-based treatments and bleach. Hair chemicals were used by a minority only.

About a fifth of the sample reported hazardous drinking and another fifth reported smoking. Painkillers were the most frequently used medication in the past three months, followed by cardiovascular medication, antacids, anxiolytics/antidepressants, cold medication, hormonal contraceptives, allergy medication, inhalers, and antibiotics. Only very few people used thyroid or diabetes medication. The BMI data indicated that on average, participants were slightly overweight.

- Insert Table 1 here -

Descriptives of psychosocial stress measures are given in Table 2. Sexual abuse and emotional neglect were the most frequently reported types of trauma. Ever having experienced the death of a loved one, a relative that was seriously ill, injured, or had been assaulted, and having something valuable lost or stolen were the most frequently encountered life events. The HCC median (IQR) of the total sample was 186.9 (507.7) pg/mg.

- Insert Table 2 here -

3.2 Sociodemographic determinants of hair cortisol

As can be seen in Table 1, age as a continuous variable was the only sociodemographic factor that was significantly associated with HCC (negative correlation). When looking at age

bands, nominally, middle-aged adults had the highest HCC, but this effect was not significant ($F(2, 136)=1.94, p=.147$). Due to the predominantly White British sample, ethnicity was dichotomised into a non-white ($n=12, HCC=497.4 (211.1) \text{ pg/mg}$) and White British group ($n=127, HCC=164.7 (473.1) \text{ pg/mg}$), and it was shown that participants in the latter group had significantly lower HCC ($\beta=-0.19, p=.023$).

3.3 Hair treatment determinants of hair cortisol

All unadjusted hair treatment results are reported in Table 1. Neither hair washing frequency nor the use of dye or bleach was associated with HCC. However, participants using heat-based treatments, such as hair straighteners or curling irons, had significantly lower HCC compared to those not using these treatments. Furthermore, HCC was significantly higher in samples reflecting the summer months ($\beta=0.24$) when compared to the winter months ($\beta=-0.18$) according to post-hoc tests ($p=.024$).

3.4 Lifestyle determinants of hair cortisol

The unadjusted lifestyle results are reported in Table 1. Hazardous drinking and smoking status were unrelated to HCC. However, the intake of a number of medications was significantly associated with higher HCC, namely painkillers, anxiolytics/antidepressants, and hormonal contraceptives. Body Mass Index was not significantly correlated with HCC; although HCC increased across BMI bands, i.e., the higher the BMI, the higher HCC, this effect was not significant ($F(2, 136)=0.49, p=.612$).

3.5 Psychosocial stress determinants of hair cortisol

The unadjusted and adjusted results regarding the psychosocial stress variables are reported in Table 2. Physical neglect was the only sub-dimension of the CTQ that was associated with HCC alterations (lower values in neglected individuals, significant by trend only). The overall frequency of lifetime or past 12 months' critical life events was not associated with HCC. Regarding specific critical life events, having placed one's spouse, in-law, or parent into a care home was paralleled by lower HCC, although again only by trend. Severity of depressive symptoms was not related to HCC.

Controlling for all of the identified confounders (age, White British ethnicity, heat-based treatments, winter and summer season, intake of painkillers, anxiolytics/antidepressants, and hormonal contraceptives) meant that men were excluded from the following analyses. Women reporting physical neglect still demonstrated a tendency towards lowered HCC. In addition, women that had ever been in war, in a combat zone, or in an area of political unrest, and those who had gone through a separation or divorce had comparably elevated HCC (the latter by trend only). By contrast, those who reported that they had ever been the victim of a serious crime showed comparably reduced HCC (trend). All other associations between psychosocial stress variables and HCC were not statistically significant ($p > .290$). When excluding women on hormonal contraceptives from the analyses, and therefore re-including men, having gone through a divorce or separation was no longer a significant determinant of HCC ($\beta = 0.13$, $p = .219$). No other statistically significant changes occurred to the results (data not shown).

3.6 Mutually adjusted analyses

When all previously identified confounders of HCC (age, White British ethnicity, heat-based treatments, winter and summer season, intake of painkillers, anxiolytics/antidepressants, and hormonal contraceptives) were entered simultaneously into one regression model, White British ethnicity, heat-based treatments, intake of anxiolytics/antidepressants, and

intake of hormonal contraceptives remained predictors of HCC, although the latter only by trend (see Table 3 for the complete model). When excluding women who were on hormonal contraceptives, and thereby re-including men, White British ethnicity ($\beta=-0.26$, $p=.018$) and using heat-based treatments ($\beta=-0.26$, $p=.020$) were the remaining predictors of HCC.

- Insert Table 3 here -

4. Discussion

The present study aimed to clarify the role of a number of sociodemographic, lifestyle, and psychosocial factors in relation to HCC in a diverse community sample. Over two thirds of the original sample were unable or unwilling to donate hair. Except for age and ethnicity, there were no associations between sociodemographic characteristics and HCC. The use of heat-based hair treatments and winter season was paralleled by lower HCC, while summer season and a number of lifestyle factors were linked with higher HCC, namely the intake of painkillers, anxiolytics/antidepressants, and hormonal contraceptives. A tendency towards lower HCC in participants reporting physical neglect during their childhood emerged, and this trend was robust against the influence of the previously identified confounding variables. A number of critical life events were related to alterations in HCC, but only when controlling for the same confounding variables. Severity of depressive symptoms was not related to HCC.

The fact that age was inversely related to HCC is at odds with some previous studies who found that the older participants, the higher their HCC (Feller et al., 2014; Stalder et al., 2013; Staufenbiel et al., 2015). One explanation for this discrepancy may be that the mean age in our sample was 50, whereas in the Stalder et al. (2013) study it was between 24 (sample I) and 31 (sample II), and Feller et al. (2014) used a sample of older adults with a mean age of 66. Moreover, the fact that age was no longer a significant predictor of HCC

when the intake of hormonal contraceptives was taken into account (mutually adjusted analyses) may suggest that the lower HCC found in older adults was partly attributable to the fact that no participants older than 50 reported the use of contraceptives.

In the Staufenbiel et al. (2015) study and in four others (Abell et al., 2016; Dettenborn et al., 2012; Feller et al., 2014; Manenschijn et al., 2013), men also had significantly higher HCC when compared to women; a finding which was not replicated here and by other previous research (Stalder et al., 2013; Wosu et al., 2015). Notably, with one exception (Staufenbiel et al., 2015), the reported mean ages of the studies reporting positive findings again suggest the use of either mainly young or older adult participants. For instance, in the largest so far published community study, measuring HCC in the Whitehall II cohort, all participants were more than 59 years old (2016). Given the marked intra-individual change in levels of sex steroids over the course of a lifespan (especially in women), and given that the hypothalamic-pituitary-gonadal axis interacts with the HPA axis on numerous levels (Bale and Epperson, 2015), one may thus speculate that differences between men and women are more likely to emerge in age-homogenous samples, that is, in samples that are confined to either pre- or post-menopausal women. Future HCC studies using samples with a wide age range may choose to additionally measure and explore a potential impact of menopausal status.

This is the first community-based study to use a wide variety of socioeconomic measures, including occupational social class, and examine their effect on HCC. No influence of this variable on HCC was detected in the present sample, and this corresponds with the fact that Abell et al. (2016) did not find HCC to differ across civil service employment grade in Whitehall II. The null-finding regarding educational attainment corresponds with previously conducted research (Staufenbiel et al., 2015; Wosu et al., 2015). These findings are especially interesting in light of the fact that socioeconomic status is often used as a proxy for chronic psychosocial stress. Likewise, being unemployed is often considered a chronic stressor (Dettenborn et al., 2010), but again, this was not reflected in heightened

HCC in the present sample. Of note, nearly all people that were not in regular employment here were pensioners rather than people currently seeking employment. Taken together, HCC seem to be robust to the influence of conventional indicators of socioeconomic status according to the reported results.

Our null-finding regarding a potential impact of hair washing frequency and the use of hair dye on HCC agrees with the majority of previous research (Stalder et al., 2013; Staufenbiel et al., 2015; Wosu et al., 2015). This is the first community study to suggest that heat-based treatments may strongly influence HCC, even when adjusting for other confounders. Future research may therefore consider excluding participants who use hair straighteners or curling irons or at least statistically controlling for this variable. The observation of lower HCC in samples collected during the winter season and higher values during summer echoes three prior studies which had reported seasonal effects on HCC (Abell et al., 2016; Braig et al., 2015; Staufenbiel et al., 2015). A number of factors, such as changes in temperature or humidity could account for this finding, but further research is warranted to pin down the source of these fluctuations.

In this sample, lifestyle variables were among the most pronounced confounders of HCC. Although as in nearly all comparable research to date, neither alcohol use nor smoking were related to HCC (Dettenborn et al., 2012; Feller et al., 2014; Manenschijn et al., 2013; Stalder et al., 2013; Staufenbiel et al., 2015; Wosu et al., 2015), the intake of a number of medications were found to be paralleled by high HCC. The fact that painkillers were linked with higher HCC resonates well with the findings of van Uum et al. (2008), who were able to demonstrate that medicated patients with chronic pain had higher HCC when compared to healthy controls. Likewise, the intake of anxiolytics and antidepressants during the three months period that was reflected in the hair samples was an important predictor of HCC. This stands in contrast to a study by Dettenborn et al. (2012), who could not detect any differences in HCC between people taking any medication versus those who were not. However, the overall prevalence of people taking medication was much lower in this

previous study, and it was therefore not possible to separate different drug classes. The use of medications with opposing impact on HCC may thus have masked any effects in this study. Finally, in the present study, women on hormonal contraceptives had higher HCC, which is in line with the findings published by Staufenbiel et al. (2015). Nevertheless, more research is clearly needed to replicate the evidence we report here. The same is true regarding BMI; our null-finding adds to a rather conflicting body of literature, showing both non-significant (Feller et al., 2014; Manenschijn et al., 2013) and positive correlations (Abell et al., 2016; Stalder et al., 2013).

The current result suggesting a trend towards attenuated HCC in individuals who experienced physical neglect as a child is compatible with previous research. For instance, both Hinkelmann et al. (2013) and Steudte et al. (2013) found lower HCC in traumatised but otherwise healthy individuals when compared to non-traumatised controls. Similarly, being the victim of a serious crime was linked with lower HCC in this sample, suggesting that such events may have also been traumatic. By contrast, those reporting separation and war-related experiences had higher HCC, mirroring previous research on a different type of stressor, namely life events (Karlen et al., 2011) and chronic stress (Herane Vives et al., 2015). Interestingly, Miller, Chen and Zhou (2007) in their meta-analysis concluded that stressors of a more distal onset and those that were no longer active (e.g., childhood physical neglect) may be associated with lower cortisol levels, whereas the opposite was to be expected with more proximal and ongoing stressors (e.g., separation/divorce and its consequences). However, since unfortunately we do not have exact dates of when life events occurred, we are unable to confirm whether timing indeed underlies the observed patterns in the present study. It is noteworthy, that only in analyses adjusted for confounders did these effects emerge, and that the sum of life events experienced during the past 12 months was unrelated to HCC. This underscores the importance of controlling for confounders in stress-related research using HCC, and suggests that future studies may be well-advised to include a more extensive measure of life events. However, no relationship

between HCC and the severity of depressive symptoms emerged even when confounders were included in the statistical models, suggesting that clinical levels of depression (as potentially indicated by the intake of antidepressants) may be a prerequisite for alterations in HCC to occur.

The present study offers a number of strengths. First, the findings add to the small body of literature looking at confounders of HCC in community samples. This seems important at this stage in research on HCC, as current knowledge mainly relies on findings in small samples of narrowly selected individuals (e.g., patients). We found the overall hair sample turnout to be low and have outlined reasons for this according to age, sex, and ethnicity. This is important information for the planning of future epidemiological studies interested in measuring cortisol. Second, it is the first study providing a closer examination of the specific effects of the most frequently prescribed classes of medications on HCC. As painkillers, anxiolytics/antidepressants, and hormonal contraceptives are consumed by a wide range of people, these findings have important implications for much of the epidemiological and clinical research undertaken using the HCC methodology. Third, we were able to study a number of potential confounders in concert with psychosocial measures that are of high relevance in research into the pathophysiology of stress-related disorders. Nevertheless, several limitations need to be acknowledged. First, although socio-demographically diverse, our sample exhibits a female preponderance that is most likely attributable to a higher volunteer bias in women with this type of research as well as to their increased ability to give hair. Second, low numbers in different ethnic groups, some of which due to some participants of Black African and Black Caribbean ethnicity's inability to give hair, as well as low numbers regarding the use of certain hair treatments and medications prevented examining the impact of some of these factors on HCC. Third, we did not assess the exact time point at which life events occurred, and this precluded us from investigating whether more distal events were differentially related to HCC when compared to more proximal events. Fourth, we did not exclude pregnant women, even though pregnancy is

linked with higher cortisol levels (La Marca-Ghaemmaghami & Ehlert, 2015), including concentrations measured in hair (Kirschbaum et al., 2009). However, since hormonal contraceptive users had markedly elevated HCC when compared to non-users, and given the age distribution of women in the sample, we do not believe our sample to contain a substantial number of pregnant women.

In sum, the present study extends previous research in community samples by for the first time reporting on the impact of a number of frequently used medications on HCC. Collectively, our findings suggest variation in HCC occurs according to age, ethnicity, use of heat-based hair treatments, season reflected in the hair sample, and medication (including hormonal contraceptives). Controlling for these confounders, we found that a number of psychosocial stressors were reflected in altered HCC. The present findings warrant replication, preferably in samples that are larger and more diverse regarding sex and ethnicity, and in clinical populations. The corroboration of the identified confounders will enable future research into stress and stress-related disorders to make full use of the many advantages offered by the HCC methodology.

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Susanne Fischer

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Conflicts of interest

None.

Contributors

SH, AP, LG, SF, MH and AJC contributed to the conception and design of the study, and SF, RD, MH and AJC contributed to the analysis and interpretation of data. SF and RD drafted the article and SH, AP, LG, SF, MH, and AJC revised it critically for important intellectual content. All authors approved the final version to be submitted.

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Table 1 Sample characteristics (potential confounders), hair cortisol concentrations according to potential confounders, and unadjusted coefficients regarding log-transformed hair cortisol concentrations. Descriptives given as mean \pm standard deviation (normally distributed variables), median (interquartile range; non-normally distributed variables), or frequencies (categorical variables). Statistics refer to bivariate Pearson or Spearman's rank order correlations, linear regressions, and univariate ANOVAs (N=139)

	Descriptive	HCC (pg/mg)	Statistic	p value
<i>Sociodemographic measures</i>				
Age (years)	50.6 \pm 14.6	-	r=-0.17	.050
18-29	14 (10%)	114.6 (847.8)		
30-59	75 (54%)	282.1 (715.1)		
60+	50 (36%)	148.8 (226.2)		
Sex			β =0.01	.941
Male	39 (28%)	191.4 (454.1)		
Female	100 (72%)	182.0 (577.1)		
Ethnicity			-	-
White British	127 (91%)	164.7 (473.1)		
Black African	1 (1%)	1162.0		

Susanne Fischer

Black Caribbean	1 (1%)	61.0		
Asian	2 (1%)	285.3		
Other	8 (6%)	562.7 (665.8)		
Relationship status			$\beta=0.03$.700
Not in a relationship	89 (64%)	183.5 (503.2)		
In a relationship	50 (36%)	195.3 (634.5)		
Educational attainment			F (2, 136)=0.41	.666
No school qualifications/up to GCSE levels	30 (22%)	209.0 (572.0)		
A levels or vocational	34 (24%)	175.3 (379.5)		
Higher qualifications	75 (54%)	183.5 (752.8)		
Employment status			$\beta=0.03$.754
Not in regular employment	54 (39%)	185.2 (441.3)		
In regular employment	85 (61%)	191.4 (532.0)		
Occupational social class			$\beta=0.06$.497
Non-manual	94 (71%)	180.1 (508.3)		

Susanne Fischer

Manual 39 (29%) 219.6 (520.7)

Hair treatment measures

Hair washing frequency (times/week) 3 (2) - $r=0.11$.191

Hair treatments

Hair dye $\beta=-0.03$.711

No 83 (60%) 186.9 (503.1)

Yes 56 (40%) 193.7 (513.8)

Bleach $\beta=-0.10$.227

No 127 (91%) 199.3 (529.4)

Yes 12 (9%) 101.4 (314.4)

Hair chemicals (e.g., relaxers) - -

No 136 (98%) 189.1 (506.5)

Yes 3 (2%) 89.8

Heat (e.g., hair straighteners) $\beta=-0.22$.010

No 124 (89%) 206.0 (526.5)

Susanne Fischer

Yes	15 (11%)	54.8 (242.4)		
Season reflected in hair sample			F (3, 135)=3.52	.017
Winter	44 (32%)	127.4 (333.3)		
Spring	41 (30%)	191.4 (520.4)		
Summer	34 (25%)	447.1 (1090.7)		
Autumn	20 (14%)	230.0 (403.0)		
<i>Lifestyle measures</i>				
Hazardous drinking (AUDIT score \geq 8)			$\beta=-0.03$.721
No	111 (80%)	191.4 (587.9)		
Yes	28 (20%)	183.0 (366.3)		
Smoker			$\beta=-0.06$.484
No	109 (78%)	186.9 (539.9)		
Yes	30 (22%)	189.3 (278.3)		
Medication intake (past 3 months)				
Topical steroids			-	-

Susanne Fischer

No	130 (94%)	189.1 (513.8)		
Yes	9 (6%)	151.3 (449.2)		
Painkillers			$\beta=0.25$.003
No	39 (28%)	101.1 (149.1)		
Yes	100 (72%)	247.5 (739.8)		
Antacids			$\beta=0.13$.120
No	106 (76%)	172.0 (471.3)		
Yes	33 (24%)	282.1 (744.7)		
Cold medication			$\beta=0.12$.173
No	114 (82%)	172.0 (479.6)		
Yes	25 (18%)	301.6 (762.5)		
Allergy medication			$\beta=0.12$.178
No	117 (84%)	179.2 (475.7)		
Yes	22 (16%)	291.9 (817.7)		
Antibiotics			$\beta=-0.05$.530
No	122 (88%)	185.2 (560.1)		
Yes	17 (12%)	212.7 (285.8)		

Inhalers			$\beta=-0.00$.995
No	119 (86%)	191.4 (508.4)		
Yes	20 (14%)	147.9 (508.6)		
Diabetes medication			-	-
No	130 (94%)	185.2 (504.4)		
Yes	9 (6%)	231.1 (1025.9)		
Anxiolytics/antidepressants			$\beta=0.21$.014
No	112 (81%)	172.0 (472.1)		
Yes	27 (19%)	317.1 (1054.2)		
Cardiovascular medication			$\beta=0.13$.129
No	104 (75%)	180.1 (444.6)		
Yes	35 (25%)	243.3 (745.6)		
Thyroid medication			-	-
No	129 (93%)	183.5 (520.6)		
Yes	10 (7%)	276.3 (442.4)		
Hormonal contraceptives			$\beta=0.27$.006
No	77 (77%)	140.8 (452.3)		

Susanne Fischer

Yes	23 (23%)	422.1 (711.5)		
Body Mass Index	27.5±6.0	-	r=0.10	.234
Underweight (<18.5)	4 (3%)	151.2 (403.9)		
Normal (18.5-24.99)	51 (37%)	146.4 (537.2)		
Overweight (≥25)	45 (32%)	199.3 (484.1)		
Obese (≥30)	39 (28%)	307.8 (583.3)		

A level=General Certificate of Education Advanced Level

AUDIT=Alcohol Use Disorders Identification Test

GCSE=General Certificate of Secondary Education

HCC=Hair cortisol concentration

Table 2 Sample characteristics (psychosocial determinants), hair cortisol concentrations according to psychosocial determinants, and unadjusted and adjusted coefficients regarding log-transformed hair cortisol concentrations; adjusted meaning that age, White British ethnicity, heat-based treatments, winter and summer season, intake of painkillers, anxiolytics/antidepressants, and hormonal contraceptives were controlled for. Descriptives given as median (interquartile range; non-normally distributed variables) or frequencies (categorical variables). Statistics refer to linear regressions (N=139 for unadjusted analyses, n=100 for adjusted analyses).

	Descriptive	HCC (pg/mg)	Unadjusted		Adjusted	
			β	p value	β	p value
Childhood trauma (CTQ; range 5-25)						
Emotional neglect	7 (6.5)	-	-0.05	.585	<0.01	>.999
Physical neglect	5 (2)	-	-0.08	.373	-0.08	.408
Emotional abuse	6 (4)	-	<-0.01	.963	0.02	.798
Physical abuse	5 (1)	-	-0.07	.416	-0.07	.431
Sexual abuse	5 (0)	-	<-0.01	.993	-0.04	.649
Any childhood trauma (CTQ)						
Emotional neglect (score \geq 15)			-0.03	.693	-0.01	.952
No	117 (85%)	191.4 (533.4)				

Susanne Fischer

Yes	20 (15%)	157.7 (430.2)				
Physical neglect (score \geq 10)			-0.14	.097	-0.17	.057
No	124 (91%)	195.3 (545.3)				
Yes	13 (9%)	79.4 (222.7)				
Emotional abuse (score \geq 13)			-0.02	.840	-0.01	.889
No	121 (88%)	186.9 (534.1)				
Yes	16 (12%)	191.9 (422.3)				
Physical abuse (score \geq 10)			-0.09	.330	-	-
No	122 (92%)	195.3 (559.4)				
Yes	11 (8%)	219.6 (249.2)				
Sexual abuse (score \geq 8)			-0.02	.819	-0.09	.364
No	114 (84%)	185.2 (621.4)				
Yes	21 (16%)	282.1 (349.9)				
Traumatic life events (lifetime)	2 (2)	-	0.01	.898	0.04	.660
Traumatic life events (past 12 months)	0 (0)	-	<-0.01	.993	-0.04	.639
Other life events (lifetime)	3 (2)	-	-0.05	.548	0.07	.447

Susanne Fischer

Other life events (past 12 months)	0 (1)	-	-0.02	.849	<0.01	.963
Any life events (lifetime)						
Loved one died			0.09	.316	0.09	.316
No	51 (37%)	151.3 (465.3)				
Yes	88 (63%)	202.9 (540.1)				
Seen something violent happen			0.04	.655	0.06	.515
No	95 (68%)	179.2 (533.3)				
Yes	44 (32%)	209.4 (486.1)				
Had serious accident			-0.01	.879	0.10	.290
No	109 (78%)	183.5 (636.7)				
Yes	30 (22%)	198.9 (380.8)				
Been in war			0.11	.187	0.20	.027
No	124 (89%)	182.2 (471.1)				
Yes	15 (11%)	282.1 (526.1)				
Lived rough			-	-	-	-
No	131 (94%)	186.9 (531.6)				
Yes	8 (6%)	231.9 (494.7)				

Susanne Fischer

Been victim of serious crime			-0.09	.278	-0.17	.062
No	77 (55%)	234.6 (721.2)				
Yes	62 (45%)	149.6 (377.9)				
Been injured with weapon			-0.04	.657	-	-
No	125 (90%)	186.9 (533.7)				
Yes	14 (10%)	156.2 (362.5)				
Hit, kicked, forced to have sex			-0.03	.712	-0.07	.490
No	84 (60%)	202.9 (546.9)				
Yes	55 (40%)	151.3 (475.8)				
Been separation, divorced			0.01	.945	0.18	.054
No	78 (56%)	183.9 (511.0)				
Yes	61 (44%)	191.4 (506.0)				
Child had serious illness, accident			-0.08	.347	0.02	.863
No	105 (75%)	199.3 (567.6)				
Yes	34 (25%)	181.4 (365.3)				
Adult child moved back into home			-0.07	.406	0.06	.567
No	119 (86%)	199.3 (535.3)				

Susanne Fischer

Yes	20 (14%)	172.0 (215.6)				
Care of grandchildren increased			-	-	-	-
No	130 (94%)	202.9 (538.5)				
Yes	9 (6%)	89.8 (191.0)				
Parent, in-law moved into home			-	-	-	-
No	131 (94%)	191.4 (534.6)				
Yes	8 (6%)	110.3 (156.1)				
Placed relative in care home			-0.15	.088	-0.01	.943
No	125 (90%)	191.4 (567.6)				
Yes	14 (10%)	76.4 (257.8)				
Problems affecting right to stay in UK			-	-	-	-
No	138 (99%)	185.2 (513.8)				
Yes	1 (1%)	421.0				
Relative seriously ill or injured			-0.06	.460	<-0.00	>.999
No	59 (42%)	234.6 (501.0)				
Yes	80 (58%)	172.0 (535.9)				
Problem with acquainted person			-0.02	.823	-0.05	.596

Susanne Fischer

No	97 (70%)	191.4 (463.6)				
Yes	42 (30%)	141.6 (541.2)				
Unemployed, job-seeking > 1 month			0.01	.951	-0.06	.541
No	101 (73%)	186.9 (535.1)				
Yes	38 (27%)	182.0 (470.8)				
Sacked from job			0.09	.315	0.10	.290
No	122 (88%)	180.1 (506.1)				
Yes	17 (12%)	313.9 (504.9)				
Had major financial crisis			-0.03	.704	0.02	.833
No	105 (75%)	186.9 (635.8)				
Yes	34 (25%)	182.0 (471.6)				
Problems with police			0.09	.281	-	-
No	123 (89%)	181.0 (509.0)				
Yes	16 (11%)	273.6 (478.4)				
Something valuable lost, stolen			0.06	.507	0.02	.798
No	75 (54%)	191.4 (409.1)				
Yes	64 (46%)	174.8 (809.1)				

Susanne Fischer

Depressive symptoms (CIS-R; range 0-4)	0 (0)	-	0.08	.346	-0.05	0.612
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CIS-R= Revised Clinical Interview Schedule

CTQ=Childhood Trauma Questionnaire

HCC=Hair cortisol concentration

Table 3 Mutually adjusted confounders of log-transformed hair cortisol concentration; multiple linear regression (women only, n=100)

	β	R^2	R^2 adj.
		0.32***	0.26
Age	-0.11		
White British ethnicity	-0.23*		
Heat-based hair treatment	-0.20*		
Winter season	0.04		
Summer season	0.13		
Painkillers	0.15		
Anxiolytics/antidepressants	0.20*		
Hormonal contraceptives	0.19 \ddagger		

\ddagger p<.10, *p<.05, ***p<.001