Title

Longitudinal association between saliva and hair cortisol concentration: a systematic comparison

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Abstract

Cortisol assays from hair have become increasingly common in psychoneuroendocrinological research as indicators of long-term output relevant to stress and health outcomes. Comparisons of hair cortisol concentration (HCC) with salivary samples have produced mixed findings, and it remains unclear which aspects of the diurnal salivary profile correspond most closely to HCC, and what time intervals between saliva and hair sampling are most relevant, taking the rate of hair growth into account. This longitudinal study aimed to evaluate the correspondence between HCC and parameters of total salivary cortisol output in the morning (CAR_{auc} and CARi) and during the rest of the day excluding the early morning period (DAY_{auc}), by systematically studying three time periods – two weeks, four weeks, and six weeks – before hair sampling. At each time period, 54 female university students (mean age: 20.85 ± 1.16 years) provided three saliva cortisol samples on day 1 at 11 am, 3 pm, at bedtime, then two samples the following day on waking and 30 minutes after awakening. Hair strand collection (1 cm nearest the scalp) took place two weeks after the last saliva sample. Results of multivariable regressions indicate that HCC was consistently associated with DAY_{auc} for all three time periods and with the aggregate DAY_{auc} across days after adjusting for age, body mass index, smoking, oral contraceptive use, hair washing frequency and hair treatments. The strongest associations were found for DAY_{auc} two weeks before hair sampling ($\beta = 0.578$, $p < 0.001$) and the aggregated DAY_{auc} across all three time periods ($\beta = 0.596$, $p < 0.001$), although the confidence intervals overlapped those for four and six week analyses. There was no significant association between HCC and either CAR_{auc} or CARi. Our study confirms that hair cortisol could be a reliable retrospective biomarker of basal and long-term cortisol output secretion at least up to six weeks earlier. The results contribute to a better understanding of the different associations between HCC and salivary cortisol in the morning and the rest of the day, while also having implications for the use of HCC as an outcome measure in intervention and treatment research.

Keywords:

Hair cortisol; Salivary cortisol; Cortisol concentration; Comparison

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis has been central to research on stress and biobehavioural processes since the pioneering work of Hans Selye, with modulation of the concentration of circulating glucocorticoids in response to mental and physical challenge being critical to the regulation of metabolic, cardiovascular, and anti-inflammatory processes (Aguilera, 2012; McEwen, 2007). The traditional sources for analysing endogenous cortisol levels in stress research are blood, urine, and saliva specimens (Turpeinen and Hämäläinen, 2013). Blood analyses can detect the secretion of cortisol bound to carrier proteins (i.e., cortisol binding protein and albumin) and its bioactive free form at a single time point (Levine et al., 2007; Russell et al., 2012). In contrast, urinary measurements cover bioactive cortisol secretion for a period of up to 12-24 hours (Burch, 1982; Russell et al., 2012). Bioactive cortisol levels at a single time point can also be measured via saliva samples. Saliva sampling is non-invasive and unobtrusive, can be carried out while people engage in everyday life activities, and has a high correlation with bioactive free serum cortisol (Kirschbaum and Hellhammer, 1994; Vining et al., 1983). Much psychoneuroendocrinological research both in laboratory and ambulatory studies have been based on salivary analyses as a reliable and feasible biomarker of acute stress (Adam et al., 2017; Hellhammer et al., 2009).

However, assays of cortisol from saliva only reflect momentary cortisol concentrations at the time of sampling rather than sustained levels and the long-term HPA axis functioning. Because salivary cortisol levels fluctuate across the day, there are analytic challenges, and several different components of the diurnal rhythm can be investigated (Adam et al., 2017; Stalder et al., 2016). Consequently, there is increasing interest in measuring cortisol from hair as an indicator of long-term output relevant to stress and health outcomes (Stalder and Kirschbaum, 2012). Based on hair growth of approximately 1 cm per month and the constant deposit of cortisol in the growing hair shaft, a hair segment of 1 cm closest to the scalp represents the cortisol output for the month preceding sample collection (Kirschbaum et al., 2009; LeBeau et al., 2011; Wright et al., 2015). Hair cortisol involves non-invasive

sampling, is less influenced by short-term situational factors than saliva, and has high intraindividual stability (Stalder et al., 2012).

These developments have led to studies comparing hair cortisol concentration (HCC) and cortisol levels in saliva to establish which aspects of diurnal profiles best relate to the integrated cortisol output measured in hair. Several studies have sampled salivary and hair cortisol concurrently, typically on the same day, with mixed results (Flom et al., 2017; Sauvé et al., 2007; Tarullo et al., 2017; van Holland et al., 2012; Vanaelst et al., 2012). For instance, a study with female school-aged students found a moderate positive correlation between concurrently measured hair cortisol concentration and total post-awakening salivary cortisol secretion ($r = 0.39$) (Vanaelst et al., 2012), whereas an earlier study with healthy middle-aged participants found no correlation between concurrently measured hair and salivary cortisol levels (Sauvé et al., 2007). Given that HCC relates to the previous weeks or months, concurrent assessments do not provide accurate temporal correspondence. Other work has compared HCC with salivary cortisol measured over previous weeks, but have varied in their findings (D'Anna-Hernandez et al., 2011; Short et al., 2016; Sugaya et al., 2020; Xie et al., 2011; Zhang et al., 2018). One study found that hair cortisol concentration was correlated only with salivary cortisol at a one-time point (i.e., 30 minutes post-awakening) two weeks before the sampling ($r = 0.40$) rather than other time points over the day. Moreover, hair cortisol was significantly and positively associated with the average cortisol concentration of the three saliva samples taken at one-week intervals before hair was cut ($r = 0.38$) (Xie et al., 2011). A study by Zhang and colleagues (2018) reported a significant correlation between hair cortisol in a 1 cm hair sample and the average of four samples across the day taken two, three and four weeks before hair was cut ($r = 0.39$). But there were no significant associations with the cortisol awakening response (CAR) or diurnal slope (range effect size: $r = 0.08 – 0.29$), and correlations with any single day were weak (range effect size: $r = 0.07 – 0.24$). Two other studies have involved relatively small samples of volunteers who collected saliva samples at three time points every day (waking, 30 minutes post-awakening, and at bedtime) over 30 days (Short et al., 2016; Sugaya et al., 2020). Both studies reported that the strongest association with HCC was obtained by averaging the area under the curve (AUC) of cortisol production over all 30 days (range effect size: $r = 0.41 – 0.61$), with no correlation with the CAR or diurnal slope.

Our study involved comparison between HCC and saliva samples obtained in the weeks before hair was cut. An issue relevant to this literature is to take account of the rate of hair growth, since there is evidence that new hair takes around two weeks to be formed in the follicle before it appears in the hair shaft above the scalp (LeBeau et al., 2011). Thus, HCC comparisons with salivary cortisol and hair cortisol would benefit from taking account of this lag time. Accordingly, the present study compared HCC from a 1 cm sample with saliva samples obtained two, four and six weeks earlier. Additionally, we deliberately obtained saliva samples relevant to waking and the CAR on a different day from those measured over later parts of the day and evening. This permitted the AUC of output over the day to be computed without contamination by the fluctuations early in the day (Clow et al., 2010), resulting in two different overall cortisol measures: a total post-awakening cortisol secretion measure and a total cortisol concentration over the rest of the day (excluding morning cortisol samples).

This study aimed to evaluate the correspondence between hair cortisol concentration in the first 1 cm of hair nearest the scalp and total post-awakening cortisol secretion (CAR_{auc}) and cortisol secretion over the rest of the day (DAY_{auc}) collected two weeks, four weeks, and six weeks before hair sampling. The time points of salivary sampling were selected to reflect the cortisol secretion at the beginning, in the middle, and at the end of the month that potentially reflected hair cortisol accumulation. We collected hair specimens two weeks after the last salivary samples to allow for hair growth. We hypothesised that hair cortisol concentration would be associated with DAY_{auc} throughout the preceding weeks (i.e., at different time points) and most strongly with the averaged (i.e., monthly) salivary DAY_{auc}. Additionally, we conjectured that CAR_{auc} at each time point and its monthly averaged value would not be associated with HCC.

2. Method

2.1. Study design, participants, and procedure

The present longitudinal study lasted seven weeks and included saliva and hair cortisol sampling. Participants completed three diurnal salivary cortisol profiles 6 (W-6), 4 (W-4), and 2 (W-2) weeks prior to hair cortisol sampling. On each week, saliva samples were obtained from 11:00 am on one day to assess cortisol output over the day, and on waking and 30 minutes later to assess the total post-awakening cortisol secretion measure. The final valid sample consist of 54 healthy female second-year predominantly white or Asian students from the Medical and Law Schools at UCL, invited via emails and flyers to participate in the study. Exclusion criteria were being pregnant, having hair shorter than 1 cm, having any chronic or acute medical conditions (e.g., cardiovascular disease, cancer, etc.), and having a regular intake of steroid medication, as these factors are known to affect cortisol secretion. Participants needed to be able to commit to all assessments, and they were compensated with £50 once all assessments were completed. The study was approved by the UCL Research Ethics Service. Participants attended the research laboratory at UCL twice. At the first visit, the study was explained in detail and written informed consent was obtained. Demographic, anthropometric, and lifestyle measures were assessed. Participants were shown how to sample their saliva and were given a pack containing five labelled Salivette tubes (Sarstedt, Numbrecht-Rommelsdorf, Germany) to take home with them. They were also given the packs for the two following sampling points (i.e., W-4 and W-2). At the second meeting (i.e., two weeks after the last saliva sampling; the W-4 and W-2 saliva sampling packs were returned to the research in the following 3-6 days after collection), a hair sample was collected, and hair-specific factors affecting hair cortisol concentration (washing frequency, hair colour, product use and hair treatment/dyeing) were assessed by a self-report questionnaire.

2.1.1 Saliva sample collection

Saliva was collected with Salivettes during three 24-hour periods: 6, 4 and 2 weeks prior to hair specimen cutting, and were timed at 11 am, 3 pm, at bedtime, at then on waking the following day and 30 minutes after awakening. Each saliva sample set (i.e., 6, 4, and 2 weeks prior to hair sampling) was collected on the same weekdays for each participant; samples were not collected during weekend days. Reminder emails and text messages were sent to encourage participants to adhere to the sampling

protocol. Participants were asked to abstain from smoking, food, medication and alcohol intake, brushing teeth, and exercise 30 minutes prior to saliva sampling. All samples were returned to UCL in the following 3-6 days and stored in a freezer at −20°C until they were sent to the Technical University of Dresden, Germany, where steroid extraction was performed. Cortisol concentrations (in nmol/L) were assessed using a time-resolved immunoassay with fluorescence detection, and the intra- and inter-assay coefficients of variation were less than 4%. The study was conducted before the recommendations for assessing the CAR were published (Stalder et al., 2022), therefore our protocol did not follow these guidelines.

2.1.2. Hair sample collection

Hair collection took place two weeks after the last saliva sampling. A scalp hair strand of 1 cm was collected from the posterior vertex position (identified as the area with most consistent hair growth) by cutting the hair as close to the scalp as possible with fine medical scissors. These were placed onto aluminium foil, labelled with the identification number, and stored in a dry, dark place (Kirschbaum et al., 2009). After the end of the collection, all hair samples were shipped to the Technical University of Dresden, Germany. The wash procedure and steroid extraction were undertaken using high-performance liquid chromatography-mass spectrometry (LC/MS) (Gao et al., 2013), with a minimum of 10 mg ± 0.5 mg of hair cut from each 1 cm hair segment, with concentrations expressed in pg/mg. Intra- and inter-assay variance coefficients for this assay were below 8%.

2.1.3. Covariates

Information on participants' age, ethnicity, weight, height, use of oral contraception, and smoking were recorded at the first visit. Body mass index (BMI) was computed as the weight (kg) ratio to the square of height (m). The use of oral contraception and smoking was measured as a binary variable. The hair-specific factors that could affect hair cortisol concentration were assessed by self-report and verified at the second visit (i.e., the time of hair sampling). These factors include the frequency of hair washes

per week and any type of hair treatment within the study period. Hair treatment was assessed with three questions combined into a binary score indicating the presence or absence of any hair treatment (Short et al., 2016).

2.2. Statistical Analysis

Data analyses were performed using IBM SPSS Statistic version 27 (SPSS Inc.). All cortisol values that lie more than three times the interquartile range below the first quartile or above the third quartile were removed. For hair cortisol, the thresholds for the interquantile range were 7.874 pg/ng and 20.884 pg/ng. For 6-W salivary cortisol collection, upper and lower limits for the interquartile ranges were 8.290-19.266 nmol/l at 11 am, 5.521-11.531 at 3 pm, 1.509-5.071 at bedtime, 8.821-21.818 at waking and 17.919-38.045 at 30 minutes after awakening. For 4-W salivary cortisol collection, upper and lower limits for the interquartile ranges were 7.939-19.568 nmol/l at 11 am, 4.505-11.044 at 3 pm, 1.319-4.011 at bedtime, 10.256-23.173 at waking and 16.236-40.015 at 30 minutes after awakening. For 2-W salivary cortisol collection, upper and lower limits for the interquartile ranges were 8.507-21.088 nmol/l at 11 am, 5.181-10.124 at 3 pm, 1.466-6.846 at bedtime, 11.037-21.640 at waking and 17.267-31.432 at 30 minutes after awakening. We excluded 11 participant from the study analyses using these criteria. The overall volume of cortisol released over the morning period (CAR_{auc}) was derived for the trapezoid formula using the waking and the 30 min post-awakening cortisol values to obtain a total post-awakening cortisol secretion measure (Chida and Steptoe, 2009; Stalder et al., 2016). We also computed a measure of dynamic increase of post-awakening cortisol (i.e., CARi) as the delta score between awakening sample and 30 minutes after awakening sample (Stalder et al., 2022, 2016). Participants with a delay of more than 15 min between waking and 30 min after waking saliva sampling were excluded from the CAR_{auc} and CARi analyses (Dockray et al., 2008). We exclude 12 participant from the study analyses for this reason. The overall volume of cortisol released over the rest of the day (DAY_{auc}) was computed by trapezoidal calculation of the 11 am, 3 pm and bedtime cortisol values (Pruessner et al., 2003). Hair cortisol, CAR_{auc}, CARi, and DAY_{auc} of the salivary cortisol data were skewed and were subjected to a natural logarithmic transformation to

normalise the distribution. The primary analyses involved multivariable linear regression to examine the relationship between the salivary cortisol parameters and hair cortisol analyses, controlling for age, BMI, smoking, use of oral contraceptives and hair-specific factors. Variance inflation factor (VIF) values and tolerance values were generated for all regression models to assess multicollinearity, and the assumption was not violated (VIF <10 and tolerance >0.1). We ran four separate models for CAR_{auc} and DAY_{auc}, corresponding to the three individual weeks and aggregated values across weeks. Additionally, to facilitate comparisons with previous studies, Pearson correlations and partial correlations between HCC and salivary measures adjusting for age, BMI, smoking, use of oral contraceptives and hair-specific factors were computed. In the models including CAR_{auc} and CARi, we added the awakening time as covariate of the partial correlation models. Ethnicity was not related to the cortisol findings, so was not included as a factor in the final models.

3. Results

Table 1 summarises the socio-demographic, health-related and hair characteristics of the sample. Participants had an age range between 20 and 24 years, and 85.2% had a BMI in the normal range (BMI < 25). Most participants were non-smokers (89.9%), and 16 used oral contraceptives.
The raw salivary cortisol levels in the five time-points across W-6, W-4, and W2 are reported in Table S1 of the Supplementary Material, and show typical diurnal profiles. Levels were moderately high at 11:00 and subsequently declined over the afternoon and evening. Cortisol concentrations were again moderately high on waking, then increased by an average of 9.64-12.05 nmol/l. The range of the mean values for CAR_{auc} across the different weeks of the study was 6.40-6.45 ln(nmol/l*min). The range of the mean value for CARi across the different weeks of the study was -1.10-3.53 ln(nmol/l). The range of the mean values for DAY_{auc} across the weeks of the study was 8.65-8.78 ln(nmol/l*min). The mean hair cortisol concentration was 2.49 ± 0.78 ln(pg/ng). The mean awakening time was 7:44 ± 1:10 at W-6, 7:59 ± 1:05 at W-4, and 8:07 ± 1:18 at W-2. The mean bedtime was 24:12 ± 1:25 at W-6, 24:18 ± 1:44 at W-4, and 24:11 ± 1:27 at W-2. No major life events that might affect participants' cortisol secretion during the seven-week study period were reported. Repeated-measures analysis of variance

indicated that the salivary cortisol parameters did not differ significantly across the three sampling time points (CAR_{auc}: F [2, 106] = 0.202, p = 0.818; DAY_{auc}: F [2, 106] = 1.641, p = 0.199). Significant associations were observed across all the single-day levels of CAR_{auc} and DAY_{auc} across the study collection period. Thus W-6 CAR_{auc} was associated with W-4 CAR_{auc} (r = 0.452, p = 0.001) and W-2 CAR_{auc} (r = 0.378, p = 0.005), while W-4 CAR_{auc} was associated with W-2 CAR_{auc} (r = 0.359, p = 0.008). Moreover, W-6 DAY_{auc} was associated with W-4 DAY_{auc} (r = 0.421, p = 0.002) and W-2 DAY_{auc} (r = 0.276, p = 0.043), and W-4 DAY_{auc} was associated with W-2 DAY_{auc} (r = 0.482, p < 0.001).

Table 2 shows the correlation coefficients between salivary cortisol parameters and HCC. The aggregated CAR_{auc} was positively correlated with hair cortisol levels (r = 0.270, p = 0.048), but there was no significant correlation between CAR_{auc} on any single day and HCC. By contrast, HCC was significantly and positively correlated with the DAY_{auc} at all three sampling points (W-6: r = 0.376; W-4: r = 0.358; W-2: r = 0.549, all p's < 0.01). The aggregated DAY_{auc} was also correlated with hair cortisol concentration (r = 0.551, p < 0.001). Adjusting the analyses for age, BMI, smoking status, the use of oral contraceptives, the frequency of hair washing and the presence of hair treatments did not substantially change any association between cortisol DAY_{auc} and hair cortisol levels, but the aggregated CAR_{auc} was no longer associated with HCC. It is notable that the strongest associations were between HCC and DAY_{auc} for samples two weeks before hair was cut (W-2) and for the aggregated DAY_{auc}. Table S2 of Supplementary Material shows the correlation and partial correlation coefficients between CARi and HCC. No significant associations were found between any CARi value (i.e., W-6, W-4, W-2, aggregated measure) and HCC. In Table S3 of Supplementary Material, we reported correlation and partial correlation coefficients between waking cortisol levels (at W-6, W-4, W-2, and the three sample aggregated measure) and HCC. Hair cortisol was significantly associated with waking salivary cortisol at W-6 (r = 0.318, p = 0.019), W4 (r = 0.282, p = 0.039) and the averaged levels of waking salivary cortisol across all the three sampling periods (r = 0.326, p = 0.016). Partial correlations partly confirmed these associations, with hair cortisol levels significantly associated with waking salivary cortisol at W-6 (r = 0.289, p = 0.047) and with the aggregate measurements of salivary cortisol at the awakening time (r = 0.564, p = 0.037).

3.1. Multivariable regressions on hair cortisol concentration

The multivariable regression models confirm that the salivary cortisol CAR_{auc} was not associated with HCC after adjusting for covariates (Table 3). In contrast, HCC was consistently associated with DAY_{auc} on all three occasions and with the aggregated DAY_{auc} across days, independently of covariates (Table 4). The standardised regression coefficients were substantially greater for W-2 (β = 0.578) and the aggregated DAY_{auc} (β = 0.596) than for W-4 or W-6 (β = 0.382); however, the confidence intervals overlapped. Additionally, the model for cortisol DAY_{auc} at W-2 accounted for 36% of variance in HCC, and the aggregated model accounted for 37% of variance, compared with 20% and 21% for W-4 and W-6, respectively.

4. Discussion

The present study explored the relationship between parameters of total salivary cortisol output in the morning and during the rest of the day assessed on three days throughout seven weeks and HCC corresponding to the same period of time. We focused on two aspects of diurnal salivary cortisol output commonly investigated in psychoneuroendocrinology, namely output in the period soon after waking, and the remainder of the day. It was found that there were consistent associations between HCC and the overall volume of salivary cortisol released over the day (excluding the early morning period), but not with the volume of salivary cortisol released over the early morning. This relationship between cortisol DAY_{auc} and HCC withstood adjustments for covariates that have been found to be related to hair cortisol concentration.

The relationship between salivary cortisol through the day and HCC is in line with previous studies in the general population that sampled these specimens over corresponding time intervals (Short et al., 2016; Sugaya et al., 2020; Zhang et al., 2018). Short and colleagues (2016) found a significant correlation (r = 0.61, p = 0.01) between HCC and the integrated salivary cortisol AUC based on three daily samples (i.e., on awakening, 30 minutes post-awakening, and at bedtime) over

one month. In a more recent study, the association between integrated salivary cortisol AUC obtained from three daily samples (i.e., on awakening, 30 minutes post-awakening, and at bedtime) over one month and HCC almost reached a significant level (r = 0.41, $p = 0.052$) (Sugaya et al., 2020). A study of young Chinese women similar to ours reported that averaged salivary cortisol AUC based on four samples (i.e., on awakening, 30 minutes post-awakening, at noon, and at 17:00) collected two, three, and four weeks before hair sampling was associated with HCC (r = 0.41, $p < 0.01$) but that only the AUC of samples collected at two and three weeks were individually correlated with HCC (Zhang et al., 2018). Our study confirms and extends these findings in two ways. First, we show that cortisol DAY_{auc} sampled over six weeks as well as more recently before hair sampling was associated with HCC. Second, our computation of salivary DAY_{auc} excluded the early morning period, so strongly indicates that output of cortisol over the day is more relevant to HCC than the early morning levels. This is consistent with our finding that HCC was not associated with the total post-awakening cortisol concentration, and is in line with the results reported by Zhang et al. (2018). In addition, other studies confirmed that there was no association between HCC and averaged daily CAR parameters over a month (Short et al., 2016; Sugaya et al., 2020).

One explanation may be that the CAR – whether assessed as the total post-awakening cortisol concentration or the increase from waking to 30 min later – is influenced by multiple short-term psychosocial factors and momentary conditions could influence morning cortisol levels substantially (Clow et al., 2010; Kramer et al., 2019; Stalder et al., 2016; Steptoe and Serwinski, 2016; Williams et al., 2005). Cortisol output over the remainder of the day may be associated more robustly with sustained lifestyle factors and experiences (Adam et al., 2017; Adam and Kumari, 2009), and therefore be more closely related to a longer-term biomarker such as HCC. Unfortunately, direct comparisons of our findings with those of many studies contrasting salivary cortisol AUC with HCC have computed the AUC through combining early morning with later time points, so may conflate systems that are regulated differently (Short et al., 2016; Sugaya et al., 2020; Zhang et al., 2018).

A central aim of this study was to clarify the temporal relationship between HCC and saliva sampling. Based on the view that hair grows around 1cm /month, we expected that HCC would be more closely associated with saliva samples collected two and four weeks rather than six weeks

earlier. But while the association of HCC with DAY_{auc} measured at two weeks was higher than for six weeks, the relationship was no stronger for four than six weeks. This could be taken as evidence that HCC is more consistently linked with saliva values obtained at the mid-point of the one month growth period. However, the confidence intervals for the associations at different time points overlapped substantially, so no firm conclusion can be drawn. A likely explanation is that the cortisol profiles at weeks two, four, and six were very similar and moderately correlated. This may also be why there was a strong association between HCC and DAY_{auc} aggregated across days. Thus these timing issues would be best clarified in participants whose cortisol levels were more variable on different weeks.

The study has a number of strengths. The longitudinal design allowed us to explore the retrospective correspondence between hair and salivary cortisol concentration, considering time as an important factor in hair growth. Moreover, distinguishing parameters of salivary cortisol secretion in the morning and the rest of the day contributed to a better understanding of the chronobiological factors relevant to the correspondence between hair and salivary cortisol concentration. However, the findings should be interpreted in light of study limitations. The timing of saliva sampling was self-reported, and inaccuracies in reporting may be particularly important early in the day (Dockray et al., 2008; Stalder et al., 2022). We calculated the cortisol CAR_{auc} based only on two samples (i.e., at awakening and 30 minutes post-awakening). In contrast, using at least three cortisol samples after awakening has been recommended to capture the CAR accurately (Stalder et al., 2016). As noted earlier, data collection was carried out before the consensus guidelines for the assessment of the CAR were published. The study design was unusual in that the collection of saliva samples to compute the DAY_{auc} was carried out on a different day to that used to assess the CAR. This was done to dissociate these two aspects of cortisol rhythm, but it does mean that estimates of cortisol output over a complete day could not be computed. We focused on the links between salivary cortisol samples and later assessments from hair, so did not include a cross-sectional comparison between saliva and hair monitored on the same day. This has limited comparisons with some previous studies that used such an approach. It should also be noted that the study sample was composed of an ethnically diverse group of well-educated female college students in London, and different findings might emerge in

other populations. The use of a female sample means that we do not know whether similar results would emerge for men.

In conclusion, we found that hair cortisol concentration was associated with the salivary cortisol DAY_{auc} collected up to 6 weeks before the hair sampling. Associations were somewhat stronger for samples collected two weeks before hair cutting, but differences were small. No association was found between hair cortisol and the cortisol awakening response. These findings suggested the use of hair cortisol as a retrospective biomarker of basal cortisol output secretion over several previous weeks. The results are relevant not only to psychoneuroendocrinological research in general, but have implications for the use of hair samples for assessing interventions and treatments aimed at managing mental ill-health or promoting psychological wellbeing. Many study designs involve pre- and post-intervention assessments. If HCC reflects cortisol secretion up to six weeks beforehand, then samples taken immediately after an intervention will not provide an accurate measure of the effects of the treatment.

Contributors

All authors contributed significantly to the conception, design, analyses or interpretation of data and were involved in revising the manuscript critically for intellectual content. The submission of this paper was approved by all authors.

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Conflicts of interest

None of the authors have any conflicts of interest to declare related to the findings of this study.

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Table 1 Demographic and hair-related characteristics of the sample (N = 54)

Characteristic	Mean ± SD or N (%)
Age	20.85 ± 1.16
Ethnicity	
White	34 (63.0)
Asian	16 (29.6)
Other (black/mixed)	4 (7.4)
Body mass index (kg/m^2)	21.95 ± 3.77
Current smoker	
Yes	6 (11.1)
No	48 (88.9)
Use of oral contraception	
Yes	16 (29.6)
No	38 (70.4)
Hair wash frequency	
1-2 times/week	9 (16.7)
3-5 times/week	34 (63.0)
6-7 time/week	11 (20.3)
Hair treatment	
Yes	4 (92.6)
No	50 (7.4)

Table 2 Bivariate and partial correlation coefficient for the different salivary parameters and hair cortisol concentration (N = 54)

Salivary cortisol parameter	W-0 Hair cortisol sample Bivariate correlation	W-0 Hair cortisol sample Partial correlation*
W-6 CAR_{auc} [ln(nmol/l*min)]	r = 0.248, $p = 0.071$	r = 0.200, $p = 0.178$
W-4 CAR_{auc} [ln(nmol/l*min)]	r = 0.230, $p = 0.094$	r = 0.227, $p = 0.122$
W-2 CAR_{auc} [ln(nmol/l*min)]	r = 0.134, $p = 0.336$	r = 0.121, $p = 0.414$
Agg CAR_{auc} [ln(nmol/l*min)]	r = 0.270, $\mathbf{p = 0.048}$	r = 0.241, $p = 0.099$
W-6 DAY_{auc} [ln(nmol/l*min)]	r = 0.376, $\mathbf{p = 0.005}$	r = 0.380, $\mathbf{p = 0.008}$
W-4 DAY_{auc} [ln(nmol/l*min)]	r = 0.358, $\mathbf{p = 0.008}$	r = 0.362, $\mathbf{p = 0.011}$
W-2 DAY_{auc} [ln(nmol/l*min)]	r = 0.549, $\mathbf{p < 0.001}$	r = 0.552, $\mathbf{p < 0.001}$
Agg DAY_{auc} [ln(nmol/l*min)]	r = 0.551, $\mathbf{p < 0.001}$	r = 0.564, $\mathbf{p < 0.001}$

Notes: CAR_{auc} = the overall volume of cortisol released over the morning period; DAY_{auc} = the overall volume of cortisol released over the rest of the day; W-6 = 6 weeks prior to hair cortisol sampling; W-4 = 4 weeks prior to hair cortisol sampling; W-2 = 2 weeks prior to hair cortisol sampling; Agg = aggregated (average of W-6, W-4 and W-2). * Partial correlation analyses were adjusted for age, BMI, smoking, use of oral contraceptives and hair-specific factors. When the model included a value of CAR_{auc}, awakening time was added as covariate of the analyses.

Table 3 Multivariable regression of hair cortisol on the salivary cortisol CAR_{auc} parameters (at each time point – W-6, W-4, W-2 – and the aggregated measure) (N = 54)

	B	95% CI	β	SE (β)	*P*
Model 1					
W-6 CAR_{auc}	0.284	[-0.122; 0.690]	0.208	0.148	0.166
Age	-0.087	[-0.287; 0.112]	-0.129	0.147	0.383
BMI	-0.013	[-0.074; 0.048]	-0.062	0.146	0.675
Smoking	0.050	[-0.670; 0.769]	0.020	0.145	0.890
Oral contraceptives	0.110	[-0.419; 0.640]	0.065	0.155	0.677
Hair wash frequency	0.031	[-0.364; 0.425]	0.024	0.154	0.877
Hair treatment	-0.578	[-1.474; 0.317]	-0.196	0.151	0.200
Model 2					
W-4 CAR_{auc}	0.329	[-0.091; 0.748]	0.230	0.146	0.122
Age	-0.087	[-0.285; 0.111]	-0.129	0.146	0.380
BMI	-0.011	[-0.071; 0.050]	-0.051	0.146	0.727
Smoking	0.054	[-0.662; 0.770]	0.022	0.145	0.880
Oral contraceptives	0.168	[-0.357; 0.694]	0.099	0.154	0.522
Hair wash frequency	0.028	[-0.362; 0.418]	0.022	0.152	0.885
Hair treatment	-0.701	[-1.576; 0.174]	-0.237	0.147	0.114
Model 3					
W-2 CAR_{auc}	0.197	[-0.284; 0.678]	0.119	0.144	0.414
Age	-0.102	[-0.303; 0.099]	-0.151	0.148	0.312
BMI	-0.014	[-0.076; 0.048]	-0.068	0.148	0.649
Smoking	0.066	[-0.666; 0.799]	0.027	0.148	0.856
Oral contraceptives	0.107	[-0.434; 0.649]	0.063	0.159	0.692
Hair wash frequency	0.117	[-0.264: 0.498]	0.092	0.149	0.538
Hair treatment	-0.643	[-1.544; 0.257]	-0.218	0.152	0.157
Model 4					
Agg CAR_{auc}	0.461	[-0.090; 1.011]	0.242	0.144	0.099
Age	-0.088	[-0.285; 0.109]	-0.131	0.145	0.372
BMI	-0.011	[-0.072; 0.049]	-0.055	0.145	0.708
Smoking	0.075	[-0.640; 0.789]	0.030	0.144	0.834
Oral contraceptives	0.108	[-0.417; 0.632]	0.064	0.154	0.681
Hair wash frequency	0.029	[-0.358; 0.415]	0.022	0.151	0.882
Hair treatment	-0.593	[-1.474: 0.287]	-0.201	0.148	0.182

Notes: CAR_{auc} = the overall volume of cortisol released over the morning period; W-6 = 6 weeks prior to hair cortisol sampling; W-4 = 4 weeks prior to hair cortisol sampling; W-2 = 2 weeks prior to hair cortisol sampling; Agg = aggregated (average of W-6, W-4 and W-2).

Table 4 Multivariable regression of hair cortisol on the salivary cortisol DAY_{auc} parameters (at each time point – W-6, W-4, W-2 – and the aggregated measure) (N = 54)

	B	95% CI	β	SE (β)	*p*
Model 1					
W-6 DAY_{auc}	0.607	[0.168; 1.046]	0.382	0.137	**0.008**
Age	-0.077	[-0.265; 0.112]	-0.114	0.139	0.417
BMI	-0.006	[-0.064; 0.052]	-0.028	0.139	0.842
Smoking	-0.046	[-0.728; 0.636]	-0.019	0.138	0.892
Oral contraceptives	0.073	[-0.428; 0.574]	0.043	0.147	0.772
Hair wash frequency	0.024	[-0.337; 0.385]	0.019	0.141	0.895
Hair treatment	-0.732	[-1.564; 0.100]	-0.248	0.140	0.083
Model 2					
W-4 DAY_{auc}	0.527	[0.125; 0.929]	0.382	0.145	**0.011**
Age	-0.029	[-0.226; 0.168]	-0.043	0.145	0.767
BMI	-0.011	[-0.069; 0.047]	-0.051	0.139	0.714
Smoking	-0.171	[-0.874; 0.532]	-0.070	0.142	0.626
Oral contraceptives	0.085	[-0.419; 0.589]	0.050	0.148	0.735
Hair wash frequency	0.060	[-0.300; 0.420]	0.047	0.141	0.738
Hair treatment	-0.803	[-1.645; 0,038]	-0.272	0.142	0.061
Model 3					
W-2 DAY_{auc}	0.901	[0.497; 1.305]	0.578	0.129	**>0.001**
Age	-0.054	[-0.224; 0.116]	-0.080	0.125	0.526
BMI	0.006	[-0.047; 0.058]	0.028	0.127	0.826
Smoking	-0.387	[-1.029; 0.254]	-0.157	0.130	0.230
Oral contraceptives	0.133	[-0.317; 0.582]	0.078	0.132	0.555
Hair wash frequency	0.176	[-0.145; 0.497]	0.138	0.125	0.275
Hair treatment	-0.548	[-1.301; 0.204]	-0.186	0.127	0.149
Model 4					
Agg DAY_{auc}	1.158	[0.655; 1.661]	0.596	0.129	**>0.001**
Age	-0.013	[-0.184; 0.159]	-0.019	0.126	0.882
BMI	0.003	[-0.049; 0.055]	0.013	0.125	0.917
Smoking	-0.350	[-0.980; 0.279]	-0.142	0.127	0.268
Oral contraceptives	0.052	[-0.394; 0.499]	0.031	0.131	0.814
Hair wash frequency	0.043	[-0.275; 0.361]	0.034	0.124	0.787
Hair treatment	-0.753	[-1.477; 0.007]	-0.249	0.125	0.052

Notes DAY_{auc} = the overall volume of cortisol released over the rest of the day; W-6 = 6 weeks prior to hair cortisol sampling; W-4 = 4 weeks prior to hair cortisol sampling; W-2 = 2 weeks prior to hair cortisol sampling; Agg = aggregated (average of W-6, W-4 and W-2).