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SHORT COMMUNICATION

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Comparison of human hair cortisol concentration stability for 1-year and 2-year test–retest intervals

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Abstract

Human hair cortisol concentration (HCC) has previously been found to be highly stable for a 1-year interval ($r = 0.73$) in terms of a product–moment correlation. The present study aimed to replicate this finding and compare HCC stability regarding 1-year and 2-year test–retest intervals. Female university students ($N = 39$) provided hair strands twice (t1 and t2) at intervals of 1 ($n = 21$) or 2 years ($n = 18$). Multiple regression analysis predicting HCC at t2 revealed a significant interaction term (HCC at t1 × time interval condition). It was determined that HCCs were substantially related for the 1-year interval but unrelated for the 2-year interval. The findings were not attributable to potential influences, such as hair treatment. The product–moment correlation showed nearly identical consistency with previous research regarding the 1-year test–retest interval. There was no significant product–moment correlation for the 2-year interval. Overall, these findings indicate that within a temporal framework of 1 year, HCCs may be stable predictors in correlational studies where the focus is on the rank orders of measured values.

KEYWORDS
biological markers, hair cortisol, human, reliability, stability

1 | INTRODUCTION

The physiological stress response is an intricate interplay of many systems and stress mediators (Joëls & Baram, 2009). As one crucial response involved, confrontation with a stressor activates the hypothalamic–pituitary–adrenal axis in humans, starting a hormonal cascade that leads to cortisol secretion (de Kloet et al., 2005). Cortisol secretion in saliva is an indicator of the acute physiological response to a stressor (Foley & Kirschbaum, 2010; Hellhammer et al., 2009; Kirschbaum et al., 2009). In addition, as cortisol is incorporated into the hair shaft during hair growth, cumulative cortisol secretion over an extended period can be retrospectively assessed using hair cortisol concentration (HCC), which is an indicator of chronic stress (Stalder et al., 2012, 2017). In this context, previous studies have shown HCC to be related to severe stress events (e.g., divorce or serious illness) during a prolonged phase of life (e.g., Karlén et al., 2011; Stalder et al., 2017; van Uum et al., 2008). HCC has also been found to be relatively stable against possible confounding factors (e.g., hair-washing frequency, age [except young children and older adults], and the use of oral contraceptives), which have shown effects only in isolated studies (Dettenborn et al., 2012; Fischer et al., 2017; Stalder et al., 2012). Thus, Stalder et al. (2017) assumed that none of these factors constitute a major confounding influence on HCC results.

With regard to sex, Stalder et al. (2017) reported in their meta-analysis a 21% higher HCC in men than in women (see also

Weckesser et al., 2021); however, Weckesser et al. (2019) did not find that sex explains the variance in the association between HCC and perceived stress. Moreover, several studies have reported no difference between sexes in the temporal stability of HCC (e.g., Chen et al., 2019; Zhang et al., 2017). Thus, while there is clear empirical evidence of sex differences in terms of absolute HHC levels (indicated by mean differences), this is not the case for correlative stability parameters. Notably, while there are studies that have focused solely on men (e.g., Herr et al., 2018), many investigations have examined all-women samples (e.g., Abdul Jafar et al., 2023; Kirschbaum et al., 2009).

With reference to the intraindividual stability of HCC values, Stalder et al. (2012) found high and statistically significant test–retest associations of HCC at a 1-year interval ($r = 0.73$, $N = 45$, most of them amateur endurance athletes). Based on further analyses, Stalder et al. (2012) concluded that, in the absence of major life events, HCC has a high level of long-term stability encompassing a strong trait component that is only to a lesser extent influenced by state-related factors. Substantial HCC test–retest relations were also found by other authors for different time intervals (e.g., Chen et al., 2019, 12-month interval; Short et al., 2016, 1-month interval; Zhang et al., 2017, 6- and 12-month intervals). However, to the best of our knowledge, no study has examined HCC stability over an extended period of 2 years and compared HCC stabilities over 1 and 2 years. Therefore, our research questions whether and to what extent HCC stability changes within intervals of 1 and 2 years. The findings to this question are relevant to further questions, such as the time interval at which HCC may be predictive of other variables (e.g., health-related outcomes). Given the previous findings on the test–retest stability of HCC, we predicted that HCC would be strongly positively related to retest HCC after a 1-year as well as a 2-year test–retest interval.

2 | METHODS

2.1 | Participants

Stalder et al. (2012) considered a test–retest stability of $r = 0.73$ as indicating a high level of long-term stability. Therefore, we conducted an a priori power analysis using G × Power 3.1 (Faul et al., 2009) around a similar effect size ($r = 0.70$ or $\rho^2 = 0.49$). According to the power analysis, a sample size of at least 21 participants would be required to detect such a relationship between HCC at the first (t1) and the second (t2) time of measurement in a multiple regression model (H1: $\rho^2 = 0.49$, H0: $\rho^2 = 0$, $\alpha = 0.05$, $1 - \beta = 0.80$, two-tailed test, number of predictors = 3). Our total sample consisted of 39 female university students ($M_{age} = 19.41$ years, $SD_{age} = 1.39$).[1] We oversampled to account for the possible exclusion of participants for potential problems, such as outlying values, an insufficient amount of hair, and measurement errors.

The participants were recruited at the University of Bern and compensated with 20 Swiss francs at each time of measurement. The first hair sample (t1) was given by 18 participants in autumn 2016 and exactly 1 year later (i.e., in autumn 2017) by another 21 participants. To exclude potential seasonal differences in HCC (Fischer et al., 2017), all 39 participants gave a second hair sample exactly 1 more year later (i.e., in autumn 2018; t2). Thus, there were two conditions regarding the distance between the first and second measurements of HCC (i.e., $n = 21$ for the 1-year interval and $n = 18$ for the 2-year interval). All hair samples were of sufficient length and included enough material for HCC to be measured.

We did not exclude any participants as outliers, as no HCC value exceeded the threshold of standardized value of 3.29 (Tabachnick & Fidell, 2007), and in this study, all absolute standardized values were below 2.42. None of the participants reported being pregnant or breastfeeding at t1 or t2. The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics commission of the University of Bern. All participants provided written informed consent after they were informed about the procedure and objectives of the study. Part of the present sample and data were collected for a pilot study on stress and parental educational background, which is reported elsewhere (Bertrams & Minkley, 2020).

2.2 | Procedure

At t1 and t2, each participant provided three thin hair strands (3–5 mm thick), which were taken from the scalp near the posterior vertex region, where the hair growth rate is most uniform (Stalder & Kirschbaum, 2012). From these strands, a 1.5 cm section of hair next to the scalp was cut off and transferred to a snap-cap vial. Assuming a hair growth rate of ~1 cm/month (Stalder & Kirschbaum, 2012), this hair segment represented a time interval of 1.5 months. The cortisol concentration (measured in pg/mg) of this interval was analysed at the Dresden LabService GmbH laboratory in Germany. The laboratory determined cortisol using a commercially available immunoassay with chemiluminescence detection (CLIA, IBL). Note that the HCC at t1 was determined immediately after the collection of hair samples; that is, the first samples were not stored for 1 or 2 years until the second collection of samples to avoid the effects of storage on HCC.

We also applied a self-report questionnaire at both measurement times to identify possible confounding differences between the two conditions (i.e., the 1-year time interval vs. the 2-year time interval). This questionnaire included questions related to stress, health, medication, and hair treatment, which were applied in a similar manner as in previous research (e.g., Stalder et al., 2012). In this questionnaire, we asked for concrete numbers (e.g., body height and weight, frequency of hair washing per week) or applied an open-response format for specific questions. For example, we asked whether medications such as hormonal contraception were currently being taken and, if so, which one.

If a contraceptive was mentioned, the current use of contraceptives was coded dichotomously with 1 ('yes') or 0 ('no') for the analysis. Analogously, dichotomous variables for the application of chemical hair treatment and hair styling methods were created. For the following questions, we asked for a direct 'yes' (coded as 1) or 'no' (coded as 0) response: 'Are you a smoker', 'During the last 6 months, have you experienced anything that you would describe as a serious life event (e.g., the death of a loved one, a serious illness, or a divorce)', and 'During the last 6 months, have you experienced anything else that you would describe as particularly stressful or demanding events or circumstances'. Since part of the present sample also participated in a different study (Bertrams & Minkley, 2020), the questionnaire also included items on psychological variables not relevant to the present study.

2.3 | Data analysis strategy

The analyses were conducted using SPSS version 28. We applied multiple regression with an interaction term (Aiken & West, 1991; Cohen et al., 2003; Hayes, 2022), which allowed us to analyse the variance in the data of all 39 participants simultaneously, instead of treating them as two separate samples. We regressed the HCC at t2 on the standardized HCC at t1, the retest time interval condition as a dichotomous categorical group variable (1 year vs. 2 years), and the product between these two predictors as the interaction term. To normalise the distributions, we log-transformed all HCC data for the analysis (Miller & Plessow, 2013; Weckesser et al., 2019). These transformations helped normalise the HCC distributions, as indicated by histograms, Q-Q plots, and Shapiro–Wilk tests. While the Shapiro–Wilk tests were statically significant prior to the log-transformations ($ps > 0.001$), indicating deviation from normal distribution, they became nonsignificant afterwards ($ps > 0.13$). To interpret the interaction, we applied a recoding procedure (Cohen et al., 2003; Hayes, 2022) that coded the 1-year interval as 0 and the 2-year interval as 1 the first time and reversed this coding the second time. We also calculated 95% confidence intervals based on bias-corrected and accelerated bootstrapping (BCa 95% CI; 10,000 bootstrap samples) for the regression coefficients (B) as estimates of the robustness of the relationships found (Field, 2014). To allow a direct comparison with the result of Stalder et al. (2012), we also report Pearson product–moment correlations for both time intervals as complementary information.

In addition, to contribute cumulative insights into HCC, we report the bivariate correlations between HCC and potentially stress-relevant variables, such as body mass index and the occurrence of serious life events (Pearson product–moment correlations or, when a categorical variable was involved, point–biserial correlations). As supplementary analyses, to rule out potential confounders, we also compared both time interval conditions with respect to group differences in the self-report measures, applying either independent samples t-tests (for continuous variables) or chi-square tests for independence (for categorical variables).

3 | RESULTS

3.1 | Main analysis

There was a significant interaction between HCC at t1 and the time interval condition ($B = -0.27$, BCa 95% CI [−0.48, −0.04], $SE\ B = 0.08$, $t = -3.56$, $p = 0.001$), qualifying significant main effects. The recoding procedure revealed that the relationship between HCC at t1 and HCC at t2 was significant for the 1-year retest interval ($B = 0.32$, BCa 95% CI [0.20, 0.48], $SE\ B = 0.06$, $t = 5.04$, $p < 0.001$) but was not significant for the 2-year interval ($B = 0.05$, BCa 95% CI [−0.03, 0.22], $SE\ B = 0.04$, $t = 1.13$, $p = 0.27$). Figure 1 illustrates this pattern. For more details on the multiple regression analysis, see Table S1 in the Supporting Information for this article.

The complementary Pearson product–moment correlations determined separately for the two time intervals were as follows: r ($df = 19$) = 0.72, BCa 95% CI [0.43, 0.91], $p < 0.001$ for the 1-year interval (indicating high stability; Zhang et al., 2017) and r ($df = 16$) = 0.31, BCa 95% CI [−0.34, 0.80], $p = 0.21$ for the 2-year interval (indicating weak stability; Zhang et al., 2017).

3.2 | Supplementary analysis

We also compared the two samples with regard to the applied self-report measures. As Table S2 (see the Supporting Information for this article) depicts, there were no statistically significant differences in the respective variables between both of the time interval conditions at the two measurement times.

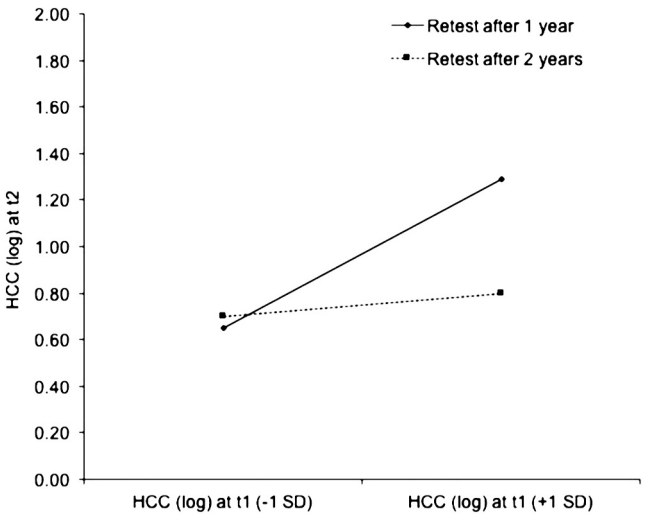

FIGURE 1 Relationship between HCC† at t1‡ and at t2‡ separately for the time interval conditions. The slopes were calculated on the basis of the regression equation (derived from the main analyses) at low (−1 *SD*) and high (+1 *SD*) values of HCC at t1. $N = 39$. †HCC = hair cortisol concentration. ‡t1 = first time of HCC measurement, t2 = retest of HCC (1 or 2 years after t1).

Table S3 displays the correlations between HCC and the assessed potentially stress-relevant variables. Only one significant correlation was found—namely, between HCC at t1 and the use of hormonal contraceptives at t2. Furthermore, there was a trend toward significance for the correlation between HCC at t1 and body mass index at t1 as well as the use of hormonal contraceptives at t1.

4 | DISCUSSION

This study compared the stability of HCCs after a 1-year interval with that after a 2-year interval. The study also replicated Stalder et al.'s (2012) study, which reported a product–moment correlation of $r = 0.73$ for a 1-year test–retest interval. Moreover, to our knowledge, the present study is the first to examine the stability of HCCs across a time interval of 2 years for direct comparison. Our main analysis revealed stable HCC for a 1-year interval in terms of the correlative relationship over and above various potentially confounding variables (e.g., hair treatment). According to Zhang et al.'s (2017) classification, the 1-year stability we found in our study can be considered 'high' and supports the validity of Stalder et al.'s (2012) finding. In contrast, the stability in HCC values completely vanished for the 2-year test–retest interval (i.e., weak stability, Zhang et al., 2017). Therefore, we conclude that HCC may be a very useful predictor of variables of interest within an interval of at least 1 year. However, based on the present findings, we recommend careful use of HCC to predict variables (e.g., indicators of physical health) that are measured beyond the 1-year frontier, since the rank order of the HCC values may have changed substantially between the first and second measurement points.

Referring to the mean values of HCC, we found a difference between the two time interval conditions at t1. This difference was nonexistent for HCC at t2. Such mean differences over time in HCC are not atypical (e.g., Stalder et al., 2012; Weckesser et al., 2021) and may reflect substantial differences between individuals in HCC based on situationally fluctuating (e.g., weekly hassles) and dispositional (e.g., person-specific adrenocortical activity) influences (Weckesser et al., 2019). Therefore, there is always the possibility that the HCC means of the two groups (i.e., t1 = 2016 and t1 = 2017) differed. Although we did not find any differences between the time interval conditions with regard to serious and stressful life events, HCC can be influenced by additional factors (e.g., anxiety disorders, systolic blood pressure, or social overload; Stalder et al., 2012, 2017), not all of which could be recorded. In principle, the timing itself (i.e., 2016 vs. 2017) could have also played a role, but we would like to refrain from speculating on which cultural or local events might have provided reasons. We are not aware of any major event, such as a pandemic, during the data collection period that could be used as an explanation, and such an event should have been reflected in different indications of serious and stressful life events between the time interval conditions.

In agreement with Stalder et al. (2012; see also Stalder et al., 2017), with a single exception, no significant correlations between HCC and potentially stress-related variables were found. Without further data, we are unable to decide whether the substantial correlation between HCC at t1 and the use of contraceptives at t2 reflects an actual relationship between hormonal stress and future behaviour or whether it is just a statistical artefact. Therefore, for a useful interpretation, theory-based future research should be carried out in this regard. However, we are sceptical that HCC determines the use of contraceptives in the distant future.

The primary limitations of this study are the relatively small sample size and the restriction of the sample to female university students, which are reasons to consider the present findings preliminary. In general, small samples are not unusual in HCC research (e.g., Short et al., 2016, with $N = 17$). The same is true for all-female samples (e.g., Kirschbaum et al., 2009). Despite recruiting a sample large enough for sufficient statistical power in the main analysis, the confidence intervals we reported would have been narrower and, thus, more precise with a larger sample. Furthermore, the extent to which the findings can be generalised is debatable, given that only young, educated females participated in the study. Stalder et al. (2012) examined a different sample of female and male participants, most of whom were amateur endurance athletes. Given the close match between the relationships for the 1-year test–retest interval in Stalder et al.'s (2012) and our study, we are confident that the relationships we found are valid. Still, it would be useful to attempt replication of the present findings with larger and more diverse samples in future research. Future studies may also closely investigate the limits of the predictive power of HCC for variables such as health outcomes in dependence of different time intervals, including intervals beyond 1 year. Based on our data, we cannot determine at which point within 1 and 2 years HCC becomes so unstable that it may no longer be considered sufficiently predictive. Future research may provide insight into this question.

To conclude, HCC has again shown high stability in terms of the rank order of values within the interval of 1 year. Therefore, for correlational studies, HCC may be a useful measure within this temporal framework, as statistical analysis and interpretation are primarily based on the rank orders of sets of values. In studies where the absolute levels of HCC are of interest, researchers should be cautious, since this and previous research (Weckesser et al., 2019) have found considerable fluctuations in HCC mean values over time.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committee of the Faculty of Human Sciences at the University of Bern (reference number: 2016-12-00002).

PARTICIPANT CONSENT STATEMENT

The participants provided their written informed consent to participate in this study.

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ENDNOTE

[1] Because we had experienced difficulties in finding males with sufficiently long hair during previous data collections, we recruited only females. In this study, we searched for individuals with relatively long hair to analyse HCC at more distant time intervals. However, due to the *washout effect*, we eventually only prepared hair segments 1.5 cm next to the scalp for analysis.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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