Sweat-inducing physiological challenges do not result in acute changes in hair cortisol concentrations

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Summary Hair cortisol concentrations (HCC) are assumed to provide a stable, integrative marker of long-term systemic cortisol secretion. However, contrary to this assumption, some recent observations have raised the possibility that HCC may be subject to acute influences, potentially related to cortisol incorporation from sweat. Here, we provide a first detailed in vivo investigation of this possibility comprising two independent experimental studies: study I (N=42) used a treadmill challenge to induce sweating together with systemic cortisol reactivity while in study II (N=52) a sauna bathing challenge induced sweating without systemic cortisol changes. In both studies, repeated assessments of HCC, salivary cortisol, cortisol in sweat and individuals’ sweating rate (single assessment) were conducted on the experimental day and at a next-day follow-up. Results across the two studies consistently revealed that HCC were not altered by the acute interventions. Further, HCC were found to be unrelated to acute salivary cortisol reactivity, sweat cortisol levels, sweating rate or the time of examination. In line with previous data, cortisol levels in sweat were strongly related to total salivary cortisol output across the examined periods. The present results oppose recent case report data by showing that single sweat-inducing interventions do not result in acute changes in HCC. Our data also tentatively speak against the notion that cortisol in sweat may be a dominant source of HCC. Further, our findings also indicate that HCC are not subject to diurnal variation. This research provides further support for hair cortisol analysis as a marker of integrated long-term systemic cortisol secretion.

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1. Introduction

Over the past decade, the analysis of cortisol in human scalp hair has received increasing attention as a promising new endocrine measure. Previous methods of cortisol assessment in blood, saliva or urine reflect short-term hormone levels and are thus poorly suited for capturing patterns of long-term cortisol secretion, e.g., in relation to chronic stress (Chrousos, 2009). By contrast, hair cortisol concentrations (HCC) are assumed to result from continuous incorporation of cortisol into slowly growing hair and to thus reflect integrated cortisol levels over the time period of hair growth (reviews: Russell et al., 2012; Stalder and Kirschbaum, 2012; Staufenbiel et al., 2013). Due to their retrospective and long-term nature, HCC should be intra-individually stable and unaffected by situational variability, e.g., through pulsatile cortisol secretion, diurnal rhythmicity or situational influences. This stability together with the ease of obtaining hair samples make HCC an attractive method for naturalistic chronic stress research. The general assumptions underlying hair cortisol analysis have now been supported by considerable evidence confirming both high test–retest reliability (Stalder et al., 2012b) as well as general validity of the method (e.g., Davenport et al., 2006; Accorsi et al., 2008; Kirschbaum et al., 2009; Stalder et al., 2010; Thomson et al., 2010; D’Anna-Hernandez et al., 2011; Manenschijn et al., 2011).

Despite these generally supportive data, however, there are still some concerns about whether HCC are indeed unaffected by acute influences (review: Sharpley et al., 2012). This is closely related to the fact that the exact mechanisms of cortisol incorporation into hair are still unclear. The ‘classical’ model assumes that lipophilic steroid hormones, like cortisol, passively diffuse into growing hair cells from blood capillaries surrounding the hair follicle (Stalder and Kirschbaum, 2012). However, besides this pathway, it also cannot be excluded that cortisol from other sources, such as sweat or sebum, may contribute to HCC (Sharpley et al., 2012; Stalder and Kirschbaum, 2012). Further, in vitro evidence has indicated that skin compartments, including hair follicles, comprise a functional equivalent of the hypothalamic–pituitary–adrenal (HPA) axis that is capable of producing cortisol (Slominski and Mihm, 1996; Ito et al., 2005; Slominski et al., 2007) and may thus also contribute toward HCC. Given that it is currently unknown how cortisol from these alternative pathways relates to long-term systemic levels, the possibility of incorporation via these routes threatens the classical model of HCC (Sharpley et al., 2012). Furthermore, it is conceivable that cortisol from alternative sources, e.g., sweat, may enter hair segments that have already emerged on the scalp which means that acute influences on HCC results cannot be excluded (Russell et al., 2014).

To date, only limited in vivo research has examined the possibility of acute influence on HCC. In two pilot studies, Sharpley et al. reported changes in HCC in response to a cold pressor test in an immediate and localized manner (Sharpley et al., 2009, 2010b). However, both studies were limited by methodological constraints, such as very small sample sizes (Ns between 3 and 5), a focus on arm and leg hair which is structurally different from scalp hair, no washing of hair prior to analyses and the absence of a control group. Hence, results have to be treated with caution. In a more recent study, cortisol in human sweat was examined in the context of HCC (Russell et al., 2014). The authors showed that significant concentrations of cortisol are present in sweat, with absolute levels being similar to salivary cortisol. Furthermore, an in vitro experiment showed that incubating hair in a hydrocortisone containing sweat-like solution for 1 h or more resulted in a significant increase in HCC that could not be reversed by isopropanol washing (Russell et al., 2014). However, the extent to which such sweat-related influences contribute to HCC variability in vivo, and thus their relevance for hair cortisol research, is currently unknown.

Based on the above data, the present study aimed to provide a first detailed in vivo investigation examining whether sweat-inducing physiological challenges result in acute changes in HCC and whether such changes are related to simultaneous systemic cortisol reactivity and/or sweat cortisol levels. To be able to distinguish between influences of systemic cortisol and sweat induction per se, we conducted two independent studies designed to induce sweat production together with a systemic cortisol reaction (treadmill challenge, study I) as well as without systemic cortisol reactivity (sauna bathing, study II). Given previous data indicating the potential value of assessing hair cortisone levels, besides HCC (Stalder et al., 2013), we also conducted additional analyses for hair cortisone levels (provided as supplementary data).

2. Methods

2.1. Participants

2.1.1. Study I
A total of 42 participants took part in this study. Twenty-six individuals were included in the intervention group and 16 individuals to the age and gender-matched control group. Participants were recruited via local advertisement or at running groups. Inclusion criteria for both groups were age above 18 years, body mass index (BMI) between 16 and 35, hair longer than 2 cm at the posterior vertex region of the head (see below), no signs of hair loss or baldness, no conditions of adrenocortical dysfunction and/or no use of glucocorticoid-containing medication. In addition, participants were only included in the intervention group if they regularly engaged in running exercise.

2.1.2. Study II
A total of 52 participants took part in this study. Thirty-two individuals were allocated to the intervention group and 20 individuals to the age and gender-matched control group. Participants were recruited via local advertisement. Inclusion criteria were identical to those of study I. In addition, participants of the intervention group were only included if they reported some previous experiences with sauna bathing.

Written informed consent was provided by all participants of both studies. The studies were conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. Participants of both studies received compensation in the form of participation credit points (students) or a small monetary reward of 10€.
2.2. Design and procedure

Fig. 1 illustrates the experimental designs of studies I and II. The studies used structurally similar protocols with different interventions (study I: treadmill challenge, study II: sauna bathing; see below) surrounded by repeated sampling of hair (twice), saliva (five times), sweat (three times) and a single assessment of individuals’ sweat rate on the experimental day. Further, to examine the stability of potentially observed acute endocrine changes, a follow-up assessment was conducted 24 h after the experimental assessment which involved further hair and saliva sampling. In both studies, participants were asked to refrain from doing any exercise 24 h prior to study day I as well as between study days I and II. Participants were instructed to refrain from eating, drinking (except water), smoking and brushing teeth 30 min prior to saliva sampling. The sampling protocols were identical for experimental and control groups, except for the fact that no sweat samples and sweating rate assessments were obtained in controls.

2.3. Experimental interventions

2.3.1. Study I

The intervention was designed to induce intense sweating and to provoke an acute systemic cortisol reaction. For this, participants absolvd an intense 30-min run on a treadmill (Tunturi T20, Tunturi Fitness, Ulm, Germany). To ensure stimulation of cortisol secretion, running speed was adjusted to reach a maximal oxygen uptake (VO₂max) of 65% (Hackney, 2006). The trial was completed at an average room temperature of 20.9 °C and at a relative humidity of 51%. Heart rate for the determination of VO₂max was recorded throughout the intervention phase using a wireless signal transmission device (Polar RS800, Polar Electro GmbH, Büttelborn, Germany). Mean VO₂max was 88.6%. The control group sat quietly in a comparable room for 30 min without any exercising. Examination times varied between 8:45 a.m. and 7:45 p.m.

2.3.2. Study II

The intervention was designed to induce intense sweating without leading to an acute cortisol response. For this, short-term moderate/high heat exposure was employed which has been shown to alter plasma cortisol levels (Brenner et al., 1998). Participants took part in a sauna bathing session in a Finnish sauna bath at 80 °C dry bulb temperature for 15 min. Average room temperature and relative humidity outside the sauna room were 24.3 °C and 61%, respectively. The control group engaged in no sauna bathing but just sat quietly for 15 min. Examination times varied between 9:30 a.m. and 7:00 p.m.

2.4. Self-report measures

In both studies, information on sociodemographic and lifestyle variables (age, sex, BMI, smoking status, regular alcohol use (consumption on ≥3 days/week), regular medication intake, use of oral contraceptives and training hours per week) and hair-specific characteristics (washes per week, hair treatment: semi-permanent color, coloration or permanent wave) were assessed using a self-developed questionnaire (as in Staler et al., 2012a). The 14-item Perceived Stress Scale (PSS; Cohen et al., 1983) was used to assess perceived stress over the past two months (time frame adjusted to the period reflected by HCC assessments).

2.5. Sample collection and preparation

2.5.1. Cortisol in saliva and sweat

The sampling of saliva and sweat for cortisol determination was performed using Salivette® sampling devices (Sarstedt, Nümbrecht, Germany). For saliva sampling, participants gently chewed on the cotton swab for 1 min (Kirschbaum and Hellhammer, 1994). For the collection of sweat, a maximum of three samples per participant were collected within the first 5 min after the intervention phase (Sw1–Sw3, see Fig. 1). Sweat samples were obtained from

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**Figure 1** Design of studies I and II. Besides different interventions, a similar experimental set up was used in the two studies with identical timing of sweat, saliva and hair sampling. Note: sweat sampling and sweat rate assessments were only conducted in participants of the intervention group.
Table 1  Descriptive information and comparisons between intervention and control groups for the two studies.

<table>
<thead>
<tr>
<th>Study I (treadmill)</th>
<th>Study II (sauna)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intervention</td>
</tr>
<tr>
<td>Age: years (M, SD)</td>
<td>(n = 26)</td>
</tr>
<tr>
<td>24.8 (6.7)</td>
<td>24.6 (3.5)</td>
</tr>
<tr>
<td>Female: n (%)</td>
<td>12 (46.2)</td>
</tr>
<tr>
<td>BMI: kg/m² (M, SD)</td>
<td>20.8 (3.1)</td>
</tr>
<tr>
<td>Smoking: n (%)</td>
<td>3 (11.5)</td>
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<tr>
<td>Regular alcohol use: n (%)</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>Regular medication: n (%)</td>
<td>2 (7.7)</td>
</tr>
<tr>
<td>Oral contraceptives: n (%)</td>
<td>7 (26.9)</td>
</tr>
<tr>
<td>Training hours/week (M, SD)</td>
<td>5.1 (2.8)</td>
</tr>
<tr>
<td>Hair washes/week (M, SD)</td>
<td>4.4 (2.0)</td>
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<tr>
<td>Hair treatment: n (%)</td>
<td>3 (11.5)</td>
</tr>
<tr>
<td>PSS score (M, SD)</td>
<td>33.9 (6.4)</td>
</tr>
<tr>
<td>Examination time (M, SD)*</td>
<td>12:55 (2:55)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; M, mean; PSS, Perceived Stress Scale; SD, standard deviation.

* Shown for main intervention day.

2.5.2. Sweat rate

The local sweat rate of the forehead was measured using the technical absorbent (TA) method (Havenith et al., 2008; Bain et al., 2011). Briefly, a TA pad (#2574, Laminated Air-laid, Technical Absorbents Ltd, Grimsby, UK) with a surface area of 6.25 cm² was prepared for each participant. Pads were bordered by a 1-cm-wide frame to avoid external sweat contamination of the measuring range. Each TA pad (without frame) was put in an airtight container and weighed prior to the trial using a precision scale (ALJ 120-4, Kern, Balingen, Germany). To assess local sweat rate, the TA pad (with frame) was maintained in a hairless position between participants’ right eyebrow and hair line for 2 min. Prior to pad application, the area was wiped dry from existing sweat. Afterwards, the TA pad (without frame) was put back in its container and weighed again. Sweat rate was calculated as the change in weight (pre- and post-application mass) divided by the surface area of the TA pad and the duration of application in units of mg per min per cm² (Bain et al., 2011).

2.5.3. Hair steroid analysis

For each of the three hair assessments, hair strands (~3 mm diameter) were cut as close to the scalp as possible from adjacent positions at the posterior vertex region of the head. The first scalp-near 2 cm hair segment was used for analyses. Wash and steroid extraction procedures followed the protocol described in Stalder et al. (2012b; study II) with minor adaptations. Briefly, samples were washed twice in 3 ml isopropanol for 3 min. For glucocorticoid extraction 7.5 mg of hair were incubated with 1.8 ml of methanol for 18 h at room temperature. The methanol was evaporated at 50 °C under a constant stream of nitrogen until the samples were completely dried. The dry residue was resuspended using 175 μl double-distilled water. 100 μl were used for analysis by a Shimadzu HPLC-tandem mass spectrometry system (Shimadzu, Canby, Oregon) coupled to an ABSciex API 5000 Turbo-ion-spray triple quadrupole tandem mass spectrometer (AB Sciex, Foster City, California) with purification by on-line solid-phase extraction. Intra and interassay coefficients of variance for HCC and hair cortisone have been shown to be between 3.7 and 8.8% (Gao et al., 2013).

2.6. Statistical analyses

Group comparisons regarding sociodemographic, health and hair-related as well as intervention-specific parameters were conducted using independent t-tests (continuous variables) or Fisher’s exact test (dichotomous variables). In study II, two participants provided insufficient sweat for analyses. In addition, two extreme sweat cortisol values (3.4 and 3.5 standard deviations above the mean, respectively) were excluded from study II data. As expected, data for HCC, sweat cortisol, salivary cortisol and examination times were not normally distributed. Box-Cox transformations most effectively reduced skewness statistics and inferential statistics were thus computed using transformed data (transformation coefficient λ = .25; Miller and Plessow, 2013). Due to the imbalance of treatment group sizes in both studies, random-intercept regression was employed to evaluate the main effects of treatment condition and time in isolation. Thereafter, we tested for the significance of the treatment × time interaction by adding the interaction term to the reference model containing the main effects only.
The area under the curve with respect to ground (AUCg) and increase (AUCi) were calculated following Pruessner et al. (2003) from saliva samples S1—S5 as composite measures of total salivary cortisol levels (AUCg) and salivary cortisol reactivity (AUGi) on the intervention day. Relationships between different endocrine measures were examined using Pearson correlations. Analyses were conducted using SPSS 21.0 for Windows (SPSS Inc., 2009) and R 3.0.1 statistical software (R Core Team, 2014). The alpha value was set to .05 with p-values referring to two-sided tests.

3. Results

Table 1 shows descriptive data of the intervention and control groups for both studies. It can be seen that the groups were well-matched on demographic, life-style and hair-related parameters (ps > .05). Due to practical reasons, later examination times occurred in sauna compared to control participants in study II (p = .001). Hence, examination time was examined as a potential covariate in this study.

3.1. Study I — treadmill challenge

3.1.1. Cortisol in saliva and sweat

Fig. 2A shows the time course of salivary cortisol concentrations through the pre and post-intervention period for the intervention and control group. Salivary cortisol levels differed significantly between the two groups ($\chi^2(1) = 37.614, p < .001$) and changed across time ($\chi^2(4) = 26.159, p < .001$). As expected, a significant group × time interaction was observed ($\chi^2(40) = 68.250, p < .001$), indicating the presence of a cortisol surge in response to the treadmill challenge that was not seen in the control group. This was further confirmed by the finding of higher AUCi values in the intervention compared to the control group ($\chi^2(1) = 20.171, p < .001$).

The analysis of sweat cortisol levels revealed that data from the three independent assessments (Sw1—Sw3) were highly inter-correlated (rs between .950 and .973, ps < .001). Hence, mean sweat cortisol levels were computed for each individual and used for further analyses. The mean (±SD) sweat cortisol concentration was 50.64 (±25.56) nmol/L, ranging from 21.22 to 116.76 nmol/L. The mean (±SD) sweat rate was 2.34 (±1.41) mg cm⁻² min⁻¹. Mean sweat cortisol concentrations were unrelated to sweat rates ($r = .039, p = .852$).

Mean sweat cortisol concentrations were positively related to salivary cortisol levels across each of the post-intervention time points (rs between .616 and .740, ps ≤ .001), although no significant associations with salivary cortisol pre-intervention (S1, $r = .168, p = .411$) or at the next-day follow-up (S6, $r = −.091, p = .666$) were observed. Fig. 2B illustrates the positive association between mean sweat cortisol levels and AUCi overall intervention-day salivary cortisol levels ($r = .719, p < .001$).

The time of examination was found to be negatively related to baseline salivary cortisol on the intervention day (S1, $r = −.625, p < .001$) and the next-day follow-up (S6, $r = −.567, p < .001$). However, no association between examination time and dynamic changes in salivary cortisol levels was observed (AUCi, $r = .178, p = .261$). Furthermore, no association between examination time and mean sweat cortisol concentrations was found ($r = −.040, p = .847$).

3.1.2. Cortisol in hair

Fig. 3 depicts mean HCC across the pre- and post-intervention period for the intervention and control group. No effect of time on HCC was observed ($\chi^2(2) = 3.705, p = .157$) but overall HCC were found to be lower in the intervention compared to the control group ($\chi^2(1) = 7.164, p = .008$). No significant group × time interaction was revealed ($\chi^2(2) = 1.08, p = .584$), indicating that groups exhibited similar patterns of HCC across time and that no HCC surge in response to the treadmill challenge was detectable.

Significant positive relationships were found between HCC across the three time points ($r_{2} = .610, r_{3} = .334, r_{23} = .736, \text{all } ps \leq .031$). Pre- to post-intervention changes in HCC were unrelated to mean sweat cortisol levels ($r = .244, p = .229$) or to sweat rates ($r = −.235, p = .257$)

![Figure 2](image-url)  
**Figure 2**  (A) Mean (±SEM) salivary cortisol concentrations across study I (treadmill run) for participants of the intervention and control group. (B) Association between the area under the curve with respect to ground (AUCg) of salivary cortisol and mean sweat cortisol levels in the intervention group.
in the intervention group. Alike, mean HCC were unrelated to mean sweat cortisol concentrations ($r = -0.055$, $p = .789$) or to sweat rates ($r = -0.360$, $p = .077$). Further, in analyses conducted on the complete sample, pre- to post-intervention changes in HCC were not found to be related to AUCc ($r = -0.047$, $p = .769$) or AUCs, salivary cortisol ($r = .059$, $p = .711$). Additionally, no associations between any HCC assessment (H1, H2, H3) and the time of examination were observed ($r_1 = .156$, $r_2 = .143$, $r_3 = .002$, all $ps > .324$). A parallel pattern of results was found for hair cortisone (see supplementary data).

### 3.2. Study II — sauna challenge

#### 3.2.1. Cortisol in saliva and sweat

Fig. 4A illustrates the time course of salivary cortisol concentrations across the pre- and post-intervention period for the intervention and control group. A main effect of time indicated that salivary cortisol levels decreased over time across both groups ($\chi^2 = 60.962$, $p < .001$). However, no overall between-group difference ($\chi^2 = 1.564$, $p = .211$) and no significant time x treatment interaction ($\chi^2 = 9.131$, $p = .058$) were observed for salivary cortisol levels. The absence of a significant salivary cortisol surge in reaction to sauna bathing was further indicated by the finding that AUC values did not differ between groups ($\chi^2 = 2.106$, $p = .147$).

The analysis of sweat cortisol levels revealed that data from the three independent samples (Sw1–Sw3) were strongly inter-correlated ($rs$ between $.588$ and $.884$, $ps < .017$). Hence, mean sweat cortisol levels were computed for each individual and used for further analyses. The mean ($\pm$SD) sweat cortisol concentration was 15.73 ($\pm$6.15) nmol/L, ranging from 6.25 to 27.5 nmol/L. The mean ($\pm$SD) sweat rate was 2.09 ($\pm$1.35) mg cm$^{-2}$ min$^{-1}$. Mean sweat cortisol concentrations were unrelated to individual sweat rates ($r = -0.333$, $p = .083$).

A comparison of mean sweat cortisol concentrations between the two studies revealed that study II sweat cortisol levels were significantly lower than those of study I ($F(1,31) = 77.575$, $p < .001$, $\eta_p^2 = .603$). No significant between-study difference in mean sweat rate was observed ($F(1,34) = .489$, $p = .487$).

Mean sweat cortisol concentrations were positively related to salivary cortisol concentrations across each of the pre and post-intervention time points (rs between $.643$ and $.688$, $ps < .001$) but not to next-day salivary cortisol (S6, $r = .140$, $p = .486$). Fig. 4B illustrates the relationship between mean sweat cortisol levels and AUCs overall salivary cortisol levels ($r = .722$, $p < .001$).

The time of examination was found to be negatively related to baseline salivary cortisol on the intervention day (S1, $r = -.292$, $p = .036$) and the next-day follow-up (S6, $r = -.411$, $p = .008$). However, no association between examination time and dynamic changes in salivary cortisol levels was observed (AUCc, $r = .019$, $p = .893$). Furthermore, no association between examination time and mean sweat cortisol concentrations was found ($r = -.051$, $p = .791$).

#### 3.2.2. Cortisol in hair

Fig. 5 visualizes mean HCC across the pre- and post-intervention period for the intervention and control group. HCC were not found to differ across time ($\chi^2 = .287$, $p = .867$) or between treatment groups ($\chi^2 = .049$, $p = .825$).
Moreover, there was no significant time × treatment interaction ($F_{10} = 4.139, p = .126$), indicating that no HCC surge in response to sauna bathing was detectable.

Significant positive relationships were found between HCC across the three time points ($r_{12} = .954, r_{13} = .961$, $r_{23} = .952$ all $p < .001$). Pre- to post-intervention changes in HCC were unrelated to mean sweat cortisol levels ($r = -.126$, $p = .532$) or to sweat rates ($r = .017$, $p = .929$). Similarly, mean HCC were unrelated to mean sweat cortisol levels ($r = .131$, $p = .515$) or to sweat rates ($r = .285$, $p = .121$). Further, pre- to post-intervention changes in HCC were not found to be related to AUC$_C$ salivary cortisol ($r = -.170$, $p = .229$) or to AUC$_S$ salivary cortisol ($r = .036$, $p = .800$). Additionally, no associations between any HCC assessment (H1–H3) and the time of examination were observed ($r_1 = .134$, $r_2 = .149$, $r_3 = .183$, all $p > .252$). An equivalent pattern of results was found for hair cortisone (see supplementary data).

4. Discussion

The current investigation provided a first systematic in vivo examination of the possibility that cortisol levels in human scalp hair may be subject to acute influences. Our results from two independent studies consistently revealed that HCC were not acutely altered by interventions that induced significant sweating both in the presence and absence of acute cortisol reactivity. These findings are at variance with earlier case report data and concur with the ‘classical’ model of hair cortisol which holds that HCC are predominantly reflective of long-term systemic cortisol levels and are robust to acute influences. Further, our findings that HCC were unrelated to sweat cortisol levels or to individuals’ sweating rate tentatively speak against an important role of sweat as a contributing source toward HCC. The additional exploration of hair cortisone levels revealed virtually identical results as for HCC (see supplementary data), suggesting that parallel conclusions can be drawn for this measure.

The main result of the present investigation is that in two independent studies using different experimental interventions, HCC were consistently found not to be affected by acute sweat-inducing physiological challenges. The current data are thus at variance with the results of two earlier case studies reporting on immediate and localized changes in cortisol levels in arm and leg hair in response to a cold pressor test (Sharpley et al., 2009, 2010b). A potential explanation for these diverging findings could be that in this research hair had been shaved off using a razor and was not washed prior to analyses. This makes it conceivable that respective HCC data may have been contaminated by cortisol from sweat or sebum. The present finding that sweat contains relatively high concentrations of cortisol (see also Russell et al., 2014) in line with this possibility. In addition, in the research by Sharpley et al., the shaving of hair using a razor may have resulted in microtrauma which means that contamination through cortisol in blood also cannot be excluded. Further, it must be noted that these earlier investigations were based on only 3 or 5 individuals and focused on arm and leg hair which is structurally different from scalp hair. These constraints were addressed in the current research and we observed a very different pattern of results that emerged with high consistency across two independent samples. This suggests that, at least concerning human scalp hair, the acute incorporation of cortisol into hair in reaction to a short-term physiological challenge seems unlikely.

The present research also provides relevant information on the possibility of diurnal influences on HCC. Two previous investigations, again conducted within small samples (Ns: 4–5), had suggested that HCC may be subject to diurnal (Sharpley et al., 2010a) and/or day-to-day variability (Esposito et al., 2012). The present data oppose these findings by consistently showing no diurnal variation in HCC assessments across the two reported studies. On the other hand, the well-known diurnal rhythm in salivary cortisol levels was clearly evident. The current hair cortisol data are thus commensurate with previous data showing high intra-individual stability of HCC (Stalder et al., 2012b) and supporting the overall validity of this method as an index of integrated long-term cortisol levels (see Russell et al., 2012; Stalder and Kirschbaum, 2012; Staufenbiel et al., 2013).

In addition to examining acute changeability of HCC, we also addressed the particular role of sweat as an additional route of cortisol incorporation into hair. Importantly, our results revealed that HCC were not associated with cortisol levels in sweat, irrespective of whether sweating was induced in the context of acute systemic cortisol reactivity or not. Further, no associations between HCC and individuals’ rate of sweating were observed. Interestingly, these in vivo data are at variance with recent in vitro evidence showing that exposing hair to a hydrocortisone-containing sweat-like solution resulted in a time-dependent increase in HCC (Russell et al., 2014). It is currently unclear how this discrepancy can be explained. However, it appears noteworthy that Russell et al. used relatively high hydrocortisone concentrations (50 ng/ml = 138 nmol/L; current two study mean: 32.5 nmol/L) and that an effect only became significant after a rather long incubation period of 1 h or more. In addition, the cutting of hair in this study may have resulted in a damaged surface that potentially enhanced the incorporation of external cortisol compared to uncut hair. Combined with the current data, this tentatively speaks against the notion that cortisol from sweat may be a dominant source of HCC under normal, real-life conditions. However, it also clearly needs to be stated that the current research only investigated the effects of one-time sweat exposure and

![Figure 5 Mean (±SEM) hair cortisol concentrations at the three sampling times of study II.](image-url)
thus future research will be needed to draw clear conclusions regarding the influence of repeated and/or long-term sweat exposure on HCC.

Besides direct implications for hair cortisol analysis, the current study also provided relevant information concerning the presence of cortisol in sweat per se. Our results revealed that three temporally close assessments of sweat cortisol levels produced virtually identical results and, more importantly, that mean sweat cortisol levels were strongly related to total salivary cortisol output across the examined period. The present data concur with the first, recently published investigation on this topic which also showed that cortisol levels in simultaneously collected sweat and saliva samples were closely related (Russell et al., 2014). Our finding that absolute sweat cortisol levels were more than three-fold higher in study I (treadmill) compared to study II (sauna) further supports the notion that sweat cortisol levels may indeed be stress reactive. Given that study I, but not study II, induced an acute salivary cortisol reaction; this marked difference in sweat cortisol levels is likely to reflect underlying differences in systemic HPA axis activity. Besides this overall close correspondence with initial sweat cortisol data, findings differed regarding the question of diurnal rhythmicity: while Russell et al. (2014) observed diurnal changes in sweat cortisol levels, the two present studies failed to reveal such a pattern. It is currently unclear what may be underlying these differences. However, given the cross-sectional nature of sweat cortisol assessments, both studies were not ideally suited for examining diurnal rhythmicity patterns. Future research employing repeated sweat assessments within individuals may help to solve this question.

The current research is subject to some limitations. First, we employed unbalanced study designs with larger intervention — compared to the control groups. However, we did not consider this a severe problem as our main interest was in intraindividual changes and as we accounted for this by using sequential hypothesis tests regarding the contribution of main and interaction effects (see Langsrud, 2003). Further, the experimental allocation was not completely randomized and thus the recruitment process may have induced some form of bias in study I given that lower overall HCC were found in the intervention group. Although an unfortunate circumstance, this does not derogate central conclusions of the present research which, again, were essentially intraindividual in nature. Besides sample characteristics, the frequency of hair sampling may also be viewed as a potential limitation of our research. The results of Sharpley et al. (2009) appeared to show a transient response which only affected cortisol levels during the first minute after stress. As the second HCC assessment in the current research was conducted 5 min post-intervention, it could be argued that the present sampling protocol may have missed a potential very rapid HCC response. However, as the present research focused on systemically stimulating interventions, we consider this possibility highly unlikely. Still, it must be stated that the present research did not explicitly study a localized challenge to scalp hair and our results are thus not a direct contradiction of those of Sharpley et al. (2009, 2010b). Hence, although it appears unlikely that localized stress to scalp hair could be a mechanism that may explain the wealth of previous HCC findings, future research systematically examining localized effects on scalp HCC may be of interest. Finally, it could also be criticized that in study I the timing of hair sampling preceded the peak of the salivary cortisol reaction and that thus the power to detect an influence on HCC may have been restricted. Here, it is important to note that group differences in the salivary cortisol response at the time of hair sampling were already present at the level of a large effect (Cohen’s $d = .95$; Cohen, 1988). Hence, a significant influence on HCC, if indeed existent, should have been detectable with sufficient power at this time point already. Further, the fact that no effect was observed at the one day follow-up also speaks against the possibility of a more slowly acting influence on HCC. Nevertheless, future replication with higher frequency of post-intervention hair sampling may still be of interest.

In sum, the present research provides highly consistent evidence from two independent studies showing that HCC are not acutely influenced by sweat-inducing interventions and are unrelated to cortisol levels in sweat, sweat rate, acute salivary cortisol reactivity or examination time. Given the in vivo nature of the present investigation, we assume that our findings represent real-life physiological processes which are of particular relevance for hair cortisol analysis as it is commonly used in psychoneuroendocrine research. With this, we provide further support for the ‘classical’ model of hair cortisol which holds that HCC provides an intra-individually stable measure primarily reflecting long-term systemic cortisol levels.

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Conflict of interest

The authors have no conflicts of interest to report.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2014.12.023.

References


