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# 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 (*N*s 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€.