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Correspondence between hair cortisol concentrations and 30-day integrated daily salivary and weekly urinary cortisol measures

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ABSTRACT

Characterization of cortisol production, regulation and function is of considerable interest and relevance given its ubiquitous role in virtually all aspects of physiology, health and disease risk. The quantification of cortisol concentration in hair has been proposed as a promising approach for the retrospective assessment of integrated, long-term cortisol production. However, human research is still needed to directly test and validate current assumptions about which aspects of cortisol production and regulation are reflected in hair cortisol concentrations (HCC). Here, we report findings from a validation study in a sample of 17 healthy adults (mean ± SD age: 34 ± 8.6 yrs). To determine the extent to which HCC captures cumulative cortisol production, we examined the correspondence of HCC, obtained from the first 1 cm scalp-near hair segment, assumed to retrospectively reflect 1-month integrated cortisol secretion, with 30-day average salivary cortisol area-under-the curve (AUC) based on 3 samples collected per day (on awakening, +30 min, at bedtime) and the average of 4 weekly 24-h urinary free cortisol (UFC) assessments. To further address which aspects of cortisol production and regulation are best reflected in the HCC measure, we also examined components of the salivary measures that represent: (1) production in response to the challenge of awakening (using the cortisol awakening response [CAR]), and (2) chronobiological regulation of cortisol production (using diurnal slope). Finally, we evaluated the test-retest stability of each cortisol measure. Results indicate that HCC was most strongly associated with the prior 30-day integrated cortisol production measure (average salivary cortisol AUC) ($r = 0.61$, $p = 0.01$). There were no significant associations between HCC and the 30-day summary measures using CAR or diurnal slope. The relationship between 1-month integrated 24-h UFC and HCC did not reach statistical significance ($r = 0.30$, $p = 0.28$). Lastly, of all cortisol measures, test-retest correlations of serial measures were highest for HCC (month-to-month: $r = 0.84$, $p < 0.001$), followed by 24-h UFC (week-to-week: r's between 0.59 and 0.68, $ps < 0.05$) and then integrated salivary cortisol concentrations (week-to-week: r's between 0.38 and 0.61, p's between 0.13 and 0.01). These findings support the contention that HCC provides a reliable estimate of long-term integrated free cortisol production that is aligned with integrated salivary cortisol production measured over a corresponding one-month period.

1. Introduction

Cortisol is a glucocorticoid hormone that is extensively examined in the context of psychoneuroendocrinological investigations because it plays a major role in regulating central and peripheral physiology and is implicated in many aspects of stress-related health and disease risk across a spectrum of physical and mental disorders. While acute cortisol reactivity comprises an important part of the adaptive response to challenge (Sapolsky, 2000), long-term changes in the secretion of cortisol (e.g., under conditions of chronic stress) may be detrimental and have been linked to an increased risk for physical and mental disease (Lupien et al., 2006;

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Chrousos, 2009). However, researchers aiming to understand the role of long-term cortisol changes as a mediator between chronic stress and disease often face methodological challenges when trying to obtain reliable estimates of long-term cortisol output. Specifically, common assessment strategies in blood or saliva only reflect acute circulating cortisol levels, which are highly volatile and influenced by a range of situational factors (e.g. Young and Breslau, 2004). Hence, to obtain reliable information on long-term secretion using these more invasive methods presents a large participant burden and likely requires a prohibitive number of repeated sampling. Another method to obtain information on longer-term cortisol secretion is urinary sampling, with urinary-free cortisol concentration (UFC) being assumed to provide an integrated index of cortisol secretion over the period of urinary sampling, usually 12 or 24 h (Remer et al., 2008). However, assessment of UFC is associated with considerable problems, such as participant non-compliance (Yehuda et al., 2003), and there is still a debate about how well urinary cortisol reflects systemically circulating free cortisol levels (Murphy, 1999).

Over the past decade, the analysis of cortisol in hair has emerged as an alternative and promising strategy for the assessment of long-term cortisol secretion (reviews: Russell et al., 2012; Stalder and Kirschbaum, 2012; Staufenbiel et al., 2013). Hair grows on average 1 cm/month (Wenning, 2000) and cortisol, like other steroid hormones, is maintained in hair at reliable levels for up to six months (Kirschbaum et al., 2009). Small amounts of cortisol are secreted by the hair follicle itself (Ito et al., 2005); however, the main proportion of cortisol found in hair is thought to be derived from the bloodstream via passive diffusion (Stalder and Kirschbaum, 2012). The overall validity of hair cortisol concentration (HCC) as an index of cumulative long-term systemic cortisol levels has been generally supported in human and animal research (Stalder and Kirschbaum, 2012). This includes indirect validation research, i.e. studies showing HCC profiles characteristic of conditions with well-described alterations of adrenocortical function, like Cushing's or Addison's Disease (e.g. Kirschbaum et al., 2009; Stalder et al., 2010; Thomson et al., 2010; D'Anna-Hernandez et al., 2011; Skoluda et al., 2012). More direct validation data comes from research examining the relationship between HCC and cortisol levels in saliva, blood or urine (e.g., Davenport et al., 2006; Sauvé et al., 2007). These studies generally revealed positive relationships between HCC and other well-established cortisol measures, yet the strength of correlations varied considerably between studies: medium to strong associations (*r*s between 0.48 and 0.90) emerged from animal research, in which other methods of cortisol sampling (e.g. feces or saliva) were often assessed repeatedly over long time periods (Davenport et al., 2006; Accorsi et al., 2008; Bennett and Hayssen, 2010). Research with humans however has revealed less strong relationships (*r*s between 0.06 and 0.57) (e.g. Sauvé et al., 2007; D'Anna-Hernandez et al., 2011; Stalder and Kirschbaum, 2012). One reason that a validation study is still needed is that most studies to date have not been specifically designed for the purpose of validating HCC measures (e.g. assessment of correspondence between cortisol measured in hair and cortisol measured in other standard sampling methods) nor elucidating which aspects of cortisol production and regulation are reflected with the HCC measure. Empirical findings used to support current assumptions about HCC as a measure of cumulative cortisol production come from studies that collected a small number of comparison cortisol samples that were obtained over a time span that was much shorter than the month-long duration that a 1 cm hair sample is thought to reflect (e.g. Steudte et al., 2011; Xie et al., 2011). Moreover, in other studies HCC and salivary cortisol concentrations were sampled concurrently (Sauvé et al., 2007; van Holland et al., 2012), which is problematic because these measures reflect cortisol concentrations across different time periods and durations.

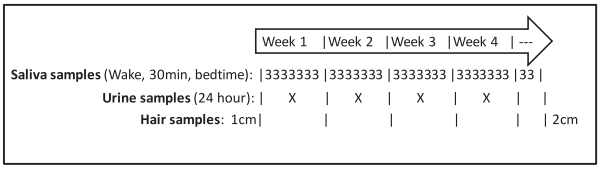

Fig. 1. Summary of study design, measures and sample collections.
A summary of the study design illustrates the measures included as well as the number and timing of cortisol samples collected during the month-long study period.

The current study aims to address the need for an in-depth human validation study to directly test and validate current assumptions about which aspects of cortisol production are reflected in HCC by examining the relationship between HCC and cortisol measured with other sampling methods, collected repeatedly, over an extended and corresponding period of time. Our primary aim was to determine the extent to which the HCC measure captures cumulative cortisol production. Thus we examined the correspondence of HCC, obtained from the first 1 cm scalp-near hair segment, assumed to retrospectively reflect 1-month integrated cortisol secretion, with 30-day average salivary cortisol area-under-the curve (AUC) (3 daily samples: on awakening, +30 min, at bedtime) and the average of 4 weekly 24-h urinary free cortisol (UFC) assessments. Each of these cortisol indices is thought to represent the unbound, and thus biologically active, fraction of cortisol in the peripheral circulation (Beisel et al., 1964; Kirschbaum and Hellhammer, 1994; Stalder and Kirschbaum, 2012). To further address whether more dynamic aspects of cortisol production and regulation are also reflected in the HCC measure, we examined HCC associations with the cortisol awakening response (CAR) and the cortisol decline over the course of the day (diurnal cortisol slope). Lastly, we evaluated the test-retest stability of each cortisol measure from month-to-month for hair and from week-to-week for salivary and UFC.

2. Methods

2.1. Participants

Twenty participants were recruited from two sites: University of California Irvine and the University of North Carolina at Chapel Hill. The samples of three individuals were excluded from the analyses because their sleep schedules were atypical (i.e. shift work) or they did not adequately adhere to the sample collection protocol. Participants were healthy adults, 10 men and 7 women aged 21–53 years ($M = 34.06$, $SD = 8.60$ years) who provided written informed consent to participate in this study. The Institutional Review Boards of the University of North Carolina School of Medicine and University of California, Irvine approved this study. Participants provided daily saliva samples, weekly 24-h urine samples, and two hair samples, over the course of the 30-day study period (See Fig. 1).

2.2. Design and procedure

Salivary Cortisol – Each individual collected three saliva samples per day for 30 days: (1) immediately on waking, 2) 30 min post-awakening, and 3) at bedtime. Saliva sampling was conducted using Salivettes (Sarstedt, Nümbrecht, Germany). Times of sampling were both self-recorded by the participant and objectively verified by use of MEMS 6 Track Cap containers (MWV Healthcare, Switzerland Ltd.). The use of such objective verification methods substantially increases validity of salivary cortisol data, particularly concerning the CAR (e.g., Kudielka et al., 2003; Broderick et al., 2004; review: Stalder et al., 2016). Participants were instructed not to go back to sleep during morning sampling period (30 min post-