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Title: Validation of an adapted procedure to collect hair for cortisol determination in adolescents

Running title: Adaption of the collection protocol for hair cortisol determination

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Abstract

Introduction: In the last decades, cortisol has been extensively studied in association to early exposure to adversity as well as in the etiology of a number of physical and mental problems. While saliva and blood samples allow the measurement of acute changes in cortisol secretion, hair samples are thought to provide a valid retrospective measure of chronic cortisol secretion over an extended period of time. Nevertheless, the existing protocol for hair collection involves considerable financial and logistical challenges when performed in large epidemiological studies.

Objective: This study aimed to validate an adapted collection protocol asking participants to sample their hair at home and to send it back to our laboratory by regular mail. Methods:

Participants were 34 teenagers between 17 and 18 years of age. They participated in two hair collections: a) at home, with the help of someone they know, and b) in our laboratory, with a trained research assistant. Results: We noted a strong correlation between cortisol ascertained from hair collected at home and at the laboratory. No mean difference in cortisol levels could be detected between the two protocols. Moreover, we showed that a wide range of hair-related, sociodemographic, lifestyle factors that may be associated with hair cortisol levels did not affect the association between cortisol measures derived from each protocol. Conclusion: Our study provides initial support that reliable measures of chronic cortisol secretion could be obtained by asking adolescents to collect a sample of their hair at home and send them to the laboratory by regular mail. This adapted protocol has considerable financial and logistical advantages in large epidemiological studies.

Keywords: Hair cortisol; Collection protocol; Chronic stress; HPA axis; Epidemiological studies.

Word count: 263

1. Introduction

Over the past 25 years, many scholars have been engaged in identifying the early roots of physical and mental health problems. Longitudinal studies initiated in early childhood, some spanning over extended periods, have provided evidence that exposure to a wide range of adverse environments - from economic deprivation to maltreatment - may shape later vulnerability to health problems in addition to, or in combination with, inherited differences in individuals' sensitivity to the environment (Shonkoff et al., 2012). While the mechanisms underlying this association remain unclear, many hypothesize that the hypothalamic pituitary adrenal (HPA) axis may be at play (Gunnar and Quevedo, 2007; Lupien et al., 2009). Cortisol has certainly been the most often studied biomarker in that regard (Doom and Gunnar, 2013).

To date, cortisol has mainly been measured from saliva or blood samples. These biospecimens have many advantages, such as reflecting responses to acute stress (experimentally-induced or not) and diurnal patterns of secretion (Russell et al., 2012). However, they also have limitations: they are susceptible to situational factors (e.g., time of day, transient variation in physical health or mood), non-adherence to the sampling protocol and requiring the collection of many samples over multiple days, which can be costly in larger samples and burdensome for participants (Adam and Kumari, 2009; Stalder and Kirschbaum, 2012). Ascertaining long-lasting disruptions of cortisol secretion in saliva or blood can also be inferred from flattened, higher or lower patterns of secretion, but these are merely proxies, essentially reflecting short-term secretion. Blood collection is invasive, may inadvertently increase the measurement of acute cortisol secretion, requires trained research assistants (Wosu et al., 2013) and both blood and saliva require storage equipment (e.g. freezers).

In the last decade, growing empirical support has indicated that hair may be a useful medium to retrospectively measure chronic cortisol secretion over an extended period of time, typically up to three months (D'Anna-Hernandez et al., 2011; Kirschbaum et al., 2009). Hair cortisol has been shown to be stable over time, and is easy to collect by minimally-trained research assistants (Adam and Kumari, 2009; Stalder and Kirschbaum, 2012). It is also less restrictive and more convenient than other procedures used for ascertaining cortisol secretion over a prolonged period (e.g., urine over 24 hours). Moreover, hair samples can be stored at room temperature without requiring storage equipment (Dettenborn et al., 2012; Stalder and Kirschbaum, 2012). Considering that the use of hair samples to ascertain chronic cortisol secretion is still at an early stage, much work remains to be done to clearly identify its potential confounders, such as hair-related (e.g., frequency of wash) and sociodemographic and lifestyle factors (e.g., sex, body mass index (BMI); Wosu et al., 2013; Rippe et al., 2016).

Hair cortisol has definite logistical advantages in measuring chronic cortisol secretion. Nevertheless, hair collection requires that a trained research assistant follow a standardized protocol, by going to the participants' home or by asking them to come to the laboratory. This implies financial and logistical burden for large epidemiological studies in which participants are dispersed over a wide territory or in isolated populations (e.g., Northern Aboriginal communities). It is still unclear, however, whether participants can reliably collect hair samples themselves (with the help of a family member or a friend) and whether these samples can be sent to the laboratory by regular mail without affecting the cortisol measurement.

The goal of the present study is to evaluate whether a reliable measurement for chronic cortisol secretion can be obtained by asking the participants to collect the hair samples themselves. First, we examined whether cortisol ascertained from hair collected by the

participants was correlated with cortisol measured in hair samples collected by a trained research assistant. We also tested the stability of cortisol concentrations across the two collection protocols to ascertain that shipping the samples by regular mail does not deteriorate cortisol. Finally, we extended earlier investigations and examined whether a range of hair-related, sociodemographic and lifestyle characteristics were associated with hair cortisol levels and whether they affected the strength of the association between cortisol obtained from the original and adapted protocols.

2. Material and methods

2.1 Sample

Participants were recruited through advertisements on the Centre for Studies on Human Stress website and on the Kijiji Montréal, a free local classified website. Adolescents between the ages of 17 and 18 years were targeted because that age corresponded to the target population in the main cohort that we intended to follow. Only those whose hair was at least 3 centimeter (cm) long were included. A total of 34 adolescents (25 females, 9 males) living in the greater Montreal area participated in the study, the majority being college students (85.3%), Caucasian (82.4%), whose parents hold at least a college degree (82.4%) and the participants considered they had sufficient income (70.6%). Adolescents too young to give an independent informed consent gave their assent and their parents provided written consent. The study was approved by the Ethics Committee of the Montreal Mental Health University Institute.

2.2 Procedures

Participants were first invited to complete a brief questionnaire during a phone interview about their sociodemographic characteristics, medical history and lifestyle habits. A collection kit was sent, which included the required material (i.e., curved scissors, hair clamps, collection

card), a short questionnaire regarding the natural state of the hair, usual care, and whether the participants used the supplied scissors to collect the hair sample. Written instructions were accompanied by several pictures describing how to identify the posterior vertex area of the head, to separate the first 3 cm proximal to the scalp with the clamp and stick it to the collection card while still holding the hair sample with the clamp, in such a way that the roots are oriented in an upward position (the instruction guide, consistent with the CDC methodology, is available upon request). Additional guidelines were offered to ensure that the collected sample was at least 1 cm in diameter. Participants were asked to return the collection kit along with the hair strand by regular mail using a prepaid, padded envelope. In the following week, an appointment was scheduled to complete the second hair collection, in our laboratory, with a trained research assistant.

Measures

2.3.1 *Cortisol*

Wash and steroid extraction procedures followed the protocol described by Kirschbaum and colleagues (2009). The first 3 cm hair segment of each sample was put into a 15 millilitre (ml) tube with 2.5 ml of isopropanol before being gently mixed on an overhead rotator. After decanting, the wash cycle was repeated and the hair was left to dry overnight. Pure methanol (1.5 ml) was added before being rotated slowly for 24 hours. The methanol was then spun down in a microcentrifuge and 1 ml was aliquoted into a clean vial. The methanol evaporated at 37 degrees Celsius under a constant stream of nitrogen until completely dry. Finally, 0.4 ml of phosphate buffer was added to the tube before it was vortexed for 15 seconds. The reconstituted sample was measured in duplicate, to assure the reliability of results, using a commercially available

luminescence immunoassay with detection (range: 0.005-4 µg/dl). Intra-assay coefficient of variation was 5.39%.

2.3.2 Hair-related, sociodemographic and lifestyle factors

Information about the natural state of the hair (e.g., color, curvature) and usual care (e.g., frequency of washing, color treatments) were obtained through the completion of a short questionnaire. Participants were also asked about sociodemographic and lifestyle factors, including sex, age, familial income, BMI, medications, cigarette and alcohol consumption.

2.4 Statistical analyses

The analyses were conducted in four steps. First, using Pearson correlations, we examined the magnitude of the association between cortisol ascertained from hair collected at home and from hair collected by a trained research assistant in our laboratory. Second, we tested whether distinct cortisol concentrations could be detected between the samples collected following each protocol, using a Student *t* test on paired samples. Third, we conducted linear regression analyses to document further whether a wide range of hair-related, sociodemographic and lifestyle factors were associated with hair cortisol levels and if these factors affected the association noted between cortisol obtained from hair collected in the original and adapted protocols, using partial correlation.

3. Results

Hair cortisol concentrations ranged from 9.12 to 118.32 pictograms per milligram (pg/mg) ($M = 33.11$, $SD = 25.84$) and from 7.68 to 105.60 pg/mg ($M = 33.01$, $SD = 24.53$) for both self-sampled and lab-based hair samples, respectively. A significant correlation between these samples was noted ($r = .91$, $p < .001$; see Figure 1). The Student paired samples *t*-test showed no significant differences in cortisol concentrations between the two samples ($t = .06$, $p = .95$).

Table 1 shows that none of the investigated sociodemographic and lifestyle factors affected the magnitude of the correlation between cortisol measurements collected at home and in the lab.

4. Discussion

This study investigated the possibility of measuring chronic cortisol secretion from self-sampled hair at the participants' home without requiring a visit from a research assistant. Preliminary evidence indicated a strong association between cortisol measured in hair samples collected at home and cortisol measured in hair samples collected in our laboratory. These results provide initial support, in a small and non-randomized sample, that this modification of the existing protocol yields reliable cortisol measures.

Self-collection of hair samples by participants offers many potential advantages. First, this procedure could greatly reduce the financial and logistical burden of conducting home visits or asking participants to come to the laboratory. This benefit is particularly salient when participants live in remote areas (e.g., Northern Aboriginal communities), are restrained by geopolitical constraints (e.g., war zones), or in large epidemiological samples with participants dispersed across large territories. Second, not only are the participants collecting their hair in a way that correlates well with samples taken by trained personnel, they can return it to the laboratory without major precautions (e.g., no dry ice). Finally, the scissors and the clamps are reusable, once disinfected, which may reduce even more the anticipated savings in the long run. Notably, however, because these preliminary findings emerge from a small and non-random sample, caution is warranted before they could be generalized to a broader population.

Third, the measurement of chronic cortisol secretion from hair does not require a strict adherence to a collection protocol because it is assumed to reflect systemic cortisol accumulated over the last three months (D'Anna-Hernandez et al., 2011; Kirschbaum et al., 2009). Thus, it is

not necessary to rely on electronic devices recording the time when the collection is performed, as recommended for saliva samples, which further reduces the cost of data collection (each cap is estimated at 130 USD). We also found that none of the considered hair-related, sociodemographic and lifestyle factors affected the strength of the association between cortisol measured from hair collected at home and cortisol measured from hair collected at the laboratory. The present study also allows to test, albeit in a small sample of volunteers, the potential confounding effect of these factors to hair cortisol levels. The natural state of the participant's hair or the hair care, such as bleaching, was not associated with cortisol concentrations. Similar findings were reported previously (Dettenborn et al., 2012; Kirschbaum et al., 2009; Stalder et al., 2012), but not consistently. For example, Hoffman and colleagues (2014) reported that some chemical processing (e.g., dye products, peroxide) and excessively shampooed hair affected cortisol measurement (Hoffman et al., 2014). Similarly, sex and the annual family income were not associated with hair cortisol, as noted in other samples (Dettenborn et al., 2012; Karlen et al., 2011; Stalder et al., 2012; Vaghri et al., 2013). The small sample size, primarily composed of females of moderate-to-high socioeconomic status may, however, have restricted the statistical power to detect these effects. Mixed findings have also been found in regards to BMI and cigarette, alcohol and drug consumption, although the available data are insufficient to draw meaningful conclusions at this point in time (Wosu et al., 2013; although see Rippe et al., 2016). The investigation further contributes to existing research aiming to identify the hair cortisol's potential confounders. Given the current state of knowledge, the best strategy remains to collect information on a wide-range of potential confounders and explore their role in preliminary analyses.

Our study provides initial support for the reliability of cortisol measures ascertained from hair collected by the participants at their home and returned by regular mail to the laboratory. These adaptations to the existing protocol represent considerable financial and logistical advantages, especially when targeting isolated populations or in large epidemiological cohorts.

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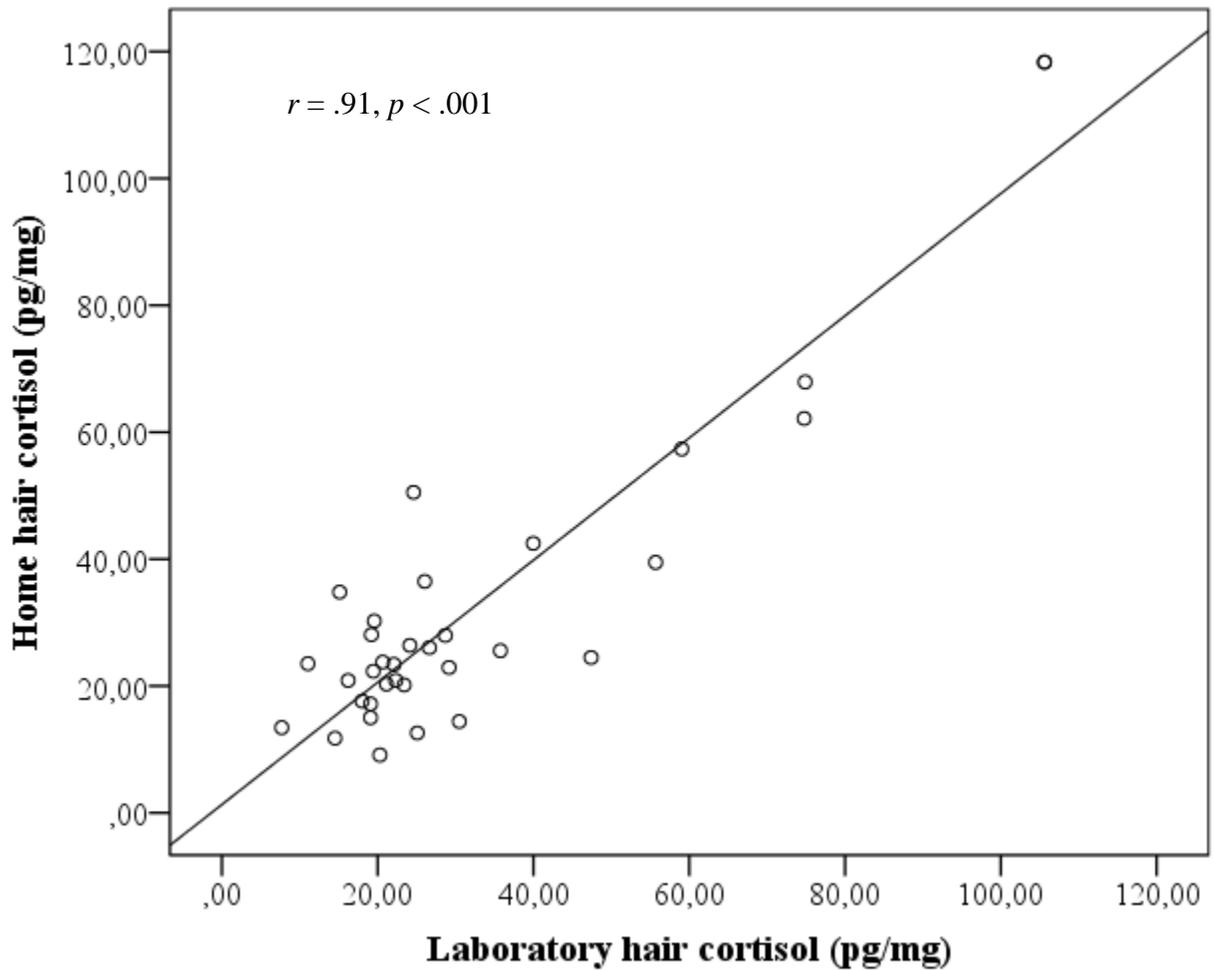
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Table 1. Associations between hair cortisol, collected at home and at the laboratory, and a wide range of potential confounders

	Home Cortisol			Laboratory Cortisol			Partial correlation between home and lab cortisol
	B	SE	<i>t</i>	B	SE	<i>t</i>	
<i>Hair-related factors</i>							
Frequency of washes	5.12	5.96	.86	9.53	5.47	1.74+	.92***
Hair bleaching (yes/no)	5.49	10.15	.54	7.75	9.59	.81	.91***
Hair curvature (yes/no)	-4.50	9.11	-.49	-2.59	8.67	-.28	.91***
Hair color (brown vs others)	14.64	10.29	1.42	10.09	9.97	1.02	.92***
<i>Sociodemographic and lifestyle factors</i>							
Sex	-4.02	10.18	-.07	-4.62	9.65	-.48	.91***
Family income	-7.49	10.53	-.71	-1.07	10.07	-.11	.91***
BMI	3.49	2.06	1.69	2.99	1.98	1.51	.91***
Smoker (yes/no)	7.15	10.53	.68	9.03	9.94	.16	.91***
Alcohol consumption (drinks/week)	.83	1.45	.57	1.38	1.36	1.02	.92***
Drugs consumption (yes/no)	-6.85	8.92	-.77	-4.55	8.51	-.53	.91***
Contraceptive pills (yes/no)	-2.52	11.94	-.21	-5.17	10.76	-.48	.93***

Note. Cortisol values were used in pg/mg. B = Unstandardized beta estimate; S.E. = Standard error; *t* = *t* statistic; BMI = Body mass index; pg/mg = Picograms per milligram. + = $p < .10$; *** = $p < .001$.

Figure 1. Association between cortisol measured from hair collected by the participants at home and at the laboratory



Notes. r = Pearson correlation; pg/mg = Picograms per milligram.