

Rapid Communication

Both Hair Cortisol and Perceived Stress during Fall Exams Decrease after the Winter Break

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Abstract

Hair cortisol concentrations have been associated with a significant number of physical and psychological stressors; however, few longitudinal studies have been reported. In this pilot work, ten male and ten female college seniors were asked to report their perceived stress levels and had one centimeter of the posterior vortex hair collected during their college fall finals. The same procedure was repeated about a month later after the winter break. Hair cortisol was extracted with methanol and measured using ELISA. Our findings indicate that hair cortisol concentrations and perceived stress levels, while not associated with each other, decreased significantly after the winter break (both with $p < 0.0001$). Hair cortisol concentrations before and after the break were associated ($p < 0.01$).

Keywords: Perceived stress; Hair cortisol concentrations; Longitudinal; Young adults

Abbreviations

HCC: Hair Cortisol Concentrations; PS: Perceived Stress; BMI: Body Mass Index; ELISA: Enzyme-Linked Immunosorbent Assay

Introduction

Several recent reviews on Hair Cortisol Concentrations (HCC) in humans document the rapidly expanding and maturing field of study [1-6]. There is a consensus that HCC increases in subjects with certain health factors (e.g., Cushing's syndrome, use of hydrocortisone, metabolic syndrome, diabetes, cardiovascular disease [7-10]) and under chronic/acute stressors (e.g., vigorous exercise, difficult employment situation, chronic pain) [11-14]. HCC associations with traumatic events and with perceived stress are complex and depend on a multitude of factors with several studies having reported contradicting results [15-20].

Several cross-sectional studies have measured HCC in younger adults. In 99 university students, HCC increase was significantly related to serious life events [16]. In 46 university students, vigorous physical activity increased HCC [21]. In a group of 24 students, HCC was lower in those who had one or two bereavement experiences relative to those with no bereavement experiences [22]. In a study with 42 students, elevated HCC was associated with lower depressive symptoms and lower perceived stress [23]. Few longitudinal cortisol studies are available to date. The first longitudinal study on cortisol was published in 1999 where blood cortisol levels were found to increase across the weeks of gestation in pregnant adolescents [24]. Also, salivary cortisol and HCC were assessed longitudinally in 21 pregnant women, and both measures rose during pregnancy as expected [25]. Saliva cortisol was measured before and after a written examination in 11 graduate students, and higher cortisol was associated with higher stress before examination and lower exam grades [26]. In 52 females, blood cortisol levels were assessed three times at 6-month intervals and cortisol was positively related to concurrent general and social

anxiety [27]. Cortisol was measured upon enrollment, immediately after enrollment, and 3 and 6 months after enrollment in 59 children. Salivary cortisol was increased 3 months after enrollment, indicating that children reacted to the challenge of school later rather than initially [28]. HCC were measured when entering school and 2 months after in 42 children; HCC were higher at the later date, especially for fearful kids [29]. In 151 patients with structural heart disease, HCC was measured at baseline and at 12-week follow-up in a randomized controlled trial of mindfulness training. At the base levels, HCC was associated with BMI, respiratory rate, and the physical summary score. After 12-weeks, HCC change was associated with the mental summary score and diastolic blood pressure [30].

Materials and Methods

Our study was designed to provide strong evidence of longitudinal HCC changes in young adults. The State University of New York College at Oswego Institutional Review Board approved this work. Traditional validated HCC assessment protocols were used [31-35], and no additional validation experiments were performed for this brief communication. The procedures are described in detail below. Of importance for longitudinal HCC measurements, intra-individual HCC stability [36] must be noted.

Questionnaire

Ten female and ten male senior chemistry students participated in this study at two different times: once during the finals week of the fall semester, when students are expected to be very busy and stressed, and again during the first week of the spring semester, when students are expected to be relaxed after a month of resting. The participant sample was very homogenous (same age students equally split by gender). Each participant signed a consent form saying they knew the benefits and risks of this study before the research project began. At both sample collection times, the students were given a questionnaire in which they had rated their Perceived Stress (PS) level on a ten-point scale, from "not at all" to "very much".

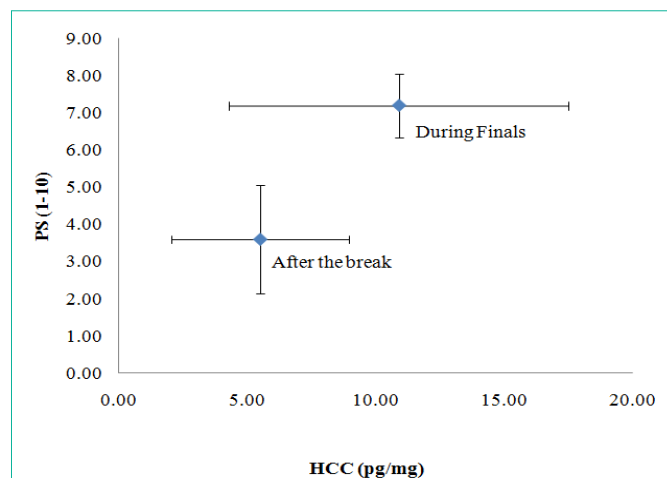


Figure 1: Longitudinal Changes in Hair Cortisol Concentrations (HCC) and Perceived Stress (PS) in College Students. HCC and PS on average decreased about two-fold after the finals (both with $p < 0.0001$). Errors are shown as one standard deviation.

Hair collection and storage

Approximately 50-100 mg hair of one cm in length from the posterior vortex was cut with surgical scissors from as close to the scalp as possible and weighed on an aluminium weighing boat. The sample was stored in an envelope at room temperature in a cabinet until extraction. The timing of measurements (about a month apart) and the fact that we studied one cm of hair closest to scalp were intentional and coincide with observations that hair in European Americans grows approximately one cm/month [1-3,6].

Hair extraction procedure

Approximately 50 mg of hair was weighed in a 15×45 mm Fisher brand vial with a rubber-lined cap. The hair was washed twice with one mL of isopropyl alcohol, vortexed for 2 minutes, and the solution was discarded. The hair was submerged into one mL of methanol, vortexed for 30 seconds, and placed into a 55°C water bath overnight. The samples were sonicated for 60 minutes. Methanol was transferred to a micro centrifuge tube and evaporated under a gentle nitrogen stream. Extracts were resuspended in 80 μ L of assay diluent that came with the Salimetrics cortisol ELISA kit and vortexed for 30 seconds immediately before ELISA procedure was performed.

For the confirmation of results, hair from all participants was pooled for the two collection times (exams and after the break), finely cut and triple- extracted (with methanol- acetone- methanol) [32].

ELISA

ELISA protocol was provided by Salimetrics. Duplicate 25 μ L samples were analyzed. The plate was read at 450 nm with a KC Junior Bio Tek plate reader. Concentrations of the controls and hair samples were determined by interpolation using 4-parameter nonlinear regression curve fit provided by KC Junior. If duplicate readings were different by more than 10%, 25 μ L of the sample were reanalyzed. The intra-assay precision was reported by Salimetrics as under 7%, the inter-assay precision under 11%, spiked recovery 97-109%, the minimal concentration of cortisol that can be distinguished from 0 was 0.007 μ g/dL in the solution, and the only cross-reactivity

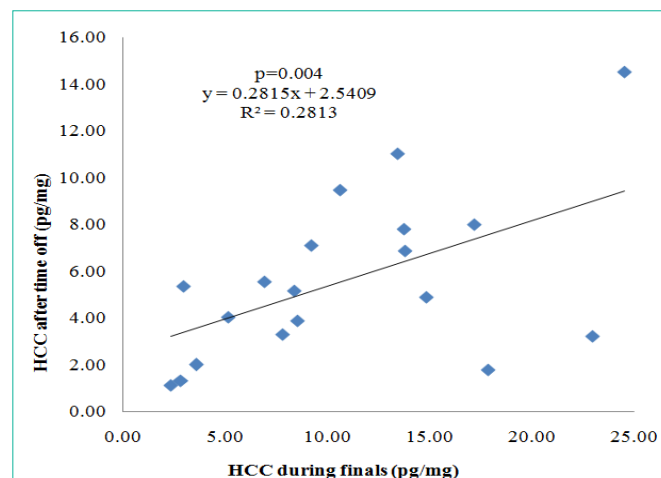


Figure 2: Intra-individual Stability of Hair Cortisol Concentrations (HCC). Individuals with higher HCC during the finals tended to have higher HCC after the time off too ($p = 0.004$).

know was with dexamethasone, an anti- inflammatory medication. Samples from an individual from the finals week and after the break were analyzed on the same ELISA plate to avoid the batch effect.

Results

With the exception of our intentional sampling of equal numbers of males and females, the study population was homogeneous: ages of all participants were very similar (seniors in a college with no non-traditional students participating), the race was 90% European American (only one African heritage female and one Asian heritage male participated, which was typical for a central New York college).

Changes in perceived stress and hair cortisol are shown in (Figure 1). Among 20 HCC and 20 PS values, one value in each set was identified as a statistical outlier in a G-test and was not included in calculations. Both, HCC and PS decreased almost two-fold from the time of finals to the time after the break.

Average HCC decreased from 10.9 pg/mg to 5.5 pg/mg. While there was a noticeable difference between individuals (yielding a high standard deviation for this measure), out of 19 participants, only one sample slightly increased in value while eighteen decreased. The change in HCC from finals to after break was found to be highly significant in a paired t-test ($t(18) = 4.64, p < 0.0001$). In an analysis of variance (ANOVA) with gender as the between-subject factor, change in HCC from exam to break did not differ as a function of gender, $F(1,17) = 0.01, p > 0.25$. Log-transformed HCC value analyses showed identical patterns.

Reported perceived stress decreased in all subjects. The average PS decreased from 7.2 to 3.6 on a ten-point scale. As with HCC, this change from finals to after break was found to be highly significant in a paired t-test ($t(18) = 10.83, p < 0.0001$). It should be noted that even though HCC and PS both decreased, neither the HCC and PS ratio of values (exam/post break) nor the change of these values over time (exam-post break) was statistically correlated to each other ($p > 0.25$). In an ANOVA with gender as the between-subject factor, change in PS from exam to break did not differ as a function of gender, $F(1,17) = 0.35, p > 0.25$.

Controlling for gender, HCC values during the final exams were significantly correlated with values after the winter break, $r = 0.67$, $p = 0.004$ (Figure 2); that is individuals who had higher HCC during the finals relative to other individuals tended to have HCC higher relative to the same individuals after the break as well.

Pooled samples of hair that were finely-cut and triple-extracted showed exactly the same HCC decrease as described above (results not shown).

Discussion

The decrease of PS and salivary cortisol in 11 graduate students after a written exam was previously documented [26]. In that regard, our results are exactly as expected. The only difference was that we measured HCC, not salivary cortisol in our study. In addition, multiple studies indicate that humans similarly handle major physiological or psychological stress, and the organismal coping with that stress can be effectively measured by the increase in HCC [1-3,6]. Thus, our results indicate that what students experience during the month leading to finals is equivalent at their young age to a major stressor or a serious life event.

The fact that HCC and PS changes were not statistically associated was not surprising. Although both went down, the amount they went down or their ratios were not correlated. In other words, all students reacted to finals (they become stressed), and while some reacted more psychologically, some reacted more physiologically; however, these two measures do not necessarily go hand-in-hand since people who react physiologically are not necessarily the same ones who react psychologically [37]. Indeed, the literature is full of examples showing that PS and even traumatic events are not necessarily associated with HCC, and sometimes patterns found in one study contradict those found in another [1-3,6].

Statistical intra-individual stability of HCC has been well documented [36]. Genetic factors and long-term stressors affect HCC; and even though stress before and during finals and time off affects HCC significantly, other continuing influences likely define whether HCC are high or low in an individual at both times of testing [36].

The weaknesses of this rapid communication include the following. 1) the study included a relatively small number of participants ($N=20$) at two different periods of time; the statistical tests performed were based on the existing sample size, as such, the fact that significant differences were still found suggests a large effect size and therefore a finding of importance. 2) The researchers relied on the Salimetrics Cortisol ELISA kits and the standard HCC determination methods without additional validation experiments; nevertheless, data in (Figure 2) indicate that our results were consistent and as expected according to the literature. 3) In all longitudinal HCC literature, to our best knowledge, there is no evidence of the change or the lack of the change of cortisol concentration in properly stored hair; such experiments must be performed in the future in the field. Also, if there was a decrease in cortisol concentration over the time of storage since our results indicate that cortisol was about two-fold higher during finals as compared to after the break, then the actual effect might have been even larger than we documented.

Conclusion

The weeks leading up to finals week are a major stressor in students' lives that is perceived by students as stress and is reflected in their HCC. In this scenario, HCC can be used as a measure of the effect of time off, indicating the disappearance of the stressor. Following this small pilot study, a significantly larger study with a significant number of participants, detailed stress questionnaires, and updated HCC measurement methodology and additional tests must be completed.

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