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Cumulative cortisol exposure increases during the academic term: Links to performance-related and social-evaluative stressors

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ABSTRACT

Objective: To examine whether cumulative cortisol production changes during a period of increased demands when cortisol and stress are assessed concurrently. The study also compared stress perceptions vs. cumulative stressful events on their respective association with cortisol output. Finally, it explored whether certain types of stressful events, those involving school/job performance or social-evaluative threat, were linked to cortisol levels across multiple weeks.

Method: The current study assessed cumulative cortisol production via hair sample in 56 undergraduates (88 % female) during both lower stress (summer break) and higher stress (academic term) periods. During the latter, both negative events (checklist) and stress perceptions were assessed weekly, and these reports were aggregated across the 10-weeks to minimize retrospective bias.

Results: Cortisol levels in hair samples were significantly higher (d = 0.84) during the academic term (M = 14.24 pg/mg, SD = 11.36) compared to summer break (M = 8.00 pg/mg, SD = 4.14), suggesting greater cumulative exposure to cortisol. Although perceived stress was not associated with cortisol levels ($r_{partial}(53) = .10$, p = 0.46), exposure to more stressful events ($r_{partial}(53) = .27$, p = 0.047), particularly events involving academic demands ($r_{partial}(53) = .37$, p = 0.006), or negative evaluation/social rejection ($r_{partial}(53) = .27$, p = 0.045), was positively associated with cumulative cortisol exposure.

Conclusions: This study demonstrates that cortisol levels in hair may be linked to cumulative exposure to stressors when measured concurrently (3 months), and that stressful events, rather than perceptions, are reflected in HPA axis activity. Real-world stressors involving performance demands and social-evaluative threat accumulate to enhance cortisol production, consistent with their acute HPA effects in the lab. Hair samples may provide a window into the past by allowing researchers to feasibly assess cortisol production before, during, and after the onset of a chronic stressor.

1. Introduction

Stressful experiences, especially persistent ones, can impart lasting effects on the body (Weiner, 1992). Miller et al. (2007) synthesized the literature on chronic stress and hypothalamic-pituitary-adrenal (HPA) axis function, concluding that exposure to chronic stress (broadly defined) was associated with alterations in HPA axis function, including greater daily cortisol output. Although the integration of a large and diverse body of research studies (and types of chronic stressors) by these authors has been seminal, their conclusions were limited by the fact that all of the studies included in their meta-analysis relied on brief measures of HPA axis function (e.g., salivary cortisol sampled over a few days) assumed to reflect ongoing HPA axis activity (before and after the study's sampling window). The exclusive use of "snapshot" measures is likely based on the assumption that daily measures validly reflect enduring patterns of HPA function, but evidence for this assumption is weak.

Studies that have examined within-person consistency of daily cortisol indices find only modest stability at best (Kuhlman et al., 2019), with the bulk of the variance in salivary cortisol explained by day-level predictors and state-dependent variables such as physical activity, social contacts, and mood (Ross et al., 2014). Chronic stress is likely to have cumulative effects on bodily systems, effects difficult to capture reliably with a single, state-dependent assessment. Furthermore, in studies of chronic stress, blood or saliva samples were often taken months if not years after the onset of the chronic stressor. For example, in the Miller et al. meta-analysis, the median time since stressor onset was 72 months (6 years!) among studies using a measure

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of daily cortisol output. Despite these limitations, cross-sectional analyses revealed that participants experiencing an ongoing stressor (of varying duration but likely present at the time of HPA assessment) tended to have higher cortisol levels (assessed over a few days at most) compared to unstressed controls (Miller et al., 2007). Whether these increased levels reflect enduring changes more likely to convey increased risk for disease, is unclear.

The present study sought to build upon this body of work by not relying on a daily measure of cortisol but instead assessing cortisol in hair - a matrix that captures cumulative cortisol production over a longer period of time (Raul et al., 2004). Rather than measuring cortisol levels sometime after stressor onset, the current study will assess cortisol in parallel with naturalistic stress. Circulating cortisol is incorporated into the hair shaft as it grows (approx. 1 cm/month; Sauve et al., 2007; Gow et al., 2010). Hair samples can provide a retrospective window into the cumulative levels of cortisol present in the body across weeks to months, minimally influenced by daily factors (Greff et al., 2019).

Several studies link chronic stress and cortisol concentrations in hair (HCC; Gidlow et al., 2016; Karlen et al., 2011; Schreier et al., 2016; Stalder et al., 2014). A recent meta-analysis found that individuals facing chronic stress displayed 22 % higher HCC levels compared to non-stressed individuals (Stalder et al., 2017), consistent with the higher daily cortisol output reported by Miller and colleagues (2007). However, of the 72 studies included in the Stalder et al. review, only three used a repeated-measured design and assessed HCC in the same sample (s) multiple times. Mayer et al. (2018) has since provided an additional prospective report of increased HCC concurrent with the onset of an ongoing stressor (medical internship). Repeated-measures designs are more powerful because an individual serves as their own pre-stress control, eliminating variance due to individual differences. In the current study, we compare HCC in college students at the end of an academic term with HCC from the same students at the beginning of the academic year. We expect that the ongoing demands of college will be associated with increased HCC compared to the summer, generally a lower stress period.

Although a growing body of work supports an association between *stressful events* and HCC (Khoury et al., 2019), the association between *perceived stress* and HCC has been less reliable. Some studies report a significant correlation between perceived stress levels and HCC (Stalder et al., 2014), but others do not (Karlen et al., 2011; Gidlow et al., 2016; Heinze et al., 2016). Stalder et al. (2017) reported no net reliable association between HCC and self-report measures of perceived chronic stress across 33 studies in their meta-analysis. Given the theoretical link between a stressful event (discrete events or ongoing circumstances) and perceived stress (Cohen et al., 1995), this inconsistency seems puzzling.

One potential explanation for this inconsistency may be limited reliability in retrospective measures of perceived stress. Self-reports of perceived stress (like other subjective self-reports) may be more vulnerable to retrospective bias than reports of objective events or circumstances. Retrospective reports tend to be disproportionately influenced by the highest levels and/or most recent levels of an experience (Redelmeier and Kahneman, 1996; Shiffman et al., 1997). Weekly assessments of stress would minimize recall bias by reducing the length of time over which a participant is asked to retrospect, producing more reliable and valid measures. The current study will assess both perceived stress and stressful events every week for multiple weeks leading up to the assessment of HCC. If perceived stress is associated with HCC, then that suggests retrospective measurement bias may have contributed to previous inconsistent findings. If perceived stress is not associated with HCC in the current study, then another explanation for the previous mixed findings must exist.

College is marked by a range of significant demands and potentially stressful experiences. Although the increase in HPA activity surrounding academic exams has been widely documented (e.g. Malarkey et al., 1995; Murphy et al., 2010; Preuss et al., 2010), less is known about the effects of other stressors that college students are likely to encounter, such as identity formation, juggling competing demands for time, navigating social relationships, adapting to new living arrangements, and managing finances. While each of these has the potential to be stressful, threats to social status or a valued aspect of the self may be especially likely to activate the HPA axis (Dickerson, 2008). Many of the stressors facing college students, both in and out of the classroom, may be of this nature, such as (potential) rejection by peers or negative evaluation by faculty. Situations high in social-evaluative threat or interpersonal rejection result in greater cortisol production acutely (Dickerson and Kemeny, 2004). In addition to acute effects in the lab, some work also demonstrates HPA reactivity to social-evaluative threats in a naturalistic context (e.g. ballroom dancing competition; Rohleder et al., 2007). However, very little work has examined the impact of social-evaluative threat on cortisol levels over an extended period of time (longer than a day or two). Our study builds on this previous work in two ways: by examining cumulative, repeated exposure to social-evaluative (among other types of) threats outside of a lab setting and (2) by examining cortisol production over a longer period of time compared to the immediate post-stressor period.

The current study will examine whether the cumulative experience of school/job stress, social/interpersonal stress (including social-evaluative threat), or other sources of stress (health, environmental, financial) are associated with cortisol production throughout a semester in college. Based on the extant literature, we predict that: 1) HCC will be higher during the academic term compared to the summer, reflecting the stress of college life, 2) students who report more stressful events or higher perceived stress throughout the term will display higher HCC, and 3) stressors that are performance-related (e.g. academics) and those that include social-evaluative threat/rejection will be most strongly associated with HCC compared to stressors that lack these elements.

2. Method

2.1. Participants

Participants were recruited via email from a larger group of sophomores taking part in a longitudinal study of resiliency during college. Based on our available resources, we could afford to recruit up to 60 participants (and analyze 120 hair samples). This yielded statistical power of 0.33 to detect a small effect but > .90 to detect a medium to large effect using a repeated-samples design. The actual sample consisted of 57 (91.2 % female) students from a small liberal arts university in the Southeast. Participants were excluded if they had chemically treated their hair in the past three months, had a chronic illness, or were taking medications known to affect cortisol levels. They were paid up to $90 dollars for their participation: ten dollars for each of the two hair samples, five dollars per weekly survey, and a $20 bonus for completing the entire study. Study protocol was approved by the university's Institutional Review Board.

2.2. Measures

Stress. Weekly stress was assessed in two ways: perceived stress and stressful events.

2.2.1. Perceived stress

Perceived stress was measured using a 4-item version of the Perceived Stress Scale (PSS; "MacArthur SES and Health Network (2020).; Cohen et al., 1983), a measure of how uncontrollable or overloaded participants found their weekly lives. Scores (0–4) were summed across all four items for weekly totals, then averaged across the number of weeks completed. Higher scores reflected greater perceived stress. The average Cronbach's alpha across the ten weeks was 0.76 (range: .59–.90). The PSS-4 has been used in successfully in previous studies of stress and well-being (King and DeLongis, 2014) and has demonstrated acceptable psychometric properties (Cohen and Williamson, 1988; Warttig et al., 2013).

2.2.2. Stressful events

Using a 19-item checklist of stressful events (adapted from Conway et al., 2015), participants indicated each week how many times they experienced an event (0 times - 5 or more times) in the past week. Weekly totals were summed to create a total events score for the 10-week study period. Due to non-normality in this variable's distribution, we transformed it using a rank-based inverse normal transformation (RIN). This type of transformation (conceptually similar to a Spearman correlation) has been shown to minimize Type I error rates while maximizing power for correlations involving non-normal data (Bishara and Hittner, 2012, 2015).

In order to examine the effects of particular types of events, events on the checklist were grouped into one of six domains: *school/job* (three items: did poorly on or failed an important exam or assignment; failed to achieve an important school-related goal that did not involve GPA; problems at work); *resources* (three items: lost money or other valuable item; property was damaged or stolen; did not have enough money to do or buy something); *environmental* (two items: was bothered by noise in my residence hall; could not find adequate quiet space to relax or have a private conversation); *health* (two items: personal illness or medical issue; event related to medical issue of family member or close friend); *interpersonal conflict* (five items: had an argument or problem with significant other, roommate, friend, family member; fight or argument among social group to which you belong) and *social evaluation/rejection* (three items: was rejected or excluded by others, was criticized by others, embarrassed myself in front of others). Total event counts for each of these domains were computed across the ten weeks. Because these data were also highly positively skewed, the same transformation (RIN) was applied to each of these variables for correlational analyses.

2.2.3. Cortisol

Cortisol production over the past three months was assessed twice (beginning and end of fall semester) via hair sample. Participants were seated comfortably while a hair sample was collected. A comb, hair clips, and scissors were sanitized using alcohol before and after use. A small section of hair (3 − 5 mm) from the middle of the back of the head (posterior vertex region) was isolated and then tied with string about an inch from the scalp to indicate the “top” of the sample. Hair was then cut as close to the scalp as possible. Following collection, samples were placed in foil wrappers and stored at room temperature until sent to the lab for analysis. After each sample was collected, it was sent to Salimetrics Ltd, Newmarket, UK for analysis by ELISA method. The three centimeters of hair proximal to the scalp were analyzed. Once cut to length, hair samples were mechanically ground using ceramic beads. A methanol extraction was performed and the samples dried in a vacuum centrifuge before resuspension and analysis. The assay yields a corrected hair cortisol concentration (HCC) value reported in pg/mg. The intra-assay coefficient of variation was 7.75 %. Cortisol data were log-transformed in order to normalize a skewed distribution.

2.2.4. Demographic, health- and hair-related variables

Participants reported their age, gender, race/ethnicity, height, weight, varsity athlete status (y/n), chronic illnesses, current and recent (in past 3 months) prescription medications, and chemical hair treatments (y/n). Body mass index (BMI) was calculated from self-reported height (inches) and weight (pounds) values.

2.3. Procedure

After study eligibility was confirmed and informed consent obtained, participants came into the lab to provide their first hair sample at the beginning of the fall semester (Time 1; late August-early September 2015) of their sophomore year. At this time, participants also provided written demographic, health, and hair-related information. Participants were asked not to chemically process or cut their hair shorter than two inches in the subsequent weeks to maintain eligibility for the study. A second hair sample was collected 11–12 weeks later, at the end of the fall semester (Time 2; late Nov-early Dec 2015). In between hair samples, participants completed weekly online surveys distributed via email for ten weeks starting the Sunday after the participants' first hair sample. The surveys were 10 − 15 min long and consisted of 33 items related to sleep habits (data not reported) and stress (events and perceptions) in the past week. After receiving the weekly email link, participants had 36 h to go online to complete the survey in Qualtrics. Any responses submitted outside the 36 -h window were not included in subsequent analyses.

3. Results

3.1. Missing data

Nearly all (56 out of 57; 98.2 %) participants who contributed a hair sample at the start of the semester (Time 1) contributed a second hair sample at the end of the semester (Time 2). The participant who did not provide a second hair sample also completed only two weekly surveys; that individual was dropped from further analysis. The majority (58.9 %) of participants completed all ten weekly surveys on time, while an additional 21.4 % missed only one week. Participants who missed more than one weekly survey (n = 11) did not differ from more adherent participants in their reported stress or cortisol levels (*p's* > 0.11). One participant did not report height or weight values.

In order to handle missing data from weekly surveys appropriately, we used hot deck imputation (Andridge and Little, 2010). This technique is much more effective that listwise deletion, pairwise deletion, or mean substitution (Hawthorne and Elliot, 2005), and is recommended for all missing data scenarios except where data are missing not at random (MNAR) and comprise more than 10 % of the sample (Roth, 1994). Hot deck imputation involves filling in missing values with values from other researcher-specified “donor” variables that match the “donee" in pre-specified ways (Myers, 2011). In our case, we imputed values from missing weeks with values from another week that was most strongly correlated with the missing week. This takes advantage of the auto-correlated nature of repeated-measures data. The pattern of results was unchanged whether we used pairwise deletion or hotdeck imputation, so we report results based on imputed data.

3.2. Descriptive statistics and covariates

Table 1 displays the descriptive statistics for the sample. Males and

Table 1
Descriptive statistics for current sample.

Variable	Mean/Frequency
Age in years	19.04 (0.38)
Gender	91.1 % female (n = 51)
Race/ethnicity	96.4 % White
BMI	22.5 (2.85)
Varsity athletes	0 %
Medication use[1]	48 % (n = 27)
oral contraceptives	30.4 % (n = 17)
psychotropic meds	17.9 % (n = 19)
PSS score (10 week avg.)	6.36 (2.39)
Total stressful events reported	39.61 (33.76)
Time 1 HCC (pg/mg)	8.00 (4.14)
Time 2 HCC (pg/mg)	14.24 (11.36)
Total (*N* = 56)	

Note. Numbers in parenthesis reflect SD unless otherwise noted.
[1] Medication use was assessed at study enrollment (Time 1).

females did not differ significantly on stress or cortisol levels (all *p's* > 0.26). Although nearly half of the students reported taking a medication (including oral contraceptives) at the time of the first hair sample, these students did not have significantly different levels of cortisol in their hair samples at either Time 1 or Time 2 (all *p's* > 0.23). Body mass was not significantly correlated with either measure of stress or with either T1 or T2 cortisol levels (all *p's* > 0.09).

3.3. Is the academic term associated with greater HCC?

Cortisol levels in hair were 78 % higher at the end of the semester (M = 14.24 pg/mg, SD = 11.36) compared to the beginning (M = 8.00 pg/mg, SD = 4.41). This effect was large (Cohen's d = 0.84) and statistically significant (paired samples $t(55) = 4.53$, $p < 0.001$).

3.4. Are stressful events or perceptions of stress associated with HCC during the academic term?

Given the overall increase in HCC during the semester, we examined whether the experience of stress was associated with cortisol production during the term, controlling for baseline cortisol levels.[1] Perceived stress (M = 6.36, SD = 2.93; range: 0.90–12.11) was not significantly associated with HCC ($r_{partial}(53) = .10$, $p = 0.46$). However, the total number of stressful events (M = 39.61, SD = 33.76; range: 0–146) was associated with HCC at the end of the term ($r_{partial}(53) = .27$, $p = 0.047$). Participants who experienced more stressful events tended to produce more cortisol throughout the semester, when accounting for their cortisol at the beginning the semester. In order to explore whether this association was driven disproportionately by the stressful events that occurred later in the term (closer to the second hair sample), we examined whether the stressful events just in weeks 9 and 10 (together) were associated with Time 2 HCC, controlling for Time 1; they were not ($r_{partial}(53) = .25$, $p = .07$).

3.5. Are certain types of stressful events more closely associated with HCC than others?

Next we examined whether particular types of stressors were more strongly linked to cortisol production. Table 2 shows the average number of occurrences reported for each event category across the ten weeks. Students who reported more school/job stressors ($r_{partial}(53) = .37$, $p = 0.006$), more social evaluation/rejection ($r_{partial}(53) = .27$, $p = .045$), and more health-related stressors ($r_{partial}(53) = .38$, $p = .005$) across the ten weeks tended to have higher cortisol levels at the end of the semester (controlling for baseline HCC). No other types of stressors (interpersonal conflict, resources, environmental) were significantly associated with HCC at the end of the term ($r_{partial}$'s < 0.22, *p's* > 0.13).

Because health-related stressors are confounded with acute illnesses that could inherently increase cortisol (i.e. due to the pathophysiology of the illness, rather than the psychological response to being ill), we examined whether a participant's own illness or an illness in a member of the participant's social network was more strongly associated with cortisol production. Participants who reported more illness in themselves also tended to have higher HCC at the end of the term ($r_{partial}(53) = .28$, $p = 0.03$), while frequency of illness in participants' family or close friends was not associated with HCC ($r_{partial}(53) = .11$, $p = 0.41$). Thus it may be that health-related stressors are associated with HCC at the end of the term because one's own illnesses inherently activate the HPA axis and/or contributes to higher psychological stress.

Finally, we wondered whether school/job, social-evaluative, or health-related stressors were independently associated with HCC at the end of the semester, controlling for the other sources of stressful events (and for baseline HCC). We conducted a stepwise linear regression with baseline HCC entered on step 1 and the total counts for each of these three types of stressors entered simultaneously in step 2. Together, school/job, social-evaluative, or health-related stressors accounted for 11.5 % more variance in end-of-semester HCC beyond baseline HCC alone (total $R^2 = 0.36$). This increase in explained variance was statistically significant (F (3,51) = 3.32, $p = 0.03$). However, none of the three types of stressors entered in step 2 were independently associated with end-of-semester HCC (beta's < .2, *p 's* > 0.23).

Table 2
Frequency of Stressful Events by Category Across 10 Weekly Reports.

Category	Average total (SD)	Median	Range (min-max)
Environmental	11.55 (16.25)	5.50	0 – 83
Health	6.59 (7.89)	5.00	0 – 51
Social evaluative threat/ rejection	6.12 (7.15)	3.00	0 – 35
School/job	6.07 (5.03)	5.00	0 – 23
Interpersonal conflict	5.86 (7.11)	3.00	0 – 33
Resources (lost/lack of)	3.68 (4.67)	2.00	0 - 28

4. Discussion

Cortisol levels increased markedly during the academic term compared to the end of summer/beginning of the term. Only a handful of previous studies have prospectively examined within-person changes in HCC across stressful and non-stressful conditions (Mayer et al., 2018; Boesch et al., 2015; Stalder et al., 2012; Iglesias et al., 2015). Our study is among the first to demonstrate that HCC increases under conditions of relatively mild chronic stress, and that the cortisol response is associated with the experience of the stressful event(s) or condition(s) themselves but not with the general perception of stress. By assessing HCC twice within the same sample of individuals, we are able to more sensitively examine the association between cumulative stress and HPA axis activity over time. Cortisol was positively associated with the number of stressful events assessed every week for ten weeks, concurrent with the production of cortisol eventually captured in the hair sample. Finally, not all types of stressful events were associated with increased HCC. Events related to one's own health, school/work life, and events involving social-evaluative threat or social rejection were the most highly associated with HCC.

Our findings are consistent with previous work that suggests a stronger link between HCC and the occurrence of stressful events themselves, rather than the general perception of stress (Stalder et al., 2017). This pattern is not unique to chronic stress and HCC; Campbell and Ehlert (2012) note a weak and inconsistent association between subjective stress responses and HPA axis reactivity (e.g. salivary cortisol) to acute lab stress, as do other researchers with regard to field studies (Hjortskov et al., 2004). If the acute HPA response is only weakly predicted by the emotional reaction to a standardized lab-based stressor, then it is not at all surprising that outside the lab, cumulative HPA activity is not associated with subjective appraisals. Although the reasons for the "psychoendocrine desynchrony" are not well understood, it could be attributed to a host of moderating factors such as psychological traits, situational characteristics, or biological predispositions (Campbell and Ehlert, 2012). To the extent that this intriguing pattern is not an artifact of study methodology, future research is needed to establish the complex pathways that comprise our cognitive, emotional, and physiological responses to stressful events.

An important strength of our study is the weekly measures of both stressful events and perceived stress. This helps to address one potential explanation for the null results that have emerged in previous studies:

[1] Time 1 and Time 2 HCC are highly correlated $r = .54$. Because data are cross-sectional, controlling for Time 1 HCC helps to address directionality; any overlap between Time 1 HCC and stress is partialed out.

retrospective bias. Of course, to completely eliminate retrospective bias, researchers would need to query stress multiple times a day for months, but this is likely to be perceived as overly burdensome by participants. Because our assessment of stress was largely coincident with the production of cortisol (although up to 2 weeks may have elapsed between the final stress assessment and the second hair sample for some participants), our work suggests that perceived stress is not associated with HCC, at least in our young, healthy sample. Stalder et al. (2017) point out that in mildly stressed populations, the range of perceived stress scores may be limited, making any associations with cortisol harder to detect. We had a 12-point range (out of possible 16) on the average weekly perceived stress score. Thus, we believe that neither a range restriction nor recall bias can adequately explain the lack of an association with HCC. Liu and Doan (2019) raise a few other potential reasons for the inconsistent association between HCC and perceived stress measured retrospectively over a month or more, including social desirability or habituation to chronic stress. Additionally, there may be a direct (perhaps non-conscious) route from the experience of a stressful event to cortisol production that is unmediated by cognitive or emotional processes.

In addition to the total burden of stressful events, particular types of events may be more likely to activate the HPA axis among college students. School/job-related stress was linked to HCC. This finding is perhaps not surprising given the nature of the sample and the setting in which our study was conducted. A body of previous work has documented increases in HPA activity during a specific examination period (one day to one week long; e.g. Malarkey et al., 1995; Murphy et al., 2010; Preuss et al., 2010). Our study extends this work by examining the accumulation of school-related stress throughout the academic term and linking it with chronic HPA activation.

Beyond the demands of work and school, our results also suggest an important role for events that involve negative social evaluation or rejection. To be sure, poor performance on an exam or assignment may also involve negative evaluation by others. The adolescent brain may be particularly vigilant for and sensitive to social feedback (Albert et al., 2013). Feelings of embarrassment peak in the late teens (Somerville, 2017). While the potency of acute negative social evaluation for activating the HPA axis has been well documented in previous lab-based work (Dickerson and Kemeny, 2004), far fewer efforts have been made to examine the presence and longer-term impact of social-evaluative threat in an ecologically-valid setting. Rohleder et al. (2007) found that ballroom dancers had elevated cortisol levels in response to a day of competition compared to a training day or control day. In another study, trait shame, an emotion closely linked to experiencing criticism or rejection, was not associated with daily cortisol levels in young women (Rohleder et al., 2008). Our work suggests that the accumulating experience of negative social evaluation (separate from interpersonal conflict more generally) does have consequences for longer-term HPA axis activity as reflected by cortisol levels in hair. Future studies are needed to understand the implications of these increased cortisol levels for the regulation of inflammation and metabolic processes in this young, healthy population.

Our study is subject to several important limitations, including a small, unrepresentative sample. Although our sample size provided adequate statistical power to detect moderate to large within-person changes in HCC, we may have been underpowered to detect small associations between perceived stress and/or certain types of stressful events and HCC. However, the results of a previous meta-analysis (Stalder et al., 2017) documented a very small and unreliable correlation between perceived stress and HCC across over two dozen studies. Thus we believe our null findings are unlikely due to Type II error. Our sample was one of convenience, predominantly White, and female. As such, the sample was not representative of the larger student body, and our results may not generalize well to other college students.

By using a self-report stressful event checklist, we may have missed important events that our participants experienced (although the checklist did include an “other event” response option). While an improvement over retrospective reports across months or years, our weekly checklist was relatively brief (19 items) and still vulnerable to some recall bias. Furthermore, we did not assess stress during the summer, and thus cannot conclude with certainty that stress levels increased from summer to school year. Previous studies have also used a similar summer-to-school-year comparison to examine immunological changes associated with stress (Segerstrom et al., 1998; Marucha et al., 1998), while Milyavskaya et al (2014) confirm that positive affect is highest and negative affect is lowest in college students during summer and winter breaks. Thus we believe that increased stress levels from summer to fall term is a reasonable (although untested) assumption. Finally, we assessed stress over 10 weeks, but 3 cm of hair could technically capture up to 13 weeks of cortisol production (depending on exact rate of hair growth). Thus, there may be one or two weeks of unmeasured life stress that contributes some noise to our data but does not account for our significant findings.

Hair is increasingly recognized as an advantageous matrix for validly assessing cortisol exposure in the previous 1–5 months (Russell et al., 2012). Although we do not currently have a definitive understanding of the physiological origins of the cortisol taken up by the hair shaft during its growth, the primary mechanism is thought to be diffusion from follicular capillaries (Cone, 1996; Greff et al., 2019). HCC levels are convergently valid with the next most “long-term” assessment of cortisol: 24 -h urinary measure (Sauve et al., 2007). Another study found a strong association between HCC and daily cortisol levels measured in saliva across a month (Short et al., 2016). The cortisol that is contained in the hair shaft is proportional to the quantity of cortisol in circulation at any given time (Greff et al., 2019). By sampling hair twice and analyzing only the most recent 3 months (i.e. the 3 cm of hair most proximal to the scalp on the posterior vertex of the head), the current study’s design minimizes many of the individual differences that influence cross-sectional analyses of cortisol levels. However, our study is unable to rule out or isolate the influence of sunlight exposure, temperature, season, or physical activity on HCC.

The current findings reveal the link between cumulative demands, especially those involving social-evaluative threat, and the HPA axis. These findings complement and extend the results of work involving acute, lab-based stressors (Dickerson and Kemeny, 2004). Although not the most frequent types of stress, events involving poor academic performance or negative social evaluation were the most strongly linked to greater cortisol production during the academic term. Over time, these sorts of events may contribute to allostatic load and reduced mental and physical well-being. Given this significance of chronic stress for understanding health outcomes, researchers investigating potential mechanisms should seek to employ measures (like hair and nail samples) that capture chronic levels of physiological functioning rather than snapshot assessments. Studies that align the timeframe of the stress and the timeframe of the physiological response provide better tests of conceptual models and are more likely to find robust effects.

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Declaration of Competing Interest

None.

References

Albert, D., Chein, J., Steinberg, L., 2013. The teenage brain: peer influences on adolescent decision-making. Curr. Dir. Psychol. Sci. 22, 114–120.
Andridge, R.R., Little, R., 2010. A review of hot deck imputation for survey non-response. Int. Stat. Rev. 78 (1), 40–64.
Bishara, A.J., Hittner, J.B., 2012. Testing the significance of a correlation with non-normal data: comparison of Pearson, Spearman, transformation, and resampling approaches. Psychol. Methods 17, 399–417.
Bishara, A.J., Hittner, J.B., 2015. Reducing bias and error in the correlation coefficient due to nonnormality. Educ. Psychol. Meas. 75, 785–804.
Boesch, M., Sefidan, S., Annen, H., Ehlert, U., Roos, L., Van Uum, S., et al., 2015. Hair cortisol concentration is unaffected by basic military training, but related to socio-demographic and environmental factors. Stress 18, 35–41.
Campbell, J., Ehlert, U., 2012. Acute psychosocial stress: does the emotional stress response correspond with physiological responses. Psychoneuroendocrinology 37, 1111–1134.
Cohen, S., Kamarch, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Soc. Behav. 24, 385–396.
Cohen, S., Kessler, R.C., Gordon, L.U., 1995. Strategies for measuring stress in studies of psychiatric and physical disorders. In: Cohen, S., Kessler, R.C., Gordon, L.U. (Eds.), Measuring Stress: A Guide for Health and Social Scientists. Oxford University Press, New York, pp. 3–26.
Cohen, S., Williamson, G., 1988. Perceived stress in a probability sample of the United States. In: Spacapan, S., Oskamp, S. (Eds.), The Social Psychology of Health. Sage, Newbury Park, CA, pp. 31–68.
Cone, E.J., 1996. Mechanisms of drug incorporation into hair. Ther. Drug Monit. 18, 438–443.
Conway, C.C., Slavich, G.M., Hammen, C., 2015. Dysfunctional attitudes and affective responses to daily stressors: separating cognitive, genetic, and clinical influences on stress reactivity. Cognit. Ther. Res. 39, 366–377.
Dickerson, S., 2008. Emotional and physiological responses to social-evaluative threat. Soc. Personal. Psychol. Compass 2, 1362–1378.
Dickerson, S., Kemeny, M., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391.
Gidlow, C.J., Randall, J., Gillman, J., Silk, S., Jones, M.V., 2016. Hair cortisol and self-reported stress in healthy, working adults. Psychoneuroendocrinology 63, 163–169.
Gow, R., Thomson, S., Rieder, M., Van Uum, S., Koren, G., 2010. An assessment of cortisol analysis in hair and its clinical applications. Forensic Sci. Int. 32–37.
Greff, M.J.E., Levine, J.M., Abuzgaia, A.M., Elzagallaai, A.A., Rieder, M.J., van Uum, S.H.M., 2019. Hair cortisol analysis: an update on methodological considerations and clinical applications. Clin. Biochem. 63, 1–9.
Hawthorne, G., Elliot, P., 2005. Imputing cross-sectional missing data: comparison of common techniques. Aust. N. Z. J. Psychiatry 39, 583–590.
Heinze, K., Lin, A., Reniers, R.L.E.P., Wood, S.J., 2016. Longer-term increased cortisol levels in young people with mental health problems. Psychiatry Res. 236, 98–104.
Hjortskov, N., Garde, A.H., Ørbæk, P., Hansen, Å.M., 2004. Evaluation of salivary cortisol as a biomarker of self-reported mental stress in field studies. Stress Health 20, 91–98.
Iglesias, S., Jacobsen, D., Gonzalez, D., Azzara, S., Repetto, E., Jamardo, J., et al., 2015. Hair cortisol: a new tool for evaluating stress in programs of stress management. Life Sci. 141, 188–192.
Karlen, J., Ludvigsson, J., Frostell, A., Theodorsson, E., Faresjö, T., 2011. Cortisol in hair measured in young adults – a biomarker of major life stressors? BMC Clin. Pathol. 11, 12–18.
Khoury, J.E., Enlow, M.B., Plamondon, A., Lyons-Ruth, K., 2019. The association between adversity and hair cortisol levels in humans: a meta-analysis. Psychoneuroendocrinology 103, 104–117.
King, D.B., DeLongis, A., 2014. When couples disconnect: rumination and withdrawal as maladaptive responses to everyday stress. J. Fam. Psychol. 28, 60–69.
Kuhlman, K.A., Robels, T., Dickenson, L., Reynolds, B., Repetti, R., 2019. Stability of diurnal cortisol measures across days, weeks, and years across middle childhood and early adolescence: exploring the role of age, pubertal development, and sex. Psychoneuroendocrinology 100, 67–74.
Liu, C.H., Doan, S.N., 2019. Innovations in biological assessment of chronic stress through hair and nail cortisol: conceptual, developmental, and methodological issues. Dev. Psychol. 61, 465–476.
MacArthur SES & Health Network. (2020) (n.d.). Retrieved from https://macses.ucsf.edu/research/psychosocial/pss4.php.
Malarkey, W., Pearl, D.K., Demers, L.M., Kiecolt-Glaser, J.K., Glaser, R., 1995. Influence of academic stress and season on 24-hour mean concentrations of ACTH, cortisol, and beta-endorphin. Psychoneuroendocrinology 20, 499–508.
Marucha, P.T., Kiecolt-Glaser, J.K., Fvagehi, M., 1998. Mucosal wound healing is impaired by examination stress. Psychosom. Med. 60, 362–365.
Mayer, S.E., Lopez-Duran, N.L., Sen, S., Abelson, J.L., 2018. Chronic stress, hair cortisol and depression: a prospective and longitudinal study of medical internship. Psychoneuroendocrinology 92, 57–65.
Miller, G.E., Chen, E., Zhou, E.S., 2007. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol. Bull. 133, 25–45.
Milyavskaya, M., Harvey, B., Koestner, R., Powers, T., Rosenbaum, J., Ianakieva, I., Prior, A., 2014. Affect across the year: how perfectionism influences the pattern of university students' affect across the calendar year. J. Soc. Clin. Psychol. 33, 124–142.
Murphy, L., Denis, R., Ward, C.P., Tartar, J.L., 2010. Academic stress differentially influences perceived stress, salivary cortisol, and immunoglobulin-A in undergraduate students. Stress 13, 365–370.
Myers, T., 2011. Goodbye, listwise deletion: presenting hot deck imputation as an easy and effective tool for handling missing data. Commun. Methods Meas. 5, 297–310.
Preuss, D., Schoofs, D., Schlotz, W., Wolf, O.T., 2010. The stressed student: influences of written examinations and oral presentations on salivary cortisol concentrations in university students. Stress 13, 221–229.
Raul, J.-S., Cirimele, V., Ludes, B., Kintz, P., 2004. Detection of physiological concentrations of cortisol and cortisone in human hair. Clin. Biochem. 37, 1105–1111.
Redelmeier, D.A., Kahneman, D., 1996. Patients' memories of painful medical treatments: real-time and retrospective evaluations of two minimally invasive procedures. Pain 66, 3–8.
Rohleder, N., Beulen, S.E., Chen, E., Wolf, J.M., Kirschbaum, C., 2007. Stress on the dance floor: the cortisol stress response to social-evaluative threat in competitive ballroom dancers. Pers. Soc. Psychol. Bull. 33, 69–84. https://doi.org/10.1177/0146167206293986.
Rohleder, N., Chen, E., Wolf, J.M., Miller, G.E., 2008. The psychobiology of trait shame in young women: extending the social self preservation theory. Health Psychol. 27, 523–532.
Roth, P.L., 1994. Missing data: a conceptual review for applied psychologists. Pers. Psychol. 47, 537–560.
Ross, K.M., Murphy, M.L.M., Adam, E., Chen, E., Miller, G.E., 2014. How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. Psychoneuroendocrinology 39, 184–193.
Russell, E., Koren, G., Rieder, M., Van Uum, S., 2012. Hair cortisol as a biological marker of chronic stress: current status, future directions, and unanswered questions. Psychoneuroendocrinology 37, 589–601.
Sauve, B., Koren, G., Walsh, S., Tokmakejian, S., Van Uum, S., 2007. Measurement of cortisol in human hair as a biomarker of systemic exposure. Clin. Investig. Med. 30, E183–E191.
Schreier, H.M., Bosquet, M.E., Ritz, T., Coull, B.A., Gennings, C., Wright, R., Wright, R., 2016. Lifetime exposure to traumatic and other stressful life events and hair cortisol in a multi-racial/ethnic sample of pregnant women. Stress 19, 45–52.
Segerstrom, S.C., Taylor, S.E., Kemeny, M.E., Fahey, J.L., 1998. Optimism is associated with mood, coping, and immune change in response to stress. J. Pers. Soc. Psychol. 74, 1646–1655.
Shiffman, S., Hufford, M., Hickcox, M., Paty, J.A., Gnys, M., 1997. Remember that? A comparison of real-time versus retrospective recall of smoking lapses. J. Consult. Clin. Psychol. 65, 292–300.
Short, S.J., Stalder, T., Marceau, K., Entringer, S., Moog, N.K., Shirtcliff, E.A., et al., 2016. Correspondences between hair cortisol concentrations and 30-day integrated daily salivary and weekly urinary cortisol measures. Psychoneuroendocrinology 71, 12–18.
Stalder, T., Steudte, S., Alexander, N., Miller, R., Gao, W., Dettenborn, L., Kirschbaum, C., 2012. Cortisol in hair, body mass index, and stress-related measures. Biol. Psychol. 90, 218–223.
Stalder, T., Tietze, A., Steudte, S., Alexander, N., Dettenborn, L., Kirschbaum, C., 2014. Elevated hair cortisol levels in chronically stressed dementia caregivers. Psychoneuroendocrinology 47 36-30.
Stalder, R., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., Kirschbaum, C., Miller, R., 2017. Stress-related and basic determinants of hair cortisol in humans: a meta-analysis. Psychoneuroendocrinology 77, 261–274.
Somerville, L.H., 2017. The teenage brain: sensitivity to social evaluation. Curr. Dir. Psychol. Sci. 22, 121–127. https://doi.org/10.1177/0963721413476512.
Warttig, S.L., Forshaw, M.J., South, J., White, A.K., 2013. New, normative, english-sample data for the short form perceived stress scale (PSS-4). J. Health Psychol. 18, 1617–1628. https://doi.org/10.1177/1359105313508346.
Weiner, H., 1992. Perturbing the Organism: the Biology of Stressful Experience. University of Chicago Press, Chicago.