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Screening for stressful life events, cortisol, and perceived stress in a school setting

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Abstract

In this cross-sectional, convenience sample study, we screened adolescents for stress using a measure of stressful life events experienced over the last 12 months, a measure of perceived stress, and salivary cortisol in a public-school setting in the Southern United States. We specifically sought to determine if cortisol followed a normal pattern of decline by collecting morning to afternoon salivary samples and further looked for an interaction effect between the variables. Statistical tests included descriptive, correlational, and multiple regression data examination with n=143 9th graders ages 14 to 16 years from two high schools in two separate small suburban towns. The majority (n = 88; 63.8%) of participants had a normal cortisol decline, with a few (n = 18; 13%) having a flattened or blunted dysregulated decline (less than .01 ug/dl change from AM to PM), or a cortisol rise from morning to afternoon (n = 32; 23.2%) or increase in cortisol from morning to afternoon). The interaction between stressful life events and cortisol regulation was significantly predictive of perceived stress β = .26, $t(131) = 3.50$, $p < .001$. Notably, the interaction accounted for significant additional variance $\Delta R^2 = .07$, $F(1,131) = 12.23$, $p < .001$. Understanding how stressful life events and perceived stress affect adolescents differently is helpful in determining assistance for coping, further assessment, and future interventions. Screening for stress in this population and collecting salivary cortisol samples in a school setting is feasible and may identify students in need of mental and emotional support.

Introduction

Stress is a necessary part of life that enables adolescents to adapt to life events during growth and development [1,2]. Adolescents are often challenged with unique stressful life events during identity development, parental conflict, educational challenges, and romantic as well as social relationships [3]. When experiencing these events, some adolescents will perceive these situations as stressful, while others may not be affected [4]. Cortisol is one of many glucocorticoid hormones released when an individual is under stress [5]. This is the body's neuroendocrine response that is produced by the hypothalamic-pituitary-adrenocortical (HPA) axis [6]. We sought to examine if schools may be a feasible place to screen for stress in adolescents by collecting survey data on stressful life events, perceived stress, and salivary cortisol. Consistent with McEwen's (1998) allostatic load model, we generated a single hypothesis (H1) that stressful life events and cortisol dysregulation would form an interaction

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that exacerbates perception of stress, while demographic correlates are controlled [7].

The current study adds a unique perspective of adolescents where data is collected from 9th grade students who are transitioning to a new school with increased academic rigor as compared to their previous middle school years. While stressful life events predict perceived stress [8-11], cortisol has been implicated as a biological substrate that modulates the expression of perceived stress [12-14]. Specifically, prior research has shown that cortisol is more strongly associated with perceived stress than the stressful life events themselves [15-18]. However, these results have been understudied in adolescents. The concern for this population is that increased and prolonged perceptions of life stress can be associated with problematic long-term outcomes over the life course such as obesity [19], anxiety, depression, general distress [20], cognitive ability [21], memory issues [22], and cardiovascular risk [23]. Therefore, the current study used a screening tool to examine adolescent stressful life events and the perception of these events experienced over the last 12 months. We also sought to determine if salivary cortisol followed a normal pattern of decline by collecting morning and afternoon salivary cortisol samples and sought to determine if there was any interaction with cortisol in those students who experienced more stressful life events and higher levels of perceived stress.

The theory used for this study was based on McEwen's Allostatic Load Model. McEwen's (1998) model postulates that daily stressors and stressful life events related to the environment at an individuals' home, school/workplace, or neighborhood, activate the Hypothalamic-pituitary-adrenal (HPA) axis, thereby releasing stress hormones such as cortisol. When an individual experiences acute stress, there is often a "fight or flight" response" (p. 171) [7]. As a result, through various neuroendocrine, neural, and neuroendocrine-immune adaptive processes (i.e. allostasis), homeostatic regulation is maintained through adaptation. However, an adolescent may respond differently depending upon their pubertal development and individual life experiences [2]. Major life events such as parental divorce, loss of a parent, moving to a new school, or traumatic occurrences can potentially disrupt allostasis. The ability to adjust to repeated stressors is determined by the way an adolescent perceives the situation [7,2]. If the perception of stress is exacerbated by other day to day activities that are chronically stressful, this may lead to problematic long-term physical or mental health issues in which these same mediators facilitate pathophysiological changes referred to as allostatic load/overload [24-26].

There continues to be a dearth of knowledge related to how stress affects an adolescent brain. Understanding of how ongoing stress leads to changes in the normal cortisol rhythms may provide a better understanding as to why some adolescents respond to stress differently. The normal diurnal cortisol rhythm is highest about 30 minutes after awakening with a decrease as the day progresses with the lowest point normally occurring after midnight [27]. Perceived stress along with living in a chaotic environment leading to disrupted sleep can produce a flat diurnal rhythm [27]. Few studies have examined stressful life events, perceived stress, and cortisol by collecting salivary samples in school settings. Bevans et al., (2008) studied n=68 children and their caregivers who reported stressful life events over the previous 12 months in addition to child exposure to early trauma [12]. Results indicated that high levels of recent trauma and frequent exposure to early life trauma was related to lower morning cortisol and higher afternoon cortisol. Bevans et al., (2008) study provides an indication that abnormal cortisol rhythms could alert a health care provider to previous stress and trauma [12]. Another study by Kelly, Young, Sweeting, Fischer, & West, 2008 examined cortisol levels by collecting two saliva samples, 30 minutes apart to examine cortisol decline on N=2,995 15-year-olds [28]. Most of these adolescents (73%) showed a decline from collection one to collection two which is what is expected in the cortisol diurnal rhythm. However, 27% of the adolescents had an increase or no change in cortisol levels from saliva collection one and two. While there are other studies examining cortisol in adolescents, there are few studies that have been conducted in school settings.

Method

Participants

The study included *n* = 143 participants in the 9th grade. See Table 1 for full demographic data. Inclusion criteria included student assent and parental consent, student ability to speak, read, and respond to measures in English, and ability to provide saliva samples for cortisol analysis. Exclusion criteria included: Student inability to speak English, student self-report of pregnancy, student self-report of clinical depression, bipolar disorder, Cushing's or Addison's disease, or physical illness with elevated temperature on the day of the study.

Procedure

This study was conducted with the approval of the university institutional review board. Informed consent was obtained from parents and assent from adolescents.

Cortisol: Participants were asked to have nothing to eat or drink an hour before saliva collection and to rinse their mouths with water for 5 minutes. A passive drool method was used to collect the specimen in a cryo vial over a 3-minute period [29]. Saliva was collected from participants in the morning (approximately 7:32 am to 10:34am) and in the afternoon (approximately 12:15 pm to 3:03 pm) to determine if the cortisol had a greater than .01 change from the morning to afternoon sample. Specific times for cortisol collection were documented on logs as participants came into the classroom. Participants were only allowed to be in the study classroom during an "elective course", therefore; the samples were collected during different courses according to the students' schedule in the morning or the afternoon.

The morning cortisol collection took place during 1st (7:32 am to 8:27 am), 2nd (8:43 am to 9:36 am), or 3rd period classes (9:41 am to 10:34 am). After lunch, students returned for a second collection during 5th (12:14 pm to 1:07 pm), 6th (1:12pm to 2:05pm) or 7th period (2:10 pm to 3:03 pm). The time between the morning and afternoon cortisol collection ranged from 1 hour, 30 minutes to 6 hours, 53 minutes with a mean of 4 hours 40 minutes, SD = 1 hr, 9 minutes. Therefore, each student had at least one hour and 30 minutes between the morning and the afternoon cortisol collection time. Samples were kept on ice until both AM and PM samples were collected and then put in 20-degree Celsius freezer while awaiting shipment to Salimetrics, LLC on dry ice overnight for duplicate analysis. The normal range for AM cortisol in adolescents is .02µg/dl to .88 ug/dl, and normal range for PM cortisol is 0 to .26 µg/dl. The diurnal rhythm is measured as the change from AM to PM (a negative number). Cortisol is expected to be higher in the morning and drop significantly in the afternoon [29]. See Figure 1. Those par-

ticipants who have a less than .01µg/dl change from AM to PM or those that show a rise in cortisol from morning to afternoon are considered to have dysregulated decline in cortisol [30].

Measures

Stressful life events (SLE's): Coddington's (1972) Life Event Scale – Adolescents (CLESA) is a self-report survey used to measure events that affect social adjustment in adolescents aged 13 to 19 years [31]. There are 50 SLE's which include 21 positive and 29 negative SLE's. Results after calculation show a SLE score that represents the number of total events occurring over the last 12 months and the impact on the participant (Life Change Unit -LCU score). Previous researchers that used the CLESA included the following psychometrics: Test-Retest reliability – α = .84 [32]; Interclass correlation - r = .63 [33]; Internal consistency/Reliability – α = .78 [34]. Reliability for the current study was α = .76.

Stressful day*:* Participants were also asked the following question: "Did anything stressful occur today? If, yes, please describe the event.

Perceived stress: Cohen's (1983) Perceived Stress Scale (PSS-10) was used to measure an adolescent's perception or current appraisal of stress over the last month. The scores were 0- never, to 4-very often, with a range of 0 to 40. Previous researchers used the PSS-10 with the following psychometrics: Reliability – α = .85 [35]; Test-Retest Correlation r = .85, [8]; Reliability – α = .81 [36]. Reliability for the current study was α=.87

Analytic Strategy and Approach

Selection of Covariates: Potential covariates were initially identified by past research and theoretical relevance. Previous studies have indicated that gender [37], sexual orientation [38], race [39], and socioeconomic status [40] influence perceptions of stress. Variables that were not significantly related to perceived stress at the bivariate level were not included in further analyses. This approach was used to limit confounding variables, while also reducing potential bias introduced by extraneous variables [41].

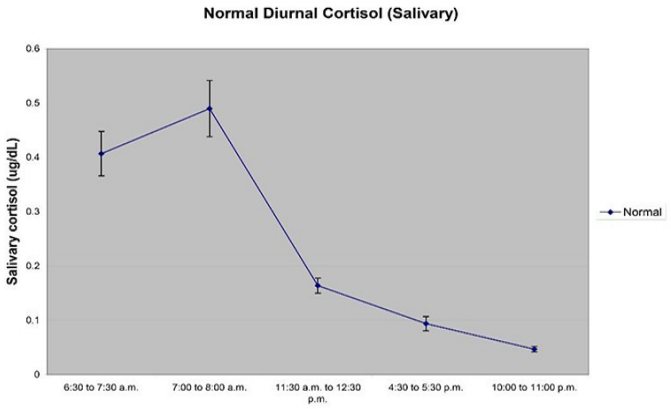
Analytic approach**:** Data was analyzed using SPSS version 23 with Hayes (2013) PROCESS macro (model 1) [42]. Potential covariates were all re-categorized as ordered binary variables. Preliminary analyses included data screening for normality and bivariate correlations. We then conducted a moderated multiple regression with perceived stress entered as a criterion variable, stressful life events entered as a predictor variable, cortisol (0 = normal decline, 1 = dysregulated decline) as a categorical moderator variable, and significant bivariate correlates of perceived stress as covariates. Simple slopes were then used to compare the relationship between stressful life events and perceived stress at normal decline in cortisol and dysregulated decline of cortisol.

Results

After removing five univariate outliers across our measures, indices of normality (skewness and kurtosis) were within acceptable ranges leaving a final sample of *n* = 138 (see Table 1 for demographic breakdown, as well as means and standard deviations). The majority (*n* = 88; 63.8%) had a normal cortisol decline, with a few (*n* = 18; 13%) having a flattened or blunted dysregulated decline (less than .01 ug/dl change from AM to PM), or a cortisol rise from morning to afternoon (*n* = 32; 23.2%; increase in cortisol from morning to afternoon). Thus, we re-categorized our participants into normal decline (*n* = 88) and dysregulated decline (*n* = 50) groups. Bivariate correlations indicated a significant positive relationship between perceived stress and the following variables: stressful life events, gender, and sexual orientation (see table 2 for coefficients).

We then conducted the specified multiple regression with gender, sexual orientation, and "stressful day" entered as covariates in the model. Results indicated a significant effect R^2 = .26, $F(6,131) = 7.75$, $p < .001$ (see Table 3). Specifically, stressful life events significantly predicted perceived stress $\beta = .20$, $t(131) = 2.45$, $p = .02$, with cortisol change from morning to afternoon being unrelated to perceived stress $\beta = -.11$, $t(131) = 1.42$, $p = .16$. However, the interaction between stressful life events and cortisol decline from morning to afternoon was significantly predictive of perceived stress $\beta = .26$, $t(131) = 3.50$, $p < .001$. Notably, the interaction accounted for significant additional variance $\Delta R^2 = .07$, $F(1,131) = 12.23$, $p < .001$. Simple slope analyses indicated an exacerbation effect in those with dysregulated decline resulting in significantly more perceived stress associated with their stressful life events $\beta = 1.32$, $t(131) = 4.57$, $p < .001$, whereas the effect was non-significant for those with cortisol that showed a decline of at least .02 ug/dl from morning to afternoon (normal rhythms) $\beta = -.004$, $t(131) = -.04$, $p = .97$. The interaction is demonstrated in Figure 2

Figure 1: Normal Diurnal Rhythm of Cortisol



Note: Permission to use this figure was granted by Salmimetrics LLC.

Figure 2: Standardized Simple Slopes

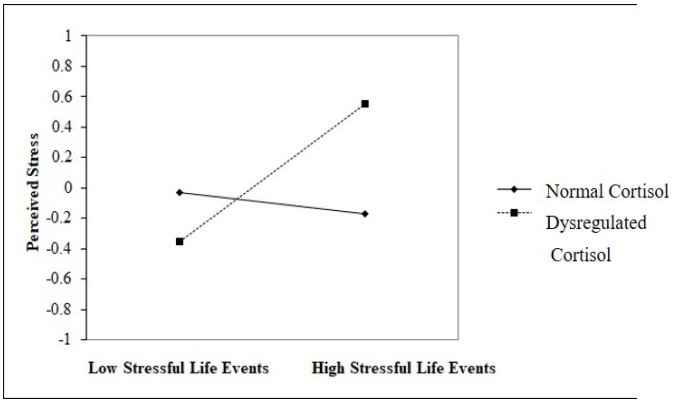


Table 1: Demographics

Demographics	*n* = 138 (%)	*M (SD)*
Gender		
Boys	48 (34.8%)	
Girls	90 (65.2%)	
Race		
White	105 (76.1%)	
Racial/Ethnic Minority	33 (23.9%)	
Sexual Orientation		
Heterosexual	118 (85.5%)	
LGBTQ	20 (14.5%)	
SES		
Self-Pay Lunch	71 (51.4%)	
Free/Reduced Lunch	67 (48.6%)	
Cortisol Diurnal Rhythm		
Normal	88 (63.8%)	
Dysregulated	50 (36.2%)	
Day Stress		
Yes	57 (41.3%)	
No	81 (58.7%)	
Age		14.25 (.51)
PSS		18.85 (7.36)
CLESA		13.01 (7.69)

Note: Day Stress: Was your day stressful? PSS: Perceived Stress Scale; CLESA: Coddington's Life Event Scale – Adolescents

Table 2: Bivariate correlations

Variable	1	2	3	4	5	6	7	8
1. PSS	----							
2. CLESA	.30**	----						
3. Cortisol	-.12	.07	----					
4. Gender	.22**	.04	-.18*	----				
5. Age	-.07	.16	.01	-.08	----			
6. Sexual Orientation	.31**	.25**	-.05	.17*	.08	----		
7. SES	.08	.20*	-.07	-.02	.14	.05	----	
8. Race	-.05	-.07	.04	.04	-.05	-.09	.34**	---
9. Day Stress	.14	.17*	.04	.03	-.01	.07	.04	-.13

Note: PSS: Perceived Stress Scale; CLESA: Coddington's Life Event Scale – Adolescents; Cortisol (0: Normal; 1: Dysregulated), Gender (0: Boys; 1: Girls), Sexual Orientation (0: Heterosexual, 1: LGBTQ), SES (0: Self-pay lunch; 1: Free/Reduced Lunch), Race (0: White, 1: Racial/Ethnic Minority), Day Stress (Was your day stressful? 0: No, 1: Yes)
* $p < .05$. ** $p < .01$.

Table 3: Moderated Multiple Regression Model Predicting Perceived Stress

Predictor	*B*	*S.E.*	*β*	*t*	*p*
Stressful Life Events	.19	.08	.20	2.45	.02
Cortisol	-1.66	1.17	-.11	-1.42	.16
Stress Life Events X Cortisol***	.52	.15	.26	3.50	.001
Gender	2.13	1.19	.14	1.79	.08
Sexual Orientation**	5.36	1.66	.26	3.23	.002
Day Stress	1.80	1.15	.12	1.57	.27

Note: Cortisol (0: Normal; 1: Dysregulated), Gender (0: Boys, 1: Girls), Sexual Orientation (0: Heterosexual, 1: LGBTQ), Day Stress (Was your day stressful? 0 = No, 1 = Yes)
** $p < .01$ *** $p < .001$

Discussion

Researchers conducting the current study sought a better understanding of how stressful life events and the perception of stress affects an adolescent's stress hormone, cortisol. We used screening instruments to examine stressful life events, perceived stress, and cortisol collection in a school setting. Consistent with our hypothesis and the allostatic stress model [7], cortisol dysregulation exacerbated the relationship between stressful life events and perceived stress even when controlling for demographic covariates.

Past examinations have shown that stress over a long period of time impacts cortisol regulation [43,44]. While we explored stressful life events broadly, it is likely that specific stressful life events have differential impacts. For instance, Luthra et al. (2009) indicated that stressors such as physical abuse, sexual abuse, and witnessing domestic violence was associated with PTSD in children, whereas exposure to accidents and disaster were not [45]. Our findings indicate stressful life events are associated with increased perceptions of stress; however, we did not control for the differing types of stressors. Consistent with the allostatic stress model, it is likely that proximity, chronicity, frequency, and intensity of the stressors influence cortisol decline [2] which, as our results indicate, further exacerbates the perceptions of stress. Therefore, it would be beneficial for future researchers to examine how different types of stressors (as well as their chronicity, frequency, and intensity) are associated with changes in the normal cortisol rhythm.

For those working with adolescents in clinics, hospitals, schools, or other settings, it is valuable to understand how stressful life events and the perception of stress can change an adolescent's basic physiological response. Collecting cortisol samples in a school setting is not a common occurrence; however, collecting salivary cortisol samples was feasible when an investigator works closely with the school nurse and school administration. The information obtained from this study provides evidence that certain adolescents may need more careful interaction with their health care providers, school nurses, psychological clinicians, and possibly even youth group leaders and other adults that work with this population regularly. It is particularly important to closely monitor those adolescents who have experienced recent stressful life events and those who there is knowledge of previous experiences with trauma from an early age. Our results also suggest the need to adopt interventions for adolescents that account for disruptions in biological processes. Interventions involving mindfulness techniques and social/emotional learning have already show promising results [46,47]. However, such interventions are not widely employed. Thus, continued development, implementation, and dissemination of evidence-based practices for adolescents exposed to stressful life events are necessary.

Limitations

While this study advances research related to the relationship between stress and cortisol dysregulation in adolescents, it is not without limitations. Primarily, the cross-sectional nature of our analysis precludes causal inferences. Moreover, longitudinal and within-subject designs are preferable for future studies examining the temporal order of effects between stress and

cortisol. Although there are other covariates that could potentially interfere with cortisol such as exercise, menstrual cycle, caffeine, smoking, and drug or food intake [48], for this study, time did not allow us to collect and analyze those additional variables.

While our sample was adequate for our present analysis, we had to combine many of our covariates into binary groups. Such a method is acceptable given sample size limitations, but not preferable given known differences across demographic groups. For instance, recent research has indicated gay and lesbian individuals exhibit notably less mental health symptoms compared to other LGBTQ groups [49]. Given sexual orientation was a significant covariate within the full model, future studies should examine the relationship between cortisol and stress across differing sexual orientation groups (as well as other relevant demographic factors). Another limitation was that only two cortisol samples were collected for this study due to school scheduling of academic courses. Although we collected the self-reported "time of awakening," it was not possible to collect saliva while students were not in the school setting. There were differences noted between the morning and afternoon cortisol collection ($\bar{x}$ = 4 hrs., 40 min, *SD* = 1 hr., 9 min., range = 1 hr., 40 min. to 6 hrs, 52 min) due to the nature of the school setting and the requirement of collecting the samples during a non-academic classroom period. Collecting samples from early morning awakening, 30 minutes after awakening, mid-day, late afternoon, and before bedtime would be the most accurate method to view the cortisol diurnal rhythm. In addition, there were several other covariates that were not examined for this study.

Conclusions

Perceptions of stress are exacerbated by the interaction of stressful life events and disrupted diurnal rhythm in adolescents. This relationship holds even when demographic correlates of perceived stress are controlled. Continued research into how differing types of stressors are related to cortisol dysregulation, and in turn, perceived stress, are warranted. Implementation of interventions designed to address stress-related issues for adolescents may be helpful in correcting dysregulations in diurnal rhythm. Such corrections will likely yield improvements in adolescent academic, social, and emotional functioning.

Statement of Human Rights – All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee at the University of Alabama at Birmingham. The IRB is a committee established under federal regulations for the protection of human subjects in research (45CFR 46). Informed consent was obtained from all participants parent or guardian, and assent was signed by all participants included in this study.

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