Cortisol Awakening Response and Youth Depression: The Impact of Anxiety, Age, and Sex

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Abstract

Though the link between dysregulation of the hypothalamic-pituitary-adrenal axis (HPA axis) and depression is well documented in adults, research with depressed youth is more inconsistent, which may be due to inattention to comorbid anxiety, age and sex. Therefore, this study examined the link between HPA-axis functioning, indexed by the cortisol awakening response (CAR), and depression, examining potential moderation by these covariates. The sample included 109 participants (46% male) between 9 and 16 years, who were administered clinically diagnostic interviews. Out of total sample, 13% (n = 14) had a current or past diagnosis of depression only, and 14% (n = 15) of the sample was comorbid for depression and current anxiety. CAR was measured via salivary cortisol collected by the participants upon waking, and 45 minutes later, across two consecutive weekdays. Results showed that although depression only was linked to heightened CAR, the link was fully mediated through time of cortisol collection. Post Hoc analyses revealed that depressed children woke significantly earlier (M = 6:55am) than any other diagnostic group (comorbid M = 8:39am; controls M = 7:38am). Although main effects of age, sex, and comorbid anxiety were not significantly associated with CAR, there was a significant interaction between age and sex such that age is positively linked to CAR in boys only. Results raise questions as to whether heightened CAR is linked directly to depression or is simply a byproduct of abnormal sleep among depressed youth and suggest that age and sex continue to be examined as covariates in pubertal samples.

Keywords: CAR, youth, childhood, adolescence, depression, HPA-axis, dysregulation, anxiety, age, sex, comorbidity, sleep

Cortisol Awakening Response and Youth Depression: The Impact of Anxiety, Age, and Sex

Depressed children and adolescents are at increased risk for affective disorders in adulthood (Harrington, et al., 1990), and youth depression causes greater life-long impairment than adult depression (Thompson, et al., 2012). Therefore, identifying the mechanism of depression throughout late childhood and adolescence is a high research priority. The link between dysregulation of the body's primary stress regulation system, the hypothalamic-pituitary-adrenal axis (HPA axis), and adult depression is well documented, though research with depressed youth is more inconsistent (Lopez-Duran, 2009; Zitman et al., 2007). One potential explanation of cross-study inconsistency is that previous work has not consistently controlled for anxiety comorbidity (Kleiman & Riskind, 2012) or demographic factors such as age and sex (Platje, et al., 2013; Pruessner, et al., 2008), which all have shown to potentially influence the association between HPA axis functioning and psychopathology. Therefore, this study examines the link between HPA-axis functioning and depression in late childhood (age 9) through adolescence (age 16), focusing on the effects of anxiety comorbidity, age, and sex. Examining these factors may be useful in identifying mechanisms underlying the link between cortisol and depression, and helping to target specific at-risk populations and implement interventions accordingly.

The hypothalamic-pituitary-adrenal (HPA) axis is responsible for modulating a series of bodily responses to stress, which culminate in the release of cortisol by the adrenal glands (Chrousos et al., 2002). Therefore, atypical cortisol release in response to stress may indicate HPA axis dysregulation and may be correlated to the development of stress related psychopathologies like anxiety and depression (Cameron, 2006). One index of the HPA-axis

functioning is the cortisol awakening response (CAR) (Kirschbaum et al., 2000). Humans follow a characteristic pattern of cortisol release throughout the day, with a surge of cortisol upon waking. Typically, cortisol levels are moderately high by the end of the sleeping period and increase until peaking 30–40 min post awakening (Adam et al., 2008; Chida & Steptoe, 2008). Salivary cortisol awakening response is an accessible biomarker providing information consistent with more procedurally difficult measures of HPA axis activity (i.e. plasma cortisol). CAR was selected as a biomarker of HPA axis regulation for this study, instead of using cortisol reactivity, because reactivity may be complicated by other developmental factors in this particular population. That is, the age range of 9-16 encompasses a broad range of cognitive development, and younger participants may have a different understanding or utilize different coping strategies during a particular psycho-social stressor than older children. These developmental differences have been shown to result in differential HPA-axis functioning across various age groups (Shapero & Steinberg, 2013).

The magnitude of the Cortisol Awakening Response is significantly associated with psychological disorders (Chida & Steptoe, 2008). Evidence exists that positively correlates Major Depressive Disorder with higher basal cortisol and CAR levels in adults (Handwerger, 2009). Among late adolescents, higher CAR during an initial visit was correlated with a significantly heightened risk of developing depression by the one-year follow-up visit (Adam et al., 2010). This finding suggests that the CAR is a prospective risk factor, and that elevated CAR may be important to the development of depression (Adam et al., 2010). A subsequent study revealed results are time sensitive, demonstrating that although higher CAR significantly predicts MDEs for up to 2.5 years following cortisol measurement, the predictive power decays over time, becoming non-significant at 2.5 years (Adam et al., 2013). This study also found that

elevated initial CAR assessment did not predict greater prospective major stressful life events, further supporting the existence of heightened CAR as a factor in the etiology of depression (Adam et al., 2013). Recent studies have also demonstrated a link between CAR and concurrent depressive symptoms in adolescents, finding that, similar to adults, heightened CAR is a correlate of depressive symptoms (Nelemens et al., 2013). Furthermore, depressed female adolescents show significantly higher CAR than same age peers (Ulrike et al., 2013). However, beyond these studies, research on the link between with depression and the Cortisol Awakening Response in adolescents is sparse.

Comorbidity between depression and anxiety is extremely common in youth (Kleiman & Riskind, 2012) and thus studying CAR in the context of comorbidity is very important. Along with depression, heightened CAR is an established precursor of anxiety disorders in youth (van Santen, et al., 2010). The association of heightened CAR with both depression and anxiety may be linked to underlying life stress such that abnormal management of stress is related to the development of both depression and anxiety, and increased CAR is positively associated with greater incidence of job and general life stressors (Wust, 2000). Additionally, synergistic effects of comorbidity may lead to further dysregulation of the HPA axis than one disorder alone. A 2004 study by Young et al. investigated the effect of depression and anxiety on ACTH, a precursor to cortisol release, and cortisol levels in response to a social stressor. It was found that only the depression-anxiety comorbid participants had higher ACTH and cortisol than controls, whereas no significant differences were present in the depression only condition. A second study by Cameron et al. (2006) had similar findings: when exposed to a psycho-social laboratory stressor, the depression only condition showed the same cortisol levels as controls, and the comorbid condition showed elevated cortisol. It remains unclear however, whether the link

between atypical CAR and depression is mostly due to the presence of anxiety among depressed youth.

Another potential factor impacting the link between CAR and depression is age. CAR emerges within the first year of life (Stalder et al., 2013), and typically increases over the course of adolescent development (Platje et al., 2013). However, how CAR may develop over childhood and adolescence in the context of depression remains unclear. Research suggests that adolescence is one of the most common periods for the onset of first depressive episode (Andrade et al., 2003; Kessler et al., 2003). Unfortunately, little information exists on the relationship between CAR and age in both normal and depressed individuals before and during puberty. This is important because there may be a development-driven shift in the link between atypical HPA-axis functioning and depression that occurs during puberty (Hankin et al., 2010). Specifically, one study shows that depression is linked to blunted HPA-axis reactivity to laboratory stressors in pre-adolescent kids, and with a hyperactive response in adolescence-similar to adults (Hankin et al., 2010; Ulrike, 2013). Thus it is possible that the link between CAR and depression varies as a function of age between the ages of 9 to 16.

Gender also plays an important role in both the manifestation of depression and the level of cortisol activity. As both depression and anxiety are stress related disorders, sex differences have been attributed to the sexual dimorphism of the HPA axis (Kokras et al., 2012;Young & Korszun, 2010). Women have been shown to be more vulnerable to anxiety and depression, and depressed women generally show greater HPA activation than depressed men (Keyes & Goodman, 2006). CAR specifically is known to be influenced by age and gender (Fries, Dettenborn, & Kirschbaum, 2009), however, how these variables affect CAR in the context of depression is unclear. Additionally, the link between heightened CAR and depression is

significantly stronger for depressed adult females than males (Pruessner et al., 2008). Therefore, we might expect an interaction between sex and depression on CAR. However, though HPA-axis activation is heightened in depressed youth populations, is has not been shown to be influenced by sex (Lopez-Duran et al., 2009) as in adults. In contrast, there is some evidence of a sex effect among youth, especially considering other indices of the HPA-axis. In a study of participants aged 10-12, depressive symptoms were linked to higher CAR only in boys (Dietrich et al., 2013). Another study found an enhanced cortisol response to psychosocial stressor in internalizing males only (Hartman, Hermanns, de Jong, & Ormel, 2013). The heterogeneous effect of sex in late childhood and adolescence may be because dimorphism of the HPA axis begins to develop at the onset of puberty. Thus, further examination of how sex differences affect the relationship between HPA activation and depression across puberty is necessary.

This research aims to study the link between depression and CAR in late childhood and adolescence. In addition, this study aims to examine potential moderators of this link, including comorbid anxiety, age, and sex. It is hypothesized that 1) depressed individuals will have higher CAR compared to non-depressed individuals and that 2) depressed individuals with comorbid anxiety will have higher CAR than subjects with depression or anxiety alone. 2) In regards to age, it is hypothesized that the link between depression and CAR increases by age. 3) It is also hypothesized that sex will moderate the link among CAR and depression primarily among males and 4) sex will moderate the link between depression and age such that sex will have no effect on CAR in depressed individuals before the onset of puberty. However, after the onset, greater CAR will be linked to depression mostly in females.

Method

This study consists of a secondary analysis based on the study titled "RAAD: Research on Adolescents with Anxiety and Depression", led by Dr. Nestor Lopez-Duran. This study evaluated the impact of stress on cognition and affect in children with depression, anxiety, and without mood disorders. We used a comprehensive clinical evaluation to assess for depressive and anxious symptoms and make subsequent diagnoses. Collection of in-lab and at-home saliva samples were used to measure cortisol reactivity, and CAR levels. Laboratory visits also involved a physiological stress task, subsequent cognitive and emotional tasks. The current analyses are based on a subsample of the RAAD data involving the clinical diagnostic data, the at home CAR sampling, and demographic data.

Participants

Participants included 109 children ranging in age from 9-16 years old (M = 12.10, SD=2.16). The sex ratio was 46.3% male (n = 64) to 53.7% (n = 74) female. Of the sample, 78.6% (n = 108) was Caucasian and 21.2% (n = 30) were considered ethnic minorities: 11% of the total sample (n = 15) identified biracial, 4.2% (n = 6) African American, 2.6% (n = 4) Asian, 2.7% (n = 4) Latino, and .9% (n = 1) other. Participants included 109 individuals who had usable CAR data out of the 138 participants in the original study. This subsample deviated significantly from the original 138 in that they were significantly older (M = 12.88, SD = 2.19; $t(245) = 2.80$, $p < .01$), however, neither the sex ratio nor race composition of the CAR subsample was significantly different than the overall sample.

Within the 109 participants in the current analyses, 13% (n = 14) of participants had a current or past diagnosis of Depression only; 10% (n = 11) of participants had a current Anxiety

disorder only, and 14% (n = 15) of the sample was Comorbid for Depression and Anxiety. Sixty-three percent (n = 69) of the CAR subsample served as undiagnosed controls.

Procedure

Clinicians administered diagnostic interviews separately to the caregiver, and then child during the initial intake visit. Both caregiver and child also provided demographic data through the completion of questionnaires. For CAR sampling, participants were asked to collect their own saliva samples at home (using standard Sarstedtt salivettes) during two consecutive weekdays within two weeks after the intake visit. Samples were obtained immediately upon waking and at 45 minutes after waking each day. Participants also kept a detailed log of collection times, unusual events, and food intake. Samples were stored in the participant's home freezer until brought into the lab, when placed in a freezer until assayed at the University Core Area Facility. Text alerts were sent to remind participants to take saliva samples, however the timing of the actual saliva collections was limited by patient report.

Measures

Clinical diagnosis was conducted via the Interview Schedule for Children and Adolescents-Diagnostic Version (ISCA-D), which is an extension of the Interview Schedule for Children and Adolescents (Sherrill & Kovacs, 2000). The ISCAD is a semi structured symptom-based diagnostic interview that involves separate interviews with the child and the parent, with the aim of assessing lifetime psychiatric history and present diagnosis. The interviews were administered by Master's level trained clinicians. Results from all interviews (100%) were discussed during diagnostic supervision group meetings with the PI and clinicians. Diagnosis

was determined via clinical consensus during these meetings based on the DSM-IV Axis 1 criteria. The ISCA-D diagnoses both lifetime and current status (Sherrill & Kovacs, 2000).

The parent of the subject was also given a demographic questionnaire to complete. A basic demographic questionnaire was created for this study to assess gender, age, and relevant background information.

Information regarding the at-home CAR sampling was recorded in an At-home Cortisol Log. Participants were instructed to take samples over the course of two consecutive "school-day" mornings: at bedtime, waking, and 45 minutes post awakening. Participants recorded the previous night's bedtime, wake-time (defined as time fully awake, before getting out of bed), first sample time (within 5 minutes of waking), and second sample time (45 minutes post first sample). Participants were instructed to not eat 45 minutes before or between the morning samples. However if participants ate or another unusual event occurred, this was recorded on the log.

Data Analysis

First, the cortisol data was cleaned. Initial means, kurtosis and skewness indicated the necessity of transforming the data. A Box-Cox Transformation with Lambda = . 26, typically used in cortisol value analysis, was employed (Miller & Plessow, 2013). The data was Windsorized at approximately 2%, meaning that cortisol values in the top 2% were forced into the 98^{th} percentile level.

Descriptive statistics were conducted on psychosocial variables and on CAR levels of each day at waking (CAR1) and 45 minutes post waking (CAR2). Next, tests were performed to predict magnitude of CAR. Specifically, the regressor method in which CAR2 is predicted by CAR1 and other predictors/covariates was employed instead of modeling change scores (CAR2-

CAR1) in order to avoid limitations associated with modeling change scores (Vickers & Altman, 2001). Using collection day as a repeated measure, mixed linear model regressions (SPSS 21) were performed. Mixed modeling was used instead of standard repeated measures ANOVA in order to model the correct covariate structure of the interrelated repeated data (Gueorguieva & Krystal, 2004; Hruschka, Kohrt, & Worthman, 2005). Covariates identified were collection time, age, sex, and the interaction between age and sex. Variables tested for predictive capability on the magnitude of CAR2 included presence of depression, comorbidity, the two way interaction between age and depression, and the three way interaction between age, sex, and depression.

Results

Descriptive Statistics

Descriptive statistics were conducted on several methodological variables in the CAR subsample across collection day. On average, the time of waking was 7:26.25am on day 1 and 7:54.13am on day 2. As participants took samples upon awakening, these times reflect wake-time as well. Mean cortisol levels for CAR sample 1 across both days were .34 ug/dl (*SD* = 0.47) and .49 ug/dl (*SD* = 1.03) for CAR sample 2.

Potential Covariates

Mixed model analysis of methodological and demographic variables including collection time, age, and sex was conducted using day as repeated effect in order to determine potential covariates. In a model adjusted for sample, time, age, sex, and age by sex, CAR sample 1 (β = .08, $t(167.67) = 2.84$, $p < .01$) and Collection Time of samples ($\beta < .00$, $t(160.60) = -4.11$, $p < .00$) were significantly predictive of CAR. Main effects of Sex ($\beta = .09$, $t(91.61) = .64$, $p = .53$) and Age ($\beta = .03$, $t(93.27) = .04$, $p = .97$) were not significantly predictive of CAR, however the

interaction of age and sex was significant (β = .04, t(91.97) = 2.08, p = .04) such that for males only, as age increased, the magnitude of CAR increased. For reference see Table 1. Subsequent analyses are conducted with covariates (collection time, age, sex, and age by sex interaction) and without covariates.

Hypothesis 1: Effect of Depression on CAR with and without covariates

In an adjusted model, controlling for covariates, a diagnosis of current or past depression did not significantly affect CAR in comparison to participants without depression (a group including controls and those with anxiety only; see Table 2; β = .14, t(92.60) = -1.46, p = .15).

In a model without controlling for covariates, there was a main effect of diagnosis on CAR magnitude such that having depression predicted a larger CAR magnitude in comparison to participants without depression (See Table 2; β = .13, t(104.01) = -2.07, p = .04).

Hypothesis 2: Diagnostic Comorbidity on CAR with and without covariates

In an adjusted model controlling for covariates, Comorbid diagnosis did not have a significant effect on CAR when compared to Depressed or Control groups: (β = .19, t(92.86) = 1.82, p = .07); (β = .15, t(91.21) = 1.06, p = .29), respectively.

In a model without controlling for covariates, there was a main effect of Depression, such that having depression predicted a larger CAR magnitude than Comorbid diagnosis (β = .18, t(104.10) = 2.09, p = .04). Again, the impact of Comorbid diagnosis on CAR was not significantly different from the control condition (β = .14, t(105.71) = .85, p = .40). For all results, see Table 3.

Post-hoc analyses of covariates

Since in hypothesis 1 and 2 diagnosis status was associated with CAR only when not controlling for covariates, we explored which covariates may be driving this effect. Post-hoc

analyses revealed significant differences in collection time by participant diagnosis, $F(3, 210) = 6.04$, $p < .00$. The Comorbid condition mean saliva collection time was similar to the Anxiety condition (both having a mean time of 8:39am), which were significantly later than the Depression condition mean time (6:55am), and the Control mean time was in between them (at 7:38am).

Hypothesis 3: Two-way interaction of Age and Depression Diagnosis with and without covariates

In an adjusted mixed model analysis controlling for covariates, the Age by Depression interaction was not significant ($\beta = .07$, $t(91.81) = .28$, $p = .78$). In a model without controlling for covariates, the Age by Depression interaction was still non-significant ($\beta = .07$, $t(98.20) = -.02$, $p = .98$). Therefore, age did not impact the link between depression and CAR. See Table 4 for reference.

Hypothesis 4: Three-way interaction of Sex, Age and Diagnosis with and without covariates

In an adjusted model controlling for covariates, the three-way interaction between Sex, Age, and Depression was not significant ($\beta = .16$, $t(86.02) = -.23$, $p = .82$). In a model not controlling for covariates, the Age by Sex by Depression interaction was still non-significant ($\beta = .16$, $t(94.46) = -.28$, $p = .78$). For reference see Table 5.

Discussion

In this study, we examined the link between depression and CAR in late childhood and adolescence, and how it is moderated by comorbid anxiety, age, and sex. We found that that depression was associated with CAR among late childhood and adolescents. However, because of the correlation between depression and wake-time, the predictive capacity of depression is

likely a function of wake-time. Additionally, comorbid anxiety, age and sex were not found to moderate the link between CAR and depression.

This study found that depression predicted a larger CAR magnitude in a 9-16 year old population. This result fits well with current literature that shows the link between depression and internalizing symptoms in youth (van Santen et al., 2011; Vrshek-Schallhorn, 2013). However, the effect of depression on magnitude of CAR was only significant when considered without covariates, suggesting that one of the covariates may be mediating the CAR-depression link in this group. Specifically, we found that when adjusted for the covariate of time, diagnosis of depression did not have a significant impact on CAR. Post Hoc analyses of collection time showed an interesting relationship between diagnosis and wake-time: the Comorbid condition mean saliva collection time was similar to the Anxiety condition (both having a mean time of 8:39am), which were significantly later than the Depression condition mean time (6:55am), and the Control mean time was in between them (at 7:38am). Due to the correlation between depression and earliest wake-time, we hypothesize that the predictive capacity of depression is likely a function of wake-time. The significant impact of wake-time on CAR has support in current literature. Evidence has suggested that the free cortisol response to awakening is influenced by awakening time in non-clinical adolescents and younger adults (Edwards et al., 2001; Kirschbaum, 2003). Also, recent research has shown total sleep time (TST) as the strongest predictor of awakening cortisol and CAR in young adults, such that lower TST has been associated with greater CAR (Kumari et al., 2009; Vargas et al., 2014). TST is determined by several sleep parameters, including wake-time. Therefore, it is plausible that depressed participants within the study were on average waking earlier and getting less total sleep time, and

producing a heightened CAR based on sleep differences rather than a mechanism intrinsic to the pathology of depression.

This information raises the question: since sleep impacts CAR, is the heightened CAR observed in depression the result of altered sleep in depressed individuals? Additionally, Anxiety and comorbid participants showed very similar mean wake-times, which were very different from the depression wake-time. Therefore, among comorbid participants, it appeared that pathology of anxiety (rather than depression) controlled their sleep schedule. It is interesting to note that sleep abnormality is a criteria in the diagnosis of depression but not anxiety. It is possible that variability in CAR is a function of variability in sleep, and that sleep explains the link between CAR and other constructs such as age, sex, depression, and anxiety.

An alternative theory is that the significant predictive capability of wake-time within this research could be due to the effect of age on wake-time. Within the population studied, there was a significantly greater incidence of depression/comorbidity as age increases, whereas controls are of greater frequency among lower ages ($t(109) = -2.53$, $p = .01$). Children diagnosed with depression or depression with comorbid anxiety were likely to be older ($M = 12.60$, $SD = 2.20$) in comparison to controls ($M = 13.79$, $SD = 1.93$). For instance, the ratio of controls to depressed children is 86%: 14% for 9-12 and 67%: 33% for 13-16 year olds which is significantly different $X^2(N = 111) = 5.19$, $p = .02$. In this way, it is possible that the greater incidence of depression in older participants within the studied sample may be the driving factor in the observed earlier wakeup time (linked to higher CAR) for older participants. A further question is what drives older participants' earlier wake time: the heightened frequency and etiology of depression itself, or high school and other obligations?

Results showed depression predicted a larger CAR magnitude than comorbid diagnosis. Additionally, prediction of CAR in comorbid individuals was not significantly different from the control condition. This is an interesting finding as it is inconsistent with past literature. It is thought that synergistic effects of comorbidity may lead to further dysregulation of the HPA axis than one disorder alone, as several studies have found that comorbid individuals have elevated HPA axis indices, in comparison to depressed individuals whose indices were comparable to controls (Young et al., 2004; Cameron et al., 2006). These studies suggested that the link between atypical CAR and depression was due mostly to the presence of comorbid anxiety in depressed youth. Our findings suggest differently. One explanation of lower CAR for anxious individuals is the presence of higher cortisol baseline values (Dietrich et al., 2013). It is possible that the same mechanism prevented a significantly higher CAR among anxious and comorbid participants. Another alternative is the existence of a nonlinear association between anxiety severity and CAR. A study has found that both high and low severity levels are associated with a lower CAR, compared with intermediate levels of severity linked to higher CAR (Wardenaar et al., 2011).

In this study, the age by depression interaction did not show significant predictive capacity for CAR magnitude. Though CAR has been found to increase over the course of adolescent development (Platje, et al., 2013, Saridjan et al., 2010), the impact of an age by depression interaction during adolescence is largely unclear. We hypothesized that the age by depression interaction would be significant, due to the possibility of a development-driven shift in the link between atypical HPA-axis functioning and depression that occurs during puberty. A factor that may have contributed to the insignificance we found was the age range of this study, 9-16, which included ages much younger than the average adolescent onset. Though recent

research has found a CAR response among infants (Stalder et al., 2013) the relationship between CAR and age though childhood has been largely understudied. The lack of a significant effect of age by depression interaction on CAR magnitude could be caused by the range of physical and psychological development encompassed by the sample. The lack of information about CAR and morning cortisol within clinical populations of this age further limits comparison of these results to the greater field.

When analyzed with and without covariates, the sex by depression interaction did not significantly predict CAR in this study. There is evidence that the link between heightened CAR and depression is significantly stronger for depressed adult females than males (Pruessner, et al., 2008), but this result has not been duplicated in depressed youth populations, where HPA activation has been shown to not be influenced by sex (Lopez-Duran et al., 2009). This may be because sex dimorphism of the HPA axis begins to develop at the onset of puberty, and therefore variance in pubertal status among the sample masked the effect of a sex by depression interaction. This is supported by the existence of a significant effect of age by sex interaction on CAR within the study. Results showed that as male age increased, the magnitude of CAR increased as well. This is reasonable, as other research has suggested a significant influence of sex-specific development on the relationship between Pituitary gland volume and HPA-axis activity and reactivity as indexed by CAR (Kaess et al., 2013). By looking at the Age by Sex interaction, this accounted for the differential impact of puberty by sex that takes place between the ages of 9 to 16. Interestingly, with and without covariates, the age by sex by depression interaction did not have a significant effect on CAR. This may be due to the differential impact depression has on HPA axis in depressed individuals only existing after the onset of puberty.

The study had several limitations. CAR was determined using only two samples per day. More samples would provide more data on the response curve as well as its regulation, which may be useful as the mechanism of CAR remains unclear. The models employed in analysis controlled for wake-up time, but no information was taken on the difference between the participant's typical wake-time schedule and actual wake-up time for the day of the test, which may have impact if abnormal. Future studies with attention to the effect of wake-up time on CAR should also attend to general sleep patterns, as well as circadian phase delay and insomnia- which have demonstrated links to CAR magnitude (Backhaus et al., 2004; Kudielka et al., 2006; Randler & Schaal, 2010). Novel evidence for the impact of light levels upon awakening on CAR suggest a need to control participants' exposure to light upon awakening, which was not attended to in the current project. Also, wake-up and sampling times were self-reported, and sampling was self-administered, and therefore adherence to correct sampling times and procedures could not be guaranteed. It is known that late collection may produce blunted CAR (Dockray et al., 2008; Griefahn & Robens, 2011; Okun et al., 2010) meaning results may reflect compliance to sampling time instead of true HPA axis activity (Clow et al., 2004), though text message alerts to encourage compliance were employed in this study. Information on menstruation and was not recorded. Although some data has suggested that the morning cortisol response is not influenced by menstrual cycle phase (Kirschbaum, 2003), future research would benefit by controlling for this potential confounding factor. Finally, the sample size of the study was relatively small, and replication of results with a larger, more diverse population may be helpful to increase generalizability of results.

These results raise questions about current understanding of the link between depression and CAR, as well as the development and psychosocial significance of CAR. Additionally, the

study suggests late childhood and adolescence is a population of special interest in regards to the link. Unlike in adults, where depression is linked with higher CAR, across late childhood and adolescence depression alone is not a strong predictor of CAR. Also, this study suggests that the impact of depression on CAR may be a function of waking time, as comorbid and anxious individuals woke later than controls, and those with depression woke earliest. Therefore, it is unclear as to whether heightened CAR is a consequence/cause of depression, or is simply a byproduct of abnormal sleep among depressed youth. Overall, this research suggests that wake-time, and possibly other measures of sleep, may have a more important impact on CAR magnitude than previously realized. More research is needed to understand the factors impacting the link between the HPA-axis and depression across late childhood and adolescence, with specific attention to the mediating role of sleep.

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Table 1

CAR Magnitude by Methodological Confounds using Day as Within Subject Effect

Confounds	**Reference**	**Beta** (Standard Error)	***T***	***P***
Time		.00	-4.11	.000**
CAR sample 1		.08	2.84	.005**
Sex	Male	.09	.64	.526
Age	*M* = 12.88	.03	.04	.967
Age X Sex		.04	2.08	.040*

Table 2

Hypothesis 1: Effect of Depression on CAR with and without covariates

Confounds	**Reference**	**Beta** (Standard Error)	***T***	***P***
With Covariates				
CAR sample 1		.23	.36	. 718
Depression	Control	.14	-1.46	.148
Age	*M* = 12.88	.03	-.17	.863
Sex	Male	.09	.61	.543
Age X Sex		.04	2.07	.041
Time		.00	-3.78	.000**
Without Covariates				
CAR sample 1		.08	3.40	.001**
Depression	Control	.13	-2.07	.041*

Table 3

Hypothesis 2: Diagnostic Comorbidity on CAR with and without covariates

Confounds	**Reference**	**Beta** (Standard Error)	***T***	***P***
With Covariates				
Car sample 1		.08	2.82	.005**
Comorbidity	Control	.15	1.063	.290
Age	*M* = 12.88	.03	-.055	.956
Sex	Male	.09	.636	.572
Age X Sex		.04	2.12	.037*
Time		.00	-3.62	.000**
Without Covariates				
CAR sample 1		.08	3.32	.001*
Comorbidity	Control	.14	.85	

Table 4

Hypothesis 3: Two-way interaction of Age and Depression Diagnosis with and without covariates

Confounds	**Reference**	**Beta** (Standard Error)	***t***	***P***
With Covariates				
CAR sample 1		.08	2.78	.006**
Age X Depression		.07	.28	.780
Depression	Control	.16	-1.42	.159
Age	*M* = 12.88	.07	-.32	.764
Sex	Male	.09	.61	.545
Age X Sex		.04	2.01	.048*
Time		.00	-3.78	.000**
Without Covariates				
CAR sample 1		.08	.41	.683
Age X Depression		.07	-.02	.981
Depression	Control	.15	-1.00	.319
Age	*M* = 12.88	.06	.65	.520

Table 5

Hypothesis 4: Three-way interaction of Sex, Age and Diagnosis with and without covariates

Confounds	**Reference**	**Beta** (Standard Error)	***t***	***P***
With Covariates				
CAR sample 1		.08	2.74	.007**
Sex X Age X Depression		.16	-.25	. 822
Sex X Depression		.34	-.40	.688
Age X Depression		.08	.41	.684
Depression	Control	.21	-.72	.477
Age	*M* = 12.88	.08	-.42	.679
Sex	Male	.33	.52	.604
Age X Sex		.16	.70	.485
Time		.00	-3.74	.000**
Without Covariates				
CAR sample 1		.21	3.40	.001**
Sex X Age X Depression		.16	-.28	.779
Sex X Depression		.33	-.48	.631
Age X Depression		.08	.32	.754
Depression	Control	.21	-1.12	.264
Age	*M* = 12.88	.08	-.54	.594
Sex	Male	.32	.53	.598
Age X Sex		.16	.81	.421