ACUTE HPA AXIS RESPONSES TO SOCIAL STRESS LONGITUDINALLY PREDICT ADOLESCENT GIRLS’ DEPRESSIVE SYMPTOMS: THE MODERATING ROLE OF SUBJECTIVE STRESS RESPONSES

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A thesis submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Psychology and Neuroscience (Clinical Psychology).

Chapel Hill
2017

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ABSTRACT

Sarah A. Owens: Acute HPA Axis Responses to Social Stress Longitudinally Predict Adolescent Girls’ Depressive Symptoms: The Moderating Role of Subjective Stress Responses
(Under the direction of Mitchell J. Prinstein)

Increases in interpersonal stress and depressive symptoms during adolescence have stimulated greater attention to stress response models of adolescent depression, but it remains unclear why only certain adolescents are vulnerable to the depressogenic effects of stress while others are not. The current study examined associations among experiences of interpersonal stress, affective reactivity, and hypothalamic-pituitary-adrenal (HPA) axis reactivity to an in-vivo psychosocial stressor as prospective predictors of depressive symptoms nine months later. Hypotheses were examined with a clinically oversampled group of 109 adolescent girls (aged 12-16) to ensure an examination of the widest possible range of prior life stress. Results indicate that adolescent girls who are most emotionally and physiologically reactive to stress and experience significant social stress are most likely to experience elevated levels of depressive symptoms longitudinally. Findings suggest that it may be critical to examine both physiological and affective stress responses when assessing risk for depression in adolescents.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>The Current Study</td>
<td>6</td>
</tr>
<tr>
<td>METHOD</td>
<td>8</td>
</tr>
<tr>
<td>Participants</td>
<td>8</td>
</tr>
<tr>
<td>Procedure</td>
<td>10</td>
</tr>
<tr>
<td>Measures</td>
<td>10</td>
</tr>
<tr>
<td>Data Analytic Plan</td>
<td>13</td>
</tr>
<tr>
<td>RESULTS</td>
<td>16</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>18</td>
</tr>
<tr>
<td>APPENDIX 1: BIVARIATE ASSOCIATIONS AMONG PRIMARY VARIABLES</td>
<td>25</td>
</tr>
<tr>
<td>APPENDIX 2: LONGITUDINAL PREDICTION OF DEPRESSIVE SYMPTOMS BY CHANGE IN POSITIVE AFFECT, CORTISOL REACTIVITY, AND INTERPERSONAL STRESS</td>
<td>26</td>
</tr>
<tr>
<td>APPENDIX 3: MEAN PLOT ILLUSTRATING THE INTERACTION OF CORTISOL REACTIVITY AND CHANGE IN POSITIVE AFFECT AT HIGH LEVELS OF INTERPERSONAL STRESS</td>
<td>27</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>28</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1 - Bivariate associations among primary variables………………………………………23

Table 2 - Longitudinal prediction of depressive symptoms by change in positive affect, cortisol reactivity, and interpersonal stress……………………………………24
LIST OF FIGURES

Figure 1 - Mean plot illustrating the interaction of cortisol reactivity and change in positive affect at high levels of interpersonal stress. .......................................................... 25
INTRODUCTION

Parallel increases in interpersonal stress and depressive symptoms during adolescence have stimulated greater attention to stress response models of adolescent depression. Stress, an individual’s biological and psychological response to challenge, consistently has been associated with major depressive disorder (Hammen, 2005; Kessler, 1997; Mazure, 1998; Monroe, Slavich, & Georgiades, 2008; Paykel, 2003; Tennant, 2002). Compared to healthy individuals, depressed patients are more than twice as likely to have experienced an instance of severe stress, and more than 80% of depressed individuals experience a severe stressor prior to depression onset (Mazure, 1998). Not all types of stressors have been prospectively associated with depressive symptoms, however. Interpersonal stressors, challenges that impact an individual’s relationships or stem from social interactions, predict the development of depression more robustly than non-interpersonal stressors (e.g. O’Neill, Cohen, Tolpin, & Gunthert, 2004; Rudolph et al., 2000). This association between interpersonal stress and depression may help explain the substantial increase in the prevalence of depression in adolescence, and the upsurge in depression prevalence for girls in particular. Over the course of adolescence, there is a marked increase in experiences of interpersonal stress (Ge, Lorenz, Conger, & Elder, 1994), with girls reporting more interpersonal stress than boys during this period (Rudolph & Hammen, 1999; Shih, Eberhart, Hammen, & Brennan, 2006). During the same period, the prevalence of depression more than doubles from 4.5% to 10% (Avenevoli, Swendsen, He, Burstein, & Merikangas, 2015). Although rates of depression increase for both girls and boys during adolescence, the gender difference in prevalence rates becomes pronounced between the ages of 11 and 15, with
lifetime prevalence rates of 14.9% for adolescent girls and 7.3% for adolescent boys (Avenevoli et al., 2015). While increases in experiences of interpersonal stress in adolescence may help explain the corresponding increase in depression prevalence, it remains unclear why certain adolescents succumb to the depressogenic effects of stress while others do not. To reduce the public health burden of depression, it is necessary to clarify how and for whom stress confers vulnerability for depression in adolescence.

There is robust evidence to suggest that stress may confer risk for depression through alterations to the hypothalamic-pituitary-adrenal (HPA) axis stress response (Guerry & Hastings, 2011; Hankin & Abela, 2005). In the face of normative experiences of stress, the HPA axis releases cortisol to facilitate the mobilization of energy reserves in preparation for a behavioral reaction to potential threats. Among adults, elevated cortisol responses to in-vivo psychosocial stressors have been associated with depressive symptoms and worsened longitudinal trajectories (Chopra et al., 2009; Ehlert, Gaab, & Heinrichs, 2001; Morris & Rao, 2014; Nestler et al., 2002), though several studies find the opposite pattern of effects (Burke, Davis, Otte, & Mohr, 2005). The precise mechanism through which HPA axis dysregulation confers risk for depression is unclear (Pariante & Lightman, 2008).

Few studies have examined the relationship between the physiological stress response and depression in adolescence, and even fewer have utilized in-vivo psychosocial stressors with this population. Additionally, the conclusions that can be drawn from these studies are limited by their cross-sectional designs. Two studies of adolescents have found positive associations between elevated cortisol responsivity to an acute stressor and depressive symptoms (Hankin, Badanes, Abela, & Watamura, 2010; Rao, Hammen, Ortiz, Chen, & Poland, 2008), while one study found adolescents with moderate to severe depression to have blunted cortisol responses...
compared to healthy controls (Harkness, Stewart, & Wynne-Edwards, 2011). While these studies are largely cross-sectional, one longitudinal study with slightly younger children (M age = 9.46 years) found that elevated levels of interpersonal stress and heightened anticipatory cortisol responses to an in-vivo social stressor interacted to predict elevated depressive symptoms one year later (Rudolph, Troop-Gordon, & Granger, 2011). Several studies found a positive association between cortisol reactivity and depression only in adolescent females, implicating HPA axis dysregulation as a potential mechanism for the sex differences in depression prevalence that emerge in adolescence (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Natsuaki et al., 2009). Due to the lack of longitudinal studies with adolescent samples, it is not possible to determine from these results whether altered cortisol responses to stress represent a risk factor for or a byproduct of depressive symptoms. This study will build on this literature by prospectively examining how patterns of HPA axis response to an in-vivo psychosocial stressor may confer risk for later depression even after controlling for baseline depressive symptoms in an adolescent sample. Moreover, the association of both blunted and elevated cortisol reactivity with depressive symptoms highlights the need for an examination of factors that might moderate the relationship between HPA axis stress responses and negative outcomes.

While cortisol output has been routinely examined as a reliable marker of physiological arousal in response to stress, physiological arousal is only one component of the stress response. As Ursin and Eriksen (2004) highlight, the subjective affective experience of a stressor is an integral part of the initial stress response and may also interact with physiological arousal in a feedback loop to attenuate, exacerbate, or prolong the stress response as a whole. In this way, an individual’s affective reactivity (their change in mood following exposure to a stressor) may
moderate the relationship between the physiological stress response and depression. There is substantial evidence to suggest that elevated affective reactivity, large increases in negative affect or large decreases in positive affect in response to a stressor, may confer risk for later depression. A meta-analysis comparing affective reactivity to psychosocial stressors between individuals with Major Depressive Disorder (MDD) and healthy controls found depressed individuals have greater affective reactivity (Burke et al., 2005). While another meta-analysis found depressed individuals to have blunted negative affective reactivity, nearly all of the cited studies used negative images like those from the International Affective Picture System (IAPS) or emotionally salient film clips rather than in-vivo psychosocial stressors to elicit affective responses (Bylsma, Morris, & Rottenberg, 2008). These image-based mood induction paradigms may not generalize to adolescents’ experiences of interpersonal stress, and do not elicit the same physiological responses as tasks involving more ecologically valid experiences of social-evaluative threat (Dickerson & Kemeny, 2004). While the majority of studies in this area have been cross-sectional, there is preliminary support for the prospective association of heightened affective reactivity with later depressive symptoms. One study of young adults found greater increases in negative affect during a venipuncture to be prospectively associated with mood disorder symptoms (McLaughlin et al., 2010), while another found elevated negative affective reactivity to mediate the relationship between interpersonal stress and depression (Charbonneau, Mezulis, & Hyde, 2009).

Despite evidence that positive affect may attenuate the physiological stress response (Tugade & Fredrickson, 2004), fewer studies have examined the impact of stress-responsive changes in positive affect on depressive symptoms. Furthermore, there is some empirical support for differences in positive affective reactivity in depression. Elevated variability in positive affect
has been associated with depressive symptoms, and individuals with past depressive episodes demonstrate greater decreases in positive affect on stressful days than healthy controls (Gruber, Kogan, Quoidbach, & Mauss, 2013; O’Grady, Tennen, & Armeli, 2010). One prospective study by O’Neill et al. (2004) found both decreased positive affect and increased negative affect in response to daily interpersonal stressors to predict an increase in depressive symptoms in college students. This evidence highlights the importance of examining the moderating role of both positive and negative affective reactivity to clarify the relationship between the stress response and later depression.

Notably, the majority of studies exploring stress responses fail to examine the combined effects of affective and physiological responses to stress, resting on the assumption that heightened affective reactivity is merely a proxy for heightened cortisol reactivity and vice versa. Although the physiological and affective stress responses are often assumed to be tightly coupled, there is a dearth of research experimentally examining the association (Mauss, Levenson, McCarter, Wilhelm, & Gross, 2005). A systematic review of studies utilizing the Trier Social Stress Test with adult samples found that approximately 40% reported no measure of subjective experience. Only 27% of studies that did report measures of subjective experience and salivary cortisol found significant correlations (Campbell & Ehlert, 2012). Given the mixed evidence for this association, it is important to examine both the unique and interactive effects of affective and physiological responses to stress on later depressive symptoms.

Finally, it is critical to examine not only physiological and affective responses to in-vivo psychosocial stressors that occur in the lab, but also actual experiences of interpersonal stressors that occur in individuals’ day to day lives. While individuals may have physiological and affective stress responses that confer risk for depression, in the absence of highly stressful
interpersonal experiences, these stress responses may not have notable effects on functioning. In order to predict the development of depressive symptoms from these experiences, the measurement of stress must not be confounded with the individual’s depressive symptoms or biased by negative mood states. Many studies rely on subjective ratings of stress from life event checklists, which may be biased by the current mood of the reporter and may lack the contextual detail necessary for measuring fine-grained differences in the severity of stressors (Hammen, 2005). There is substantial evidence to suggest that interviews which use contextual information to generate objective ratings of stress severity result in less recall bias and mood bias than checklist methods (e.g. McQuaid, Monroe, Roberts, Kupfer, & Frank, 2000). In order to assess the relationship between stress responses and later depression, it is critical to measure experiences of stress in the intervening period as precisely and objectively as possible. This study builds on prior literature by measuring not only responses to an in-vivo psychosocial stressor, but also objective ratings of experiences of interpersonal stress over a nine-month period using a semi-structured interview.

The Current Study

This study aimed to build on the current knowledge of the association between HPA axis responses to stress and depression by examining the relationship between affective and physiological reactivity to stress and prospective longitudinal outcomes. The current study examined the role of associations among actual experiences of interpersonal stress, affective reactivity, and HPA axis reactivity to an acute, in-vivo psychosocial stressor in the development of depressive symptoms over a nine-month span. Given the marked increase in stress during the adolescent transition and corresponding increase in depression prevalence, hypotheses were
examined with a clinically oversampled group of adolescent girls to ensure an examination of the widest possible range of prior life stress.

In keeping with prior research by O’Neill et al. (2004) and cross-sectional studies of depressed adolescents’ cortisol responses to stress, we predicted that, in the context of significant interpersonal life stress, individuals who experienced elevated affective reactivity and elevated cortisol reactivity would experience more deleterious outcomes than those who had blunted or elevated patterns of cortisol response alone. Although prior studies have focused on negative affective responses to stress, we also predicted that large decreases in positive affect in response to stress would interact with elevated cortisol reactivity to predict worsened depressive symptoms at nine months in the context of significant interpersonal stress, given the literature suggesting that positive affect may buffer against the deleterious effects of stress.
METHOD

Participants

Recruited participants included 220 female adolescents between the ages of 12 and 16 ($M_{age} = 14.13$ years, $SD = 1.40$). Participants were recruited from a wide range of community and clinical placements, including inpatient psychiatric units, outpatient mental health agencies, high schools, and the local community via flyers, radio, and mass e-mail advertisements. Inclusion criteria for the study included (a) female gender, (b) baseline age between 12 and 16, (c) caregiver available to participate, and (d) mental health concerns (e.g. mood and adjustment disorders, substance use, disruptive behavior disorders) in the prior two years. A qualifying history of mental health concerns was determined based on parent report of their adolescent’s prior diagnosis or treatment, or a brief screening interview (KSADS) administered by a trained researcher. Adolescents were excluded for current psychosis, intellectual disability, and pervasive developmental disorders. Approximately 63.6% of participants identified as Caucasian, 23% as African-American, 2.1% as Latino American, 1.7% as Asian-American, and 9.6% identified as multi-racial or belonging to another group. At baseline, approximately half of the adolescents lived with two parents or caregivers, while the remainder reported living in a single-parent household. Approximately 57% reported current medication use, including antidepressants, stimulants, antipsychotics, antihistamines, antibiotics, anxiolytics, anticonvulsants, and hormonal birth control.

A total of 199 (90.5%) of these participants were available for follow-up phone call assessment of depressive symptoms 9 months later at Time 2, and 146 (73.4%) of these
participants were available for the Life Stress Interview follow-up assessment at Time 2. No significant differences were revealed for any of the constructs measured in this study between adolescents who participated in both time points and adolescents who did not participate in follow-up assessments at Time 2. No significant differences were revealed for any of the constructs measured in this study between adolescents who had complete data and adolescents with missing data, with one exception. Adolescents who were missing data for baseline positive affect (n = 7) had lower levels of interpersonal stress, $M = -0.31$, $SD = 0.23$, than adolescents who had data for baseline positive affect, $M = 0.02$, $SD = 0.40$; $t(120) = -2.12$, $p < .05$, $d = 1.01$.

Preliminary analyses revealed that adolescents who reported taking oral contraceptives at baseline had significantly blunted cortisol responses to the stress task, $M = -0.63$, $SD = 0.78$, compared to adolescents who were not taking oral contraceptives, $M = 0.10$, $SD = 1.02$; $t(204) = 4.52$, $p < .001$, even after controlling for baseline levels of depression and experiences of interpersonal stress, consistent with prior work (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009; Kirschbaum, Pirke, & Hellhammer, 1995). Thus, adolescents who reported using oral contraceptives ($n = 18$) were excluded from further analyses. In addition, participants were excluded if data regarding oral contraceptive use were missing ($n = 1$) to provide a conservative test for analyses. The rationale for eliminating girls on the basis of oral contraceptive use is as follows. First, oral contraceptive use leads to low, stable levels of estradiol and progesterone among OC users (Fleischman, Navarrete, & Fessler, 2010). Second, progesterone naturally potentiates the HPA stress response. Experimental suppression of ovarian function using a GnRH agonist leads to blunted cortisol responses to stress, an effect which is reversed by addback of progesterone (Roca et al., 2003). Therefore, taken together, it can be expected that
OCs will suppress HPA axis output by preventing ovulation-related progesterone production. Thus, oral contraceptive users were excluded, and the final sample size for all analyses was 110.

Procedure

Participants attended the baseline visit with a caregiver. During the baseline visit, participants completed a series of self-report questionnaires (e.g. demographics, depressive symptoms). Approximately three hours after arrival, participants watched an emotionally neutral film clip before providing an initial saliva sample to ensure that cortisol levels reflected a resting baseline of hypothalamic-pituitary-adrenal axis functioning. Participants then underwent a modified Trier Social Stressor Task (TSST; Kirschbaum, Pirke, & Hellhammer, 1993, see below) and provided additional saliva samples.

Nine months after the baseline visit, participants completed a questionnaire over the phone to assess depressive symptoms (MFQ; Costello & Angold, 1988, see below). On a separate call, also approximately nine months after the baseline visit and generally following the symptom assessment, a different trained researcher conducted a semi-structured phone interview (LSI; Rudolph & Flynn, 2007, see below) with each participant to assess experiences of stress.

Measures

Depressive Symptoms. Depressive symptoms were assessed with the Mood and Feelings Questionnaire (MFQ; Costello & Angold, 1988), a 33-item self-report measure of depressive symptoms in children and adolescents between the ages of 8 and 18. Participants were asked how true (0 = not true, 1 = sometimes true, 2 = mostly true) each statement about depressive symptoms (e.g. “I felt miserable or unhappy”) had been for them in the previous two weeks. Data were analyzed using a mean score of all items, with higher mean scores indicating more
depressive symptoms. The MFQ had high internal consistency across time points (Cronbach’s α = .95 for both baseline and nine months).

*Life Stress.* The Youth Life Stress Interview (LSI; Rudolph & Flynn, 2007), an adapted version of the Child Episodic Life Stress Interview (Rudolph & Hammen, 1999) was used to assess the severity, frequency, and type of participants’ experiences of stress during the nine months between baseline and follow-up. This semi-structured interview was conducted by extensively trained researchers who used probes to gather detailed factual information about the precise timing and contextual features of stressful experiences relating to school and academics, behavioral problems, family relationships, peer relationships, and romantic relationships. The interviewer then provided a detailed narrative of each event and its surrounding context to a team of 3-6 expert raters. These raters used a consensus process to assign an objective stress rating on a 5-point scale to represent how the event would impact a typical adolescent under the same circumstances, with higher scores representing greater stressfulness. Data such as the adolescent’s subjective experience of the stressor were masked to prevent biases in objective ratings. Consensus ratings were also used to categorize each event as interpersonal (affecting an adolescent’s relationship or involving an interaction between the adolescent and another individual) or noninterpersonal. Using these data, a mean interpersonal stress severity score was then calculated for each participant. Events rated 1 (*no negative impact/stress*) were excluded.

To assess reliability, two independent teams of raters double coded 30% of participant interviews. Approximately 30% of cases were randomly selected and re-rated by two independent teams, and high reliability was found for both ratings of episodic stress impact (intraclass correlation coefficient = .95) and interpersonal vs. noninterpersonal event content (Cohen’s *K* = .92).
**Affective Response.** Affect was measured at baseline (approximately 2 hours after arrival to the lab, 50 minutes prior to the stress task) and immediately post-stress task with a modified version of the Positive and Negative Affect Schedule for Children (PANAS-C; Laurent et al., 1999). The original PANAS-C is a 27-item self-report measure assessing negative and positive dimensions of affect in children and adolescents. Participants are asked to rate their present identification with a list of feelings using a scale ranging from not at all (0) to extremely (100). In the modified version used for this study, 12 items were taken from the Negative Affect scale of the PANAS-C (Frightened, Nervous, Afraid, Scared, Mad, Miserable, Gloomy, Lonely, Ashamed, Sad, Guilty, Disgusted) for the purpose of brevity, and two additional items (Annoyed and Angry) were added to better capture what Watson and Clark describe as the “hostility” dimension of affect (Watson & Clark, 1999). Three items were taken from the Positive Affect scale of the PANAS-C to capture basic positive affect (Calm, Happy, Joyful). Positive and Negative Affect scales were kept independent rather than collapsed into a single measure of affective response, as there is substantial evidence to suggest that positive and negative affect are orthogonal constructs rather than ends of a continuum (e.g. Kercher, 1992). Composite scores were created for the Positive and Negative Affect subscales, based on the mean of the item scores, with higher scores representing greater self-reported positive and negative affect respectively. Both factors demonstrated good internal consistency (Cronbach’s $\alpha = .81$ for positive affect, .86 for negative affect).

**HPA Axis Response.** The TSST is a social-evaluative stress task that has been shown to significantly increase salivary cortisol across diverse populations. Approximately three hours after arrival in the laboratory, participants watched an emotionally neutral film clip to ensure that baseline cortisol reflected HPA-axis activity at rest. Participants were then instructed to spend
one minute preparing a three-minute audition speech about why they should be selected to star in a fictional reality show about teens’ ability to form friendships. After the preparation period, participants were led into an adjoining room with an undergraduate male judge and instructed to give the speech while facing a video camera and a screen displaying their live image. Participants were informed that the judge could not answer questions and would be evaluating their audition throughout. Judges were trained to refrain from providing feedback of any kind, but instructed to prompt the participant to continue if she ceased before the three-minute limit.

To measure HPA axis responses to stress, cortisol samples were collected using salivettes 20 minutes after baseline in order to capture cortisol levels at rest, 20 minutes after the start of the TSST to capture cortisol levels pre-stressor, and 20 minutes after the conclusion of the TSST to capture peak cortisol levels. Saliva samples were frozen and stored at -25°C before being transported on dry ice to the Behavioral Endocrinology Laboratory at Pennsylvania State University (Salimetrics, PA) for analysis. Each saliva sample was assayed for cortisol with a 510-k cleared high-sensitivity enzyme immunoassay with a sensitivity range of 0.007 ug/dl to 1.2 ug/dl. All values were log transformed to correct for skew prior to analyses, as is common practice. For a subset of participants (n=30), baseline cortisol was collected using passive drool rather than salivettes. For these participants, baseline cortisol was treated as missing to ensure consistency across cortisol assessments. Missing values were handled using full information maximum likelihood method on M-plus.

Data Analytic Plan

Given the small sample size used in these analyses, several diagnostic tests were conducted to explore the integrity of the analyses. As analyses with small sample sizes have the potential to be disproportionately influenced by single cases, regression diagnostics were
conducted to ensure that no single case exerted undue influence on parameter estimates and to confirm the appropriateness of the proposed model for the data. There was no evidence to suggest that any case had undue influence on parameter estimates; all |DFFIT| statistics and all |DFBetas| were less than 1. The assumption of multicollinearity was assessed using VIF. There did not seem to be any concerning multicollinearity, as all VIF values were below 2. Additionally, all tolerance values are above the cutoff of 0.2, confirming the lack of multicollinearity. The assumption of normality of residuals was examined using a P-P plot and a graph of residuals by percentile. The residuals appeared to be fairly normally distributed, with the exception of Time 2 depressive symptoms. To account for this heteroscedasticity in the dependent variable, all analyses were computed using Huber-White robust standard errors.

Descriptive statistics and correlations (or t values, for gender and medication usage) were computed for all study variables. A logarithmic transformation was applied to cortisol values and interpersonal stress, noninterpersonal stress, positive and negative change in affect, and cortisol reactivity were all centered prior to analysis.

We hypothesized several interaction effects among interpersonal stress, cortisol reactivity, and change in affect would predict adolescents’ depressive symptoms at Time 2. A hierarchical multiple regression analysis was conducted with adolescents’ Time 2 depressive symptoms as the dependent variable (see Table 2). After controlling for adolescents’ baseline depressive symptoms in the first step, corticosteroid use, psychotropic medication use, baseline affect and baseline cortisol level were entered in the second step. Cortisol timing (time between saliva collection and waking) was also entered in this step to account for diurnal fluctuations in cortisol (Lovallo & Thomas, 2000), but did not affect prediction of depressive symptoms and was thus removed from the final model. The three main effects were centered and also entered in
the second step: interpersonal stress, cortisol reactivity (i.e., the difference score between pre-Trier and post-Trier cortisol levels), and change in affect (i.e., the difference score between pre-Trier and post-Trier positive or negative affect). All two-way interactions between the three primary variables of interest were entered in the third step, and a three-way interaction was entered in the fourth step. Positive and negative affect were examined in separate models.
RESULTS

Means and standard deviations for the primary variables of interest are presented in Table 1. Pearson correlations were conducted to examine bivariate associations among primary variables (see Table 1). Cortisol reactivity was not associated with change in negative affect, but was modestly negatively associated with change in positive affect, such that individuals who experienced a greater HPA axis response to the TSST also reported a larger drop in positive affect following the task. Interpersonal stress was positively correlated with depressive symptoms at both time points, as expected. There was a moderate level of stability for depressive symptoms over time.

A hierarchical multiple regression analysis was conducted to examine whether a three-way interaction among stress, cortisol reactivity, and change in affect predicted adolescents’ depressive symptoms at Time 2. Given the high degree of correlation between positive affect and negative affect, regressions were run analyzing the effects of each separately. The same was done for interpersonal stress and noninterpersonal stress, resulting in four regressions total.

After controlling for medication usage and baseline depressive symptoms, the three-way interaction among interpersonal stress, cortisol reactivity, and change in positive affect was statistically significant (see Table 2). At Step 1, Time 1 depressive symptoms explained 22% of the variance in Time 2 depressive symptoms. The addition of baseline positive affect, change in positive affect, baseline cortisol, cortisol reactivity, interpersonal stress, and medication usage to the model in Step 2 did not significantly improve the prediction of depressive symptoms at Time 2, although interpersonal stress did significantly predict depressive symptoms at Time 2. At Step
3, the two-way interaction between change in positive affect and interpersonal stress significantly predicted depressive symptoms at Time 2. With the addition of the three-way interaction, the full model explained 36% of the variance in depressive symptoms at Time 2.

To probe the significant three-way interaction, the effect of cortisol reactivity on depressive symptoms was examined at specific values of interpersonal stress and change in positive affect (Preacher, Curran, & Bauer, 2006). For individuals with significant interpersonal stress (one standard deviation above the mean) and large decreases in positive affect in response to the TSST (one standard deviation below the mean), the simple slope of cortisol reactivity on depressive symptoms was 0.12 and marginally significant (p=0.09). This effect became significant at 1.6 standard deviations below the mean for change in positive affect. Thus, individuals who experience significant interpersonal stress and demonstrate substantial decreases in positive affect in response to acute stress demonstrate more positive relationships between cortisol reactivity and later depressive symptoms, controlling for initial depressive symptoms. In contrast, the slopes for adolescents with low levels of interpersonal stress were not significantly different from zero regardless of change in positive affect. Similarly, for adolescents with low change in positive affect but high levels of interpersonal stress, the slope between cortisol reactivity and Time 2 depressive symptoms was nonsignificant.

Contrary to expectation, after controlling for medication usage and baseline depressive symptoms, none of the interactions among interpersonal stress, cortisol reactivity, and change in negative affect significantly predicted depressive symptoms at nine months, and there were no significant main effects in the model. In keeping with prior literature, noninterpersonal stressful events did not predict depressive symptoms at nine months, nor did noninterpersonal stressful events interact with any of the main predictor variables to predict depressive symptoms.
DISCUSSION

This study fills a critical gap by prospectively examining how elevated affective and physiological responses to an in-vivo psychosocial stressor interact with experiences of significant interpersonal stress to confer risk for later depression, even after controlling for baseline depressive symptoms. The majority of research associating stressful interpersonal experiences with depression has focused on the role of HPA axis dysregulation, but few studies have used HPA axis responses to in-vivo stressors to prospectively predict changes in depressive symptoms. Furthermore, most examine the impact of the HPA axis stress response in isolation, despite evidence that affective stress responses may ameliorate or exacerbate HPA axis responses to stress. These results indicate that adolescent girls who are most emotionally and physiologically reactive to stress and experience significant social stress are most likely to experience elevated levels of depressive symptoms longitudinally.

In particular, girls who experienced large decreases in positive affect (i.e. calmness, happiness, and joyfulness) and large increases in cortisol in response to an interpersonal stressor had the largest increases in depressive symptoms nine months later, but only if they also experienced a high average level of interpersonal stress in that time period. Notably, no effect was found for changes in negative affect in response to stress. Additionally, in keeping with prior literature, experiences of noninterpersonal stress did not predict changes in depressed mood, highlighting the importance of social stressors in particular in the etiology of depression.

While previous research has often assumed that elevated or blunted HPA axis responses to interpersonal stress confer risk for depression, these findings clarify that it may be necessary
to examine the affective response as well to determine who is at risk. A study by Moons, Eisenberger, and Taylor (2010) found that different affective responses to stress were associated with different profiles of physiological response, with some affective responses corresponding to increases in cortisol but not increases in proinflammatory cytokines, and vice versa. Given research demonstrating that HPA hyperreactivity may lead to depression by moderating inflammatory processes (e.g. Pariante & Lightman, 2008; Slavich & Irwin, 2014), affect may be essential to determine the conditions under which HPA axis hyperreactivity may be relevant to the longitudinal prediction of depression. The girls in our study who experienced heightened cortisol responses to stress and large decreases in positive affect may have been most vulnerable to heightened depressive symptoms longitudinally due to their unique profiles of proinflammatory response, moderated by their HPA axis and affective responses. While this study did not measure immune markers, these results underscore the importance of examining affective responses as moderators of physiological stress responses. Future research should explore how affective responses, HPA axis responses, and immune responses to stress might dynamically interact to confer risk for depression.

This study is among the first to identify positive affective reactivity to a stressor as a novel vulnerability factor for depressive symptoms via its impact on the physiological stress response. These findings are consistent with the broaden-and-build theory posited by Fredrickson (2001), which posits that the sustenance of positive affect under conditions of stress facilitates adaptive coping in part through the attenuation of physiological stress responses (Tugade & Fredrickson, 2004). This study builds on cross-sectional literature associating positive affective variability with depressive symptoms (Gruber et al., 2013), and studies finding that those with a history of depression demonstrate larger decreases in positive affect on high stress days
compared to healthy controls (O’Hara, Armeli, Boynton, & Tennen, 2014). Susceptibility to fluctuations in positive affect may leave individuals less capable of flexibly and adaptively responding to stress, while the maintenance of positive emotion under conditions of stress may contribute to effective emotional regulation and the subsequent downregulation of physiological stress responses. One prior study found that decreases in positive affect in response to interpersonal stress were associated with greater endorsement of disengagement and elevated substance use as strategies for coping with stress, while changes in negative affect were not (O’Neill et al., 2004). Given these associations, large decreases in positive affect in response to stress may reflect the use of maladaptive coping strategies, which may exacerbate negative effects of high physiological reactivity to interpersonal stress. Furthermore, the use of maladaptive coping strategies in response to interpersonal stressors may reflect limited social support seeking and limited social problem-solving skills, which may contribute to increases in experiences of interpersonal stress. Given that affective responses were not assessed throughout the stress induction paradigm, it cannot be concluded whether the affective responses recorded preceded the physiological stress responses and heightened them, proceeded from the physiological responses, or interacted with the physiological stress responses in a dynamic feedback loop. Furthermore, the conclusions that can be drawn from this study about the mechanisms by which the affective response contributed to elevated risk for depression are limited by the fact that the use of coping strategies were not measured. Further research should examine how affective responses to stress might interact with adaptive and maladaptive coping strategies to ameliorate or exacerbate the negative effects of stress.

Additionally, blunted positive affect can be considered within the RDoC framework as reduced responsiveness to reward and a reduction in appetitive functioning. While this study is
limited by the fact that we did not assess for reward motivation, it is possible that elevated
cortisol responses to stress coupled with a large decrease in positive affect represents an inability
to sustain positive anticipation of reward under conditions of stress, and that this difference in
reward processing confers increased risk for depression. This is in keeping with research that
finds depressed individuals to have diminished responses to anticipated reward (McFarland &
Klein, 2009).

Contrary to expectation, we did not find the interaction between negative affective
reactivity and cortisol reactivity to the stressor to predict later depressive symptoms at any level
of mean interpersonal stress. Prior research examining the association between negative affective
reactivity and depression has been largely cross-sectional and mixed, with some studies finding
no difference in negative affective reactivity between depressed individuals and healthy controls
(Croes, Merz, & Netter, 1993; Gotthardt et al., 1995; Morris, Rao, Wang, & Garber, 2014;
O’Grady et al., 2010), some finding increased negative affective reactivity in depressed
individuals (Husky, Mazure, Maciejewski, & Swendsen, 2009; van Winkel et al., 2015; Young,
Lopez, Murphy-Weinberg, Watson, & Akil, 2000), and some finding reduced affective reactivity
in depressed individuals (Peeters, Nicolson, Berkhof, Delespaull, & deVries, 2003). The null
findings for negative affect may be due in part to the fact that there was less variance in negative
affective reactivity (SD=14.41) than positive affective reactivity (SD=27.48), making an effect
more challenging to detect. Further research is needed to clarify whether there are differences in
negative affective reactivity for depressed individuals, and whether differences in negative
affective reactivity represent a risk factor for depressive symptoms.

Our finding that interpersonal stressors rather than noninterpersonal stressors were
associated with depression longitudinally highlights the relevance of negative social interactions
in the etiology of depression, and underscores the importance of interpersonal experiences around the adolescent transition in particular. Numerous studies have demonstrated that experiences of interpersonal stress, like social loss and rejection, predict the onset of depression more than non-interpersonal stressful experiences (Hammen, 2005; Slavich, O’Donovan, Epel, & Kemeny, 2010; Slavich, Thornton, Torres, Monroe, & Gotlib, 2009). It is unclear from these findings whether the increasing importance of interpersonal experiences during the adolescent transition might elevate the impact of interpersonal stressors on adolescent mental health, and future research should assess whether age moderates these effects. Additionally, as there is evidence to suggest that girls experience more interpersonal stress than boys and report greater emotional responses to this stress (Rudolph, 2002), future research should examine whether there are gender differences in the association between interpersonal stress, physiological stress responses, and affective stress responses.

Continued study of the impact of interpersonal stressors on later depressive symptoms would benefit from the inclusion of more comprehensive assessment approaches. While the rigorous coding of the Youth Life Stress Interview provides objective consensus ratings of the stressfulness of each reported life event, it is still possible that participants’ reporting of the events might have been somewhat influenced by their depressive symptoms at the nine-month follow-up. The highly structured interview is conducted during a phone call several days apart from the assessment used to determine depressive symptom severity at nine months to facilitate the independence of these reports; however, it should be noted that the proximity between these two calls prevented interviewers from ensuring their complete independence. In order to further limit the effects of any potential reporting bias due to mood symptoms at nine months, all analyses were conducted using mean stress severity scores rather than sum scores. Analyses
therefore exclusively utilized objective rater-assigned severity scores, preventing any potential artificial inflation of scores due to over-reporting of stressful events by depressed individuals. Future research would benefit from the inclusion of experience sampling methods to allow for additional fine-grained examination of interpersonally stressful experiences. Additionally, the inclusion of experience sampling methods would allow for an examination of affective responses to stressful experiences in daily life. While it is likely that the Trier Social Stress Task does not generalize to every experience of interpersonal stress that adolescents experience, the use of an in-vivo psychosocial stress task to elicit physiological and affective stress responses and prospectively predict longitudinal outcomes represents a novel addition to the adolescent depression literature, and a marked improvement in methodological rigor over cross-sectional and self-report designs. Future research should examine whether these associations hold in predicting more short-term changes in depressive symptoms, and whether this interaction remains predictive over years. Additionally, although examining interactions with adrenal hormones and menstrual cycle hormone fluctuations is beyond the scope of this study, there is some evidence to suggest that the hypothalamic-pituitary-gonadal axis is also stress responsive and interacts with HPA axis activity. Future research should examine how HPA axis responses to stress might change with the pubertal transition, and experimentally examine the associations of HPA-HPG axis crosstalk with longitudinal outcomes.

Overall, the results of this study offer compelling evidence to suggest that the interplay of subjective and physiological stress responses to interpersonal stressors may predict the development of depressive symptoms in adolescent girls. These findings highlight the importance of examining affective and physiological reactivity together as interactive components of a dynamic system, rather than considering them to be redundant measures of a
categorical stress response. While clinical work may eventually use biomarkers to assess risk for depression, this research highlights the importance of placing such biomarkers in the context of subjective emotional experiences.
## APPENDIX 1: BIVARIATE ASSOCIATIONS AMONG PRIMARY VARIABLES

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<tr>
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<th>1</th>
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<td>1. Depressive Symptoms</td>
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<td>2. Baseline Positive Affect</td>
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<td>4. Baseline Negative Affect</td>
<td>.26**</td>
<td>-.23*</td>
<td>.10</td>
<td>-</td>
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<td>.17</td>
<td>-.46**</td>
<td>-.09</td>
<td>-</td>
<td></td>
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<td>6. Baseline Cortisol</td>
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<td>-.08</td>
<td>.01</td>
<td>-.02</td>
<td>.07</td>
<td>-</td>
<td></td>
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<td>7. Δ Cortisol</td>
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<td>.05</td>
<td>-.24**</td>
<td>-.02</td>
<td>.06</td>
<td>-.25**</td>
<td>-</td>
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<td>8. Mean Interpersonal Stress</td>
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<td>.09</td>
<td>-.02</td>
<td>.14</td>
<td>-.03</td>
<td>-.18</td>
<td>-</td>
<td></td>
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<tr>
<td>9. Mean Noninterpersonal Stress</td>
<td></td>
<td>.31**</td>
<td>.01</td>
<td>.01</td>
<td>.12</td>
<td>.10</td>
<td>-.14</td>
<td>.34**</td>
<td>-</td>
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<td>Time 2</td>
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<td></td>
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<td>10. Depressive Symptoms</td>
<td>.47**</td>
<td>-.04</td>
<td>-.17</td>
<td>.12</td>
<td>.03</td>
<td>.11</td>
<td>.04</td>
<td>.29**</td>
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<td>Means</td>
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<td>-34.17</td>
<td>5.05</td>
<td>10.50</td>
<td>-.94</td>
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<td>Standard Deviations</td>
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<td>25.37</td>
<td>25.38</td>
<td>5.70</td>
<td>12.57</td>
<td>.21</td>
<td>1.06</td>
<td>.40</td>
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<td>.37</td>
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</table>

* p < .05; ** p < .01; *** p < .001
APPENDIX 2: LONGITUDINAL PREDICTION OF DEPRESSIVE SYMPTOMS BY CHANGE IN POSITIVE AFFECT, CORTISOL REACTIVITY, AND INTERPERSONAL STRESS

Time 2 Depressive Symptoms (MFQ)

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<tr>
<th>Predictors</th>
<th>Step Statistics</th>
<th>Final Statistics</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ΔR²</td>
<td>b (se b)</td>
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<td>Step 1</td>
<td></td>
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<tr>
<td>Time 1 MFQ</td>
<td>.221***</td>
<td></td>
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<tr>
<td>Step 2</td>
<td>.069</td>
<td></td>
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<tr>
<td>Baseline Positive Affect</td>
<td>-.001 (.01)</td>
<td>-.002 (.001)</td>
</tr>
<tr>
<td>Δ Positive Affect</td>
<td>-.002 (.002)</td>
<td>-.003 (.001)*</td>
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<tr>
<td>Baseline Cortisol</td>
<td>.210 (.158)</td>
<td>.194 (.151)</td>
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<tr>
<td>Δ Cortisol</td>
<td>.033 (.031)</td>
<td>.054 (.029)</td>
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<tr>
<td>Mean Interpersonal Stress</td>
<td>.177 (.088)*</td>
<td>.131 (.087)</td>
</tr>
<tr>
<td>Corticosteroid Use</td>
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<td>-.037 (.124)</td>
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<tr>
<td>Psychotropic Medication Use</td>
<td>.014 (.062)</td>
<td>.047 (.061)</td>
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<tr>
<td>Step 3</td>
<td>.035</td>
<td></td>
</tr>
<tr>
<td>Δ Cortisol x Mean Interpersonal Stress</td>
<td>.009 (.093)</td>
<td>-.087 (.103)</td>
</tr>
<tr>
<td>Δ Cortisol x Δ Positive Affect</td>
<td>-.001 (.001)</td>
<td>-.001 (.001)</td>
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<tr>
<td>Δ Positive Affect x Mean Interpersonal Stress</td>
<td>-.007 (.003)**</td>
<td>-.010 (.003)**</td>
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<tr>
<td>Step 4</td>
<td>.032*</td>
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<tr>
<td>Δ Cortisol x Δ Positive Affect x Mean Interpersonal Stress</td>
<td></td>
<td>-.007 (.003)*</td>
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<tr>
<td>Total R²</td>
<td>.357*</td>
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* p < .05; ** p < .01; *** p < .001; MFQ = Depressive Symptoms
APPENDIX 3: MEAN PLOT ILLUSTRATING THE INTERACTION OF CORTISOL REACTIVITY AND CHANGE IN POSITIVE AFFECT AT HIGH LEVELS OF INTERPERSONAL STRESS

High Interpersonal Stress

Depressive Symptoms at 9 Months

Cortisol Reactivity

- High (+1 SD ΔPositive Affect)
- Low (-1 SD ΔPositive Affect)
REFERENCES


McFarland, B. R., & Klein, D. N. (2009). Emotional reactivity in depression: diminished responsiveness to anticipated reward but not to anticipated punishment or to nonreward or avoidance. *Depression and Anxiety, 26*(2), 117–122. https://doi.org/10.1002/da.20513


