

RUNNING HEAD: Adolescent emotion and cortisol

*Transactions among adolescent trait and state emotion
and diurnal and momentary cortisol activity in naturalistic settings*

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Summary

In a community sample of 52 adolescents, multilevel growth curve modeling was utilized to examine whether within-person changes in momentary mood states, and individual differences in trait emotional functioning, were related to adolescent cortisol levels in naturalistic settings. Salivary cortisol levels were measured 7 times a day on 2 typical weekdays in conjunction with diary reports of adolescent mood states. Questionnaire reports of trait emotional functioning (depression, anxiety, and anger) were obtained, as were reports of demographic, developmental, and health control variables. After accounting for the effects of time of day and a wide range of control variables, within-person increases in state negative mood (worry/stress and anger/frustration) were significantly associated with within-person increases in cortisol. When examining trait emotional functioning, adolescents with higher levels of depressive symptoms had slightly lower basal cortisol levels, and adolescents with higher levels of trait anger had a significantly stronger cortisol response to awakening. Several developmental effects were found - adolescents at higher stages of pubertal development had daytime basal cortisol curves that were more elevated, had a steeper diurnal decline, and showed a lesser cortisol awakening response, and cortisol responses to worry/stress increased with age. Cortisol levels were also higher at moments adolescents were alone rather than with others, an effect that declined significantly with age. Results suggest that ongoing transactions occur between adolescents' everyday emotional experiences and their cortisol levels, and that adolescent cortisol activity is modified by age/pubertal stage and by trait emotional functioning.

Key Words: Emotion, HPA axis, Cortisol, Multilevel Modeling, Adolescence, Naturalistic

Introduction

In this study, sophisticated data collection and analytic techniques are employed to examine associations between state and trait emotion and cortisol levels in normal adolescents going about their everyday lives, controlling for the effects of time of day and a wide variety of health and lifestyle factors. Given the relative lack of research on the normal functioning of the hypothalamic-pituitary-adrenal (HPA) axis in adolescents, identifying the extent to which adolescent cortisol levels vary with their mood states and with trait differences in emotional functioning is of considerable normative interest; such findings are also of great relevance to researchers interested in identifying risk factors for the development of emotional pathology.

Consistent associations have been found between the functioning of the HPA axis and the presence of major depression (MDD) in adult populations (Chrousos & Gold, 1992; Yehuda et al., 1996). Alterations in emotional functioning are a primary feature of MDD, and both state and trait affect are associated with HPA axis activity even in healthy adults (Polk et al., 2005). As a result, there has been considerable interest in identifying individual differences in HPA axis activity as a potential risk factor for the development of emotional disorders (Granger et al., 1996; Goodyer, Herbert, & Tamplin, 2003). Rates of onset of depression and other emotional disorders increase dramatically in adolescence (Burke et al., 1990, 1991), and many adult episodes of MDD have their initial symptom onset during this time period (Costello et al., 2002). As a result, adolescence is a critical time period in which to study biopsychosocial processes related to risk for emotional disorder.

Researchers studying associations between HPA axis activity and emotional pathology in adolescence have theorized that adolescents with elevated basal cortisol levels and/or a tendency toward greater HPA reactivity to social and emotional challenges may be at greater risk for the development of internalizing problems (including depression and anxiety disorders) (Granger et al., 1996; Goodyer, Herbert & Altham, 1998). Associations between basal cortisol levels and

depression have however been far less consistent in adolescents than in adults, with some studies finding no association, others finding associations only in particular subgroups or at particular times of day, and still others finding associations between higher basal cortisol levels and depression only when additional factors (such as levels of DHEA) are taken into account (Birmaher & Heydl, 2001; Goodyer et al., 2001; Kaufman et al., 2001; Dahl, 2002; Angold, 2003). In the few adolescent studies examining reactivity, several offer evidence in favor of greater cortisol reactivity as a risk factor for the development of internalizing problems (Granger et al., 1996; Goodyer et al., 2000); others provide more mixed evidence, with associations between internalizing symptoms and reactivity depending on gender and the nature of the stressor (Klimes-Dougan et al., 2001).

Most prior studies of adolescent emotional functioning and cortisol have focused on high-risk samples (Goodyer et al., 2000), clinic-referred samples (Granger et al., 1994, 1996), or community samples selected to fit particular diagnostic profiles (Klimes-Dougan et al., 2001), and have utilized laboratory-based stress paradigms. According to the tenets of developmental psychopathology, however, it is important to understand the normal functioning of emotional, social and biological systems in order to identify their role in the development of psychopathology (Cicchetti & Rogosch, 2002). That is, it is hard to identify what represents “abnormal” or “risky” patterns of cortisol activity when little is known about the typical or expected functioning of the HPA axis in adolescents. Virtually no research exists on the normal responding of the HPA axis to social and emotional challenges experienced by adolescents in their everyday lives (for exceptions see Flinn & England, 1995 and Schneiders et al., 2005). This lack of research on cortisol activity in normal adolescents in their everyday environments is surprising, given that the rapidly changing physical, social, and emotional worlds of adolescents present them (and researchers) with a rich array of social and emotional stressors.

Several prior studies have used an experience sampling approach to measuring cortisol reactivity to everyday events and emotions in adult populations (Nicolson, 1992; van Eck et al., 1996a; Smyth et al., 1997). These studies, in which adults were randomly beeped during their daily lives to provide diary reports of mood followed by salivary cortisol samples, found experiences of stress, distress, and negative emotion to be associated with higher momentary cortisol levels. No published studies, however, have yet taken this approach to studying associations between adolescent emotion and cortisol. There are several advantages to this naturalistic approach: it allows examination of reactivity to events and emotions that actually occur in participants' lives, and thus may have increased salience; it allows simultaneous measurement of basal cortisol rhythms; it allows investigation of individual differences in amount of exposure to stressful events; and provides minimal disruption of typical routines compared to bringing participants to a lab setting (de Vries, 1992). Finally, in adults, individual differences in reactivity to familiar stressors encountered in daily life have been found to be of greater relevance for understanding psychopathology than reactivity to novel, laboratory-based stressors (van Eck et al., 1996b). The downside of the naturalistic study of adolescent cortisol activity is that it presents numerous logistical and statistical challenges, including the necessity of controlling for variability in wake-times, bed-times, menstrual cycles, medication use, and other extraneous factors that may alter HPA axis functioning. These challenges, however, may be at least partially met with sophisticated measurement and modeling approaches.

Normative Patterns and Developmental Trends in Diurnal Cortisol Activity

The neurophysiology of the HPA axis is reviewed in detail in numerous other sources (Kirschbaum & Hellhammer, 1989; Johnson, Karmilaris, Chrousos & Gold, 1992; Lovallo & Thomas, 2000); only a few key facts are reviewed here. Cortisol levels are typically highest in the morning upon awakening, increase 50-60% (on average) in the first 30-40 minutes post-awakening, drop rapidly in the next few hours, and then more slowly to reach a low point around

midnight (Weitzman et al., 1971; Kirschbaum & Hellhammer, 1989; Pruessner et al., 1997).

Approximately 62%-72% of the variation in cortisol levels gathered across the waking day is explained by time of day in adults (Adam & Gunnar, 2001; Adam, 2005). There is, however, significant and potentially meaningful between-person variability in the elevation and slope of the cortisol diurnal rhythm (Smyth et al., 1997; Adam & Gunnar, 2001) and in the size of the cortisol awakening response (Pruessner, 1997; Pruessner, Hellhammer & Kirschbaum, 1999).

Given the changes that occur in emotional, social and biological domains during adolescence (Spear, 2000 a, b; Cicchetti & Rogosch, 2002; Netherton et al., 2004), age and pubertal stage-related trends in both basal cortisol activity and cortisol reactivity to stressors may be important to examine and control. A number of studies suggest that a small increase in basal cortisol levels occurs across mid- to late adolescence, perhaps especially in adolescents girls (Kenny, Gancayo, Heald, & Hung, 1966; Kenny, Preeasambat & Migeon, 1966; Kiess et al., 1995; Lupien et al., 2001; Walker, Walder & Reynolds, 2001; Netherton et al., 2004). To my knowledge there are no prior studies examining developmental trends in the cortisol response to awakening or cortisol reactivity to stressors across the adolescent years.

Measuring Cortisol Dynamics Using Multilevel Growth Curve Modeling

Given a thorough sampling protocol, multiple parameters of cortisol activity can be examined in everyday settings. These include the elevation and slope of the diurnal cortisol curve, the size of the cortisol awakening response (CAR), and cortisol responses to momentary mood states. The data collection and analytic approach used in this paper (repeated measurement of mood and cortisol and use of multilevel growth curve modeling) permit the simultaneous modeling of all of these aspects of ambulatory cortisol activity, along with an examination of how trait emotion and other individual difference variables influence these cortisol parameters.

The advantages of using multilevel growth curve analysis to model cortisol data are described in detail in Adam and Gunnar (2001) and in Hruschka, Kohrt and Worthman (2005). In the context of the current study, these advantages include: added statistical and interpretive power due to use of a within-person, repeated-measures design (Raudenbusch & Liu, 2001); adjustment for within-person and within-day correlations among cortisol levels (see Hruschka et al., 2005); high tolerance for missing data and for within- and between-subject variations in the timing of sampling (an advantage over ANOVA methods); simultaneous modeling of multiple cortisol parameters (elevation, slope, cortisol awakening response); the ability to partition state and trait variation in cortisol levels; and the ability to examine the effects of both time-varying, state covariates (such as mood, or situation at the time of each beep), and non-time-varying, trait, or individual difference covariates (including trait emotional functioning and demographic, developmental and medical control variables) on cortisol levels and diurnal activity.

Using this approach, this study will address the following primary questions: First, are there within-person associations between adolescent mood states and adolescent cortisol levels, controlling for the effects of time of day? More specifically, are adolescent cortisol levels higher during moments they are experiencing higher levels of negative emotion? Second, are individual differences in adolescent basal cortisol activity, the cortisol response to awakening, and cortisol responses to negative mood states related to individual differences in trait emotional functioning? This study will also report briefly on the effects of a variety of demographic and health control variables, and on whether developmental variables (age and pubertal stage) are related to individual differences in adolescents' diurnal cortisol rhythms, responses to awakening, or responses to momentary mood states.

Method

Participants

The data for this study were collected as a follow-up to a multidisciplinary study of child and adolescent functioning in middle-to-high-income two-parent working families (the Sloan Family Study). A small subset of families who had completed the Sloan Family Study were asked if they would like to participate in an additional “Physical Stress Study”, in which we would examine how the stresses of their everyday lives affected their “physical stress and health”. Families were selected because they had a) already completed the Sloan Family Study procedures b) had signed a form indicating an interest in participating in additional research.

Adolescents who participated in the follow-up Stress Study were significantly older [by .67 years; $t(495)=2.71, p=.007$], and in a higher grade level [by .64 grades; $t(492)=2.53, p=.012$]. They lived in homes with a higher family income, $t(316)=2.31, p=.022$ and a higher parent educational status [$t(309)=2.57, p=.011$ for mothers, $t(253)=2.89, p=.004$ for their partners]. Included adolescents were not, however, significantly different from non-participants on racial/ethnic status, levels of depression, anxiety, or anger, parent marital or cohabitational status or levels of parent depression, anxiety, or anger (p 's > .05). Of the adolescents who completed the procedures, two were excluded because their health surveys indicated that they used steroid-based asthma medications and two were excluded because they completed less than 50% of the requested samples, leaving 52 adolescents (28 boys, 24 girls). Although this is a modest sample size, the advantage of a repeated measures approach such the one used in this study is that the statistical power is bolstered not only by the number of participants, but also by the large number of observations obtained for each person (Raudenbush & Liu, 2001).

The characteristics of the participating adolescents on the primary demographic and study variables are noted in Table 1. Gender differences were also tested: There were no significant differences between male and female adolescents on any of the variables except level of pubertal development and body mass. As expected given sex differences in pubertal timing (Peterson, 1988), adolescent girls were significantly more advanced in their stage of pubertal development

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(girls 3.6, boys 3.0; $t(50) = 3.85, p = .000$). Adolescent boys were however significantly higher in body mass than adolescent girls (girls 21.0, boys 23.15; $t(50) = -2.39, p = .021$).

Procedures and Measures.

Participating adolescents completed four types of measures: 1) repeated salivary cortisol samples, which were gathered on two typical weekdays in conjunction with 2) momentary diary reports of everyday events and emotions, 3) adolescent survey self-report of trait emotional functioning (depression, anxiety, and anger expression) and 4) adolescent survey self-report of physical health, health behaviors and medication use. Questionnaire, cortisol and diary data were also collected from the parents of the adolescents; these findings are reported elsewhere (Adam, 2005). Because we had had prior in-person contact with these families, participants were recruited and informed consent was obtained from adolescents and their parents by phone, and materials were sent to and collected from participants by courier.

Diary reports and saliva sampling. This study employed the Experience Sampling Method (ESM; Chikszentmihalyi & Larson, 1987), which involves participants completing diary reports of where they were, who they were with, and what they were thinking and feeling during the course of their everyday lives when prompted to do so by a specially programmed watch. This method has been used extensively in adolescent populations; prior validity studies have shown good response rates for adolescents, with very little systematic bias introduced by non-response (Larson, 1989). The current study expands upon this procedure by having adolescents provide samples of saliva in relation to each diary report, which are later assayed for salivary cortisol.

Adolescents were asked to complete 7 diary-saliva sample pairs across the day from morning to evening on each of two typical, consecutive weekdays. We asked participants to avoid special days such as graduation, birthdays, or days when important tests were scheduled. Participants were given a reminder phone call the evening before their first day of participation, during which the procedures were reviewed in detail, and any questions were answered. We also

inquired about participants' health on the evening before their participation; those with any active illness (e.g. influenza, common cold) were rescheduled for a later date.

On each of the two scheduled weekdays, diary-sample pairs were provided by participants on the following schedule: in the morning *immediately after waking* (participants were instructed to place materials on a bedside table and to take samples "as soon as their eyes opened and before their feet hit the floor"); *40 minutes after waking* (to capture the cortisol response to awakening); *immediately before bedtime*; and *four times during the day* when signaled by a specially programmed watch. The watch signals were semi-random – that is, they were randomly scheduled within four intervals across the day, with intervals chosen to avoid sampling immediately after lunch or dinner hours (Quigley & Yen, 1979). The advantage of these semi-random beeps is that participants are unable to anticipate the timing of those beeps, and are thus more likely to be engaged in the natural flow of their activities. Participants were signaled again to provide a saliva sample 20 minutes after each beeped diary entry, as it takes 20 to 30 minutes after a stressor for cortisol levels to reach their peak in saliva (Kirschbaum & Helhammer, 1989).

In summary, participants were asked to provide 7 diary-sample pairs each day on each of two weekdays, for a total of 14 diary-sample pairs per person. Due to budgetary constraints, electronic monitoring of compliance with these sampling procedures was not possible. All of our written and verbal instructions to participants, however, emphasized that accurate timing of morning samples, and accurate reporting of all sampling times was essential. The reminder phone call to participants the evening before their first day of sampling re-emphasized these details. Studies examining compliance with this type of ambulatory protocol in adults have shown reasonable (>80% of samples completed on time) electronically verified compliance rates (Jacobs et al., 2005).

Salivary cortisol sampling and assay procedures. The saliva sampling procedure involved chewing a stick of Trident[®] gum to stimulate saliva, then expelling the saliva through a small

straw into a sterile cryogenic vial. Participants refrigerated samples in their home refrigerators at the end of each sampling day, a protocol which has been followed in numerous published field studies (e.g. Kertes & Gunnar, 2004; Stetler, Dickerson & Miller, 2004). Although cortisol levels are not affected by the motion and temperature variations accompanying a normal postal trip by regular mail (Clements & Parker, 1998), we had participants return samples to us by courier. Samples were frozen at -20 degrees when they reached our lab, and were later sent on dry ice to Salimetrics (State College, PA), where they were frozen at -70 degrees until assay.

All samples were assayed in duplicate for salivary cortisol by enzyme immunoassay using the Salimetrics LLC salivary cortisol assay (Salimetrics, State College, PA). The test uses 25 μ l of saliva (for each singlet determination), has a range of sensitivity from .007 to 1.8 μ g/dl, and average intra- and inter-assay coefficients of variation were less than 5% and 9% respectively. Due to a strong positive skew in the distribution of the cortisol values, a normalizing natural logarithmic transformation was performed on the cortisol data prior to analysis.

Survey reports of trait emotion. Adolescents completed a survey providing information on their own typical or trait emotional functioning (including symptoms of depression, anxiety, and anger), and a variety of other stable aspects of individual and family functioning. The current study focuses on the adolescent emotional functioning variables.

Depressive symptoms. Adolescent depressive symptoms were measured using the Center of Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977), a 20-item self-administered questionnaire assessing the frequency and severity of depressive symptoms experienced the preceding week. Adolescents indicated how often they experienced cognitive, behavioral, affective and somatic symptoms of depression on a scale from 0 (*rarely or none of the time/ less than day*) to 3 (*most or all of the time/ 5-7 days*) resulting in a scale from 0 to 60 with higher scores reflecting greater distress ($\alpha = .89$). Validity studies suggest that the CES-D is a valid

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measure of depressive symptomatology in community samples (Weissman et al, 1977; Amenson & Lewinsohn, 1981; Roberts & Vernon, 1983).

Anxiety and anger. Adolescent anxiety and anger were examined using the anxiety and anger scales of the Taylor's Measures of Dysphoria, Anxiety, Anger and Self-esteem (Taylor & Tomasic, 1996). Adolescents reported how often they perceived themselves as "feeling on the edge, like something awful is going to happen", "feeling nervous for reasons I can't put my finger on", "having trouble concentrating" and the extent to which they feel that "my anger is unpredictable", "I get more angry than I should" and "I express my anger easily". Adolescents noted the extent to which these statements apply to them on a scale of 0 (*never*) to 4 (*very often*).

Adolescent health survey. Adolescents also reported on a variety of health and lifestyle variables. These included: presence of physical and/or mental illnesses; types of medications used; consumption of caffeine and alcohol; type and frequency of nicotine intake; typical exercise pattern; oral contraceptive use; presence of pregnancy; timing of menstruation in relation to cortisol collection; height and weight (for determination of body mass index, BMI); bedtimes, wake-up times and hours of sleep on the days of testing. Stage of pubertal development was reported using the Pubertal Development Scale (Peterson, 1988). Each of these variables is suspected to have an influence on cortisol levels (see Kirschbaum & Hellhammer, 1989; 1994; Kirschbaum et al., 1999; Kudielka & Kirschbaum, 2002; Netherton et al., 2004). It is therefore important to measure these variables and statistically control for their effects when significant in order for the associations of interest to be properly revealed.

Having a chronic health condition, being pregnant, or taking steroid-based medications for asthma or allergy were exclusion factors for the study. For all other health variables, associations with each of the cortisol parameters were examined, and statistically controlled when significant associations were found. Health or lifestyle variables not significantly associated with cortisol parameters are not included in the final set of models.

Family demographic characteristics. Parents completed a survey reporting on family demographic characteristics as well as a variety of other aspects of individual and family functioning that are not analyzed here. For the purposes of these analyses, mother report of family income, whether the child lived in a traditional two-parent family, and average parent education levels were examined as potential demographic control variables in all analyses, and were retained in the model only if they were found to be significant.

ESM emotion data reduction. The momentary emotion or mood section of the ESM diary asked participants how they felt at the time of the beep, and asked them to rate the extent to which they were experiencing each of 22 different mood states. Most were rated on 4 point unipolar scales ranging from Not at all to Very much, including Cheerful, Lonely, Nervous, Cooperative, Angry, Responsible, Frustrated, Competitive, Strained, Worried, Caring, Irritated, Relaxed, Stressed, Proud, Friendly, Hardworking and Productive. Four of the mood states were rated on 7-point bipolar scales, including Happy-Sad, Weak-Strong, Passive-Active, and Excited-Bored. In order to reduce the number of variables used in data analyses and reduce the possibility of Type 1 error, a principal component analysis (with varimax rotation) was performed on within-person z-scores of these variables, to ensure that all variation on these factors was based on within-person changes in mood state rather than between-subject differences (see Larson & Delespaul, 1992). Five factors emerged: Positive-Happy (happy, strong, active, excited); Negative-Stress (stressed, worried, strained, nervous); Negative-Angry (frustrated, irritable, angry); Positive-Social (friendly, cooperative, proud, caring) and Positive-Productive (responsible, hardworking, productive).

Because of prior research on the importance of social relationships for cortisol (Adam, Klimes-Dougan & Gunnar, 2005) and the importance of being alone and loneliness on adolescent emotion and physiological stress (Csikszentmihalyi & Larson, 1984, pp. 176-197; Cacioppo, Hawkley, & Berntson, 2003) we also examined associations between being alone (vs.

with others) at the time of the beep and cortisol levels. While the effects of other common events/situations encountered by adolescents in their daily lives could be examined in relation to cortisol levels, such analyses are beyond the scope of the current paper.

Data Analysis.

HLM analyses. To answer most of study questions, Hierarchical Linear Modeling (HLM) growth curve analysis was used (Bryk & Raudenbusch, 1992), with the effects of time of day and mood state on cortisol levels modeled at Level 1, and the effects of stable individual difference variables, including trait emotion, modeled at Level 2. In order to more accurately estimate the shape of each person's basal cortisol curve, and thus more accurately identify the extent to which individual data points deviated from their basal pattern, the cortisol data from the two days of measurement were combined. By increasing the number of cortisol data points available across the waking day for each individual, this approach provides an improved estimate of each individual's typical (rather than day-specific) diurnal rhythm and also increases the available degrees of freedom in the Level 1 model. Most prior studies using a multi-level modeling approach to examine associations between emotion and cortisol have also combined data across days (e.g. van Eck et al., 1996a; Smyth et al., 1997). A dummy variable for day of measurement was however included at Level 1, to account for the possibility of systematic differences in cortisol levels across days and correlated within-day error. Use of a 3-level approach which models days separately did not substantially alter the results (see Stetler et al., 2004 and Hruscka et al., 2005 for 3-level models). The HLM analysis proceeded in three major steps:

Step 1 -- Modeling diurnal cortisol patterns. First, at Level 1, each person's cortisol values were predicted by the time of day of each sample, to provide estimates of the average shape of each person's diurnal (daytime) cortisol rhythm across the two days of sampling. Time of day values were expressed as number of hours since awakening for each person each day. As change in cortisol levels across the day is typically not linear, a curvilinear model was utilized. The best

fit was obtained by a 2nd degree polynomial function, including both linear (time since waking) and quadratic (time since waking squared) terms for time of day. The time of day variables were centered in the middle of the day (8 hours post-awakening) in order to reduce multicollinearity between the linear and quadratic terms (see Neter, Wasserman & Kutner, 1990, pp. 315-316). The 40-minute cortisol response to awakening sample was indicated with a 0 1 dummy variable, with the coefficient on that dummy variable reflecting the size of the cortisol awakening response. A simplified presentation of the Level 1 model of basal cortisol activity (where i represents the individual, and j represents the repeated-measures occasion, or beep) is thus:

$$\text{LNCORT}_{ij} = \beta_0 + \beta_1 * \text{Time}_{ij} + \beta_2 * \text{Time}_{ij}^2 + \beta_3 * \text{CAR}_{ij} + \beta_4 * \text{Day}_{ij} + e_{ij}$$

Step 2 -- Associations between trait emotion and diurnal cortisol. The influence of the trait emotion variables (depressive symptoms and trait anxiety and anger), and of the demographic, developmental and medical control variables on diurnal cortisol rhythms were then tested, by conducting analyses of the associations between these variables and the Level 1 β coefficients. First, in an exploratory analysis, each of the trait emotion variables and control variables was entered on its own as a Level 2 predictor of the set of Level 1 β coefficients. Any variable that was significantly associated with a Level 1 coefficient ($\beta_0, \beta_2, \beta_3, \beta_4$) was entered simultaneously in the Level 2 model for that coefficient. Variables that were not significantly associated with cortisol activity were not retained in the model in order to preserve degrees of freedom.

Step 3 -- Associations between state emotion and momentary cortisol levels. Next, to examine the effects of within-person changes in mood state on cortisol levels, deviations of adolescents' observed cortisol values from their fitted diurnal curves were predicted from adolescents' mood states at the time of each cortisol sample. To do this, each of the mood state factors were added to the Level 1 model (after the linear and quadratic time of day variables, cortisol response to awakening, and day of testing variables). Only those mood state factors that

significantly predicted cortisol levels were retained in the model. In addition, a dummy variable indicating whether or not the adolescent was alone at the time of each beep was also added.

Whether the trait emotion variables and the demographic, medical, and developmental control variables modified the associations between momentary mood and cortisol was then examined through exploratory analyses, and any significant modifiers of the momentary mood-cortisol associations were retained. A simplified form of the full Level 1 and Level 2 models to be tested follows (each L1 mood variable and each L2 individual difference variable retained in model would actually have its own coefficient):

$$\text{Level 1: } \text{LNCORT}_{ij} = \beta_0 + \beta_1 * \text{Time}_{ij} + \beta_2 * \text{Time}_{ij}^2 + \beta_3 * \text{CAR}_{ij} + \beta_4 * \text{Mood}_{ij} + \beta_5 * \text{Alone}_{ij} + \beta_6 * \text{Day}_{ij} + e_{ij}$$

$$\text{Level 2: } \beta_0 = \gamma_{00} + \gamma_{01} * \text{Demographic}_i + \gamma_{02} * \text{Developmental}_i + \gamma_{03} * \text{Medical}_i + \gamma_{04} * \text{TraitAffect}_i + r_{0i}$$

$$\beta_1 = \gamma_{10} + \gamma_{11} * \text{Demographic}_i + \gamma_{12} * \text{Developmental}_i + \gamma_{13} * \text{Medical}_i + \gamma_{14} * \text{TraitAffect}_i + r_{1i}$$

$$\beta_2 = \gamma_{20} + \gamma_{21} * \text{Demographic}_i + \gamma_{22} * \text{Developmental}_i + \gamma_{23} * \text{Medical}_i + \gamma_{24} * \text{TraitAffect}_i + r_{2i}$$

and so on for β_0 through β_n where n is the number of Level 1 β 's

Given that each individual completed up to 14 momentary samples, the degrees of freedom at Level 1 are sufficient to model the multiple time of day and mood variables described, and the person-level sample size of 52 allows modeling of 4 or 5 Level 2 variables simultaneously. The data for male and female adolescents were analyzed together, but gender effects were examined by entering a gender dummy in each Level 2 model. Although these analytic procedures are complicated, an important point of this paper is that a thorough understanding of cortisol activity in naturalistic settings requires sophisticated data collection and analytic strategies such as these.

Results

Diary and Saliva Sample Completion Rates.

For the $N=52$ participants included in the analyses, the average number of completed ESM-cortisol pairs available was 10.04 out of the requested 14 pairs (71%). When cortisol

values were present, but the associated ESM data were missing, missing ESM values (less than 5%) were replaced with the each person's mean for that ESM variable.

Adolescent Diurnal Cortisol Activity.

Adolescents in this study showed the expected strong diurnal rhythm in cortisol levels – levels were highest in the morning, dropped most rapidly across the morning hours, then continued to decline more slowly to near zero values at bedtime. As noted above, the effects of time of day on cortisol were fit using a quadratic function. While the quadratic term is not significant in all models, it was retained in the model because there was significant between-person variability in this parameter -- it was important for the fit of some people's diurnal curves but not for others. Using this model, 68% of the variation in participants' cortisol levels was accounted for by the linear and quadratic time of day variables, with cortisol dropping, on average, 11% for every hour after awakening (see Table 2). The dummy variable for the cortisol response to awakening accounted for an additional 4% of the variance (for a total of 72%). On average, cortisol levels were 64% higher 40 minutes post-awakening than they were at the time of waking. The dummy variable representing day of testing was not significant.

Whether the elevations and slopes of adolescents' diurnal cortisol curves and their cortisol responses to awakening are related to the trait emotion variables and the demographic and health control variables are also presented Table 2. Only variables with significant effects on diurnal cortisol patterns were retained; variables in the final model were entered simultaneously and represent the independent effect of each variable.

Trait emotion and diurnal cortisol. Diurnal cortisol curves were found to be significantly less elevated (as indicated by the intercept parameter, centered at midday) among adolescents with higher levels of self-reported depressive symptoms, although this effect size was relatively small (1% decrease per scale point). The cortisol response to awakening was significantly larger for adolescents with higher levels of trait anger. Notably, a one point increase in self-reported

anger (on a 5 point scale) was associated with a 33% increase in the size of the cortisol response to awakening. Levels of anxiety were not associated with any of the diurnal cortisol parameters.

Demographic, developmental and health controls and diurnal cortisol. Curves were significantly more elevated among adolescents at higher levels of pubertal development; levels were 23% higher for every point increase on the 5-point pubertal development scale. Diurnal curves were also significantly higher among adolescents with greater self-reported nicotine use. As the form of nicotine use reported was cigarettes (by 4 participants, or approximately 8% of the sample), this effect reflects tobacco use -- diurnal cortisol curves are 1% higher for each additional cigarette typically used each day. The slope of the diurnal cortisol curve was significantly steeper for adolescents at higher stages of pubertal development, and significantly flatter among adolescent girls using oral contraceptives. The cortisol awakening response was found to be significantly lower for adolescents at higher pubertal stages. There were no significant effects of gender, the other demographic variables, number of caffeinated beverages per day, number of alcoholic beverages per week, number of hours of exercise per week, phase of menstrual cycle, wakeup and bedtimes, or hours of sleep on the days of testing on cortisol.

Effects of Mood State on Momentary Cortisol Levels.

Whether or not it is possible, controlling for time of day, to observe associations between changes in mood states and changes in cortisol levels in adolescents was a central question of this study. As shown in Table 3, both the negative-worry and the negative-angry mood state factors were significantly associated with within-person increases in cortisol in the full model. The other three mood state variables (positive-happy, positive-social, and positive-productive) did not show significant associations with cortisol. As noted in Table 3, there is on average a 5% increase in cortisol for every *SD* increase in worry, and an 8% increase for every *SD* increase in anger. There were no significant modifiers of the effects of anger on cortisol. The effect of

worry on cortisol was however significantly modified by adolescent age (but not pubertal stage), with stronger associations between worry and cortisol present for older adolescents.

There was also a significant effect of being alone on cortisol – cortisol levels were, on average, 14% higher when adolescents were alone than when they were with others. The being alone effect was modified by the age of the adolescent – in this case, being alone has less of an effect (by 8% per year of age) on cortisol levels in older adolescents than in younger. The full model presented in Table 3, including the time of day variables, the cortisol response to awakening, being alone, state emotion, trait emotion, and the demographic, developmental and medical control variables accounted for 77% of the total observed variance in cortisol levels.

Discussion

The functioning of the HPA axis, and its transactions with the social world and with behavior are complex. As a result, appropriately complex research designs and analytic strategies are required. The current study combines a well-planned ambulatory data collection protocol with statistical approaches that take best advantage of the data, in order to describe the naturalistic associations between adolescent trait and state emotion and cortisol activity, taking into account the influence of multiple demographic, developmental and health control variables. Associations were found between individual differences in trait emotional functioning and adolescents' diurnal cortisol rhythms, and within-person changes in adolescent mood state were associated with within-person changes in adolescents' cortisol levels as they went about the course of their everyday lives. The shape of adolescents' diurnal cortisol rhythms, and the associations between trait and state emotions and cortisol, were also found to be modified by developmental and health control variables.

Trait Influences on Adolescent Diurnal Cortisol Activity

One contribution of this study is the descriptive finding that basal cortisol activity follows a similar pattern in normal adolescents as it does in adults – the average adolescent diurnal profile

is characterized by high wakeup values, a strong (64%) increase in cortisol levels in response to awakening, followed by a rapid decline in levels over the next few hours, then a gradual decline to near-zero values in the evening. Although other studies have examined diurnal cortisol profiles in adolescents (primarily in clinical samples), no prior studies have examined such a wide range of factors predicting individual differences in basal cortisol activity in normal adolescents. This study identified several individual difference variables associated with adolescent basal cortisol patterns, including aspects of trait emotional functioning as well as developmental and medical factors.

Trait emotion and diurnal cortisol. In the current study, higher levels of depressive symptoms on the CESD are associated with adolescents having less elevated diurnal cortisol profiles. This finding that is at odds with the adult literature suggesting that cortisol levels tend to be more elevated, at least for some subsamples of depressed adults (Chrousos & Gold, 1992). Prior evidence for associations between basal cortisol levels and depression in adolescence has however been mixed, with some studies finding higher basal cortisol levels, and some finding no or negative associations (Dahl & Ryan, 1996; Kaufman, et al. 2001; Angold, 2003). The current study used a continuous measure of depressive symptoms, rather than DSM-IV diagnostic status, and most adolescents' symptom levels on the CESD fell in the subclinical range. In follow-up analyses, when adolescent report of having a *diagnosed major depressive disorder* was added to the model, self-reported MDD was in fact marginally associated with *higher* cortisol levels [$t(47)=1.793, p=.079$]. This potential discrepancy between depression-cortisol associations within the normal vs. clinical range of functioning deserves further examination. In general, however, the current findings add to the growing body of evidence suggesting that associations between HPA axis functioning and depression may take a different form in adolescents than in adults. Whether these differences are due to unique features of adolescent neurobiology (Spear,

2000 a, b), or due to adolescents having a shorter history of exposure to depressive symptoms and their experiential triggers, remains to be studied in future research.

Although prior research and theory have suggested that children and adolescents with anxiety disorders may show heightened cortisol reactivity, particularly to familiar stressors (Gunnar & Vazquez, 2006), in the current study trait levels of anxiety were not significantly associated with aspects of diurnal cortisol activity or cortisol reactivity to momentary negative emotion. The fact that this study did not explicitly distinguish between negative emotions generated by novel as opposed to familiar stressors may play a role in this result, as this distinction proved important in revealing associations between adult anxiety and cortisol (van Eck et al., 1996a). On the other hand, it may be that trait anxiety is simply not associated with individual differences in cortisol activity in adolescents when anxiety is in the subclinical range. While trait differences in anxiety levels between adolescents are not associated with differences in basal cortisol activity, other findings in this study show that moment-to-moment changes in anxious mood *do* matter for changes in cortisol levels: within-person increases in anxious mood significantly predict within-person increases in cortisol levels.

Trait levels of anger in adolescents were not associated with the elevations or slopes of their diurnal cortisol rhythms, but a strong positive association was found between trait levels of anger in adolescents and the size of their cortisol response to awakening. Typically, in adults, the size of the cortisol response to awakening has been associated with the level of chronic strain to which the individual is exposed (Schulz et al., 1998; Pruessner, Helhammer & Kirschbaum, 1999; Wüst et al., 2000; Steptoe et al., 2001). One prior study in adults examined trait anger and found a similar effect – higher post-awakening cortisol among adults with greater tendencies toward anger expression (Steptoe et al, 2000). Whether higher trait anger contributes to a higher morning cortisol response, a larger increase in post-awakening cortisol contributes to increased anger experience, or both are signs of a less effective central emotion-regulatory mechanism

remains to be clarified. One study conducted in adults (Wüst et al., 2000) and one in children (Bartels et al., 2003) has found a moderately strong genetic component to the cortisol awakening response; the extent to which the anger-awakening response association is accounted for by a genetic trait or by more situational or developmental factors also remains to be determined. As will be described in more detail below, in addition to the effects of anxious mood, significant associations between anger and cortisol and were also found at the state or momentary level.

Control variables effects. Among the medical control variables, adolescent tobacco use was associated with more elevated diurnal cortisol curves, use of oral contraceptives was associated with flatter diurnal cortisol curves, and in the final model higher adolescent BMI was associated with a greater cortisol response to awakening. While not the focus of this study, these findings speak to the importance of carefully identifying and controlling for the impact of medical/lifestyle factors when modeling cortisol activity in naturalistic settings.

Given the focus of this paper on cortisol activity during adolescence, the pubertal stage effects are of more substantive interest – adolescents at higher stages of pubertal development had more elevated diurnal cortisol curves, a steeper diurnal cortisol decline from wakeup to bedtime, and a smaller cortisol response to awakening. The effect of pubertal stage on the elevation of the diurnal curve is of particular interest in that it suggests the possibility of a developmental increase in cortisol levels across adolescence, consistent with several prior studies (Kiess, et al., 1995; Kenny et al., 1966 a, b; Lupien et al., 2001; Walker et al, 2001; Netherton et al., 2004; Shirtcliff et al., 2005). It is worth noting that adolescent age was also a significant predictor of the elevation and of the slope of the diurnal rhythm (in the same direction as pubertal stage), but only when pubertal stage was not in the model. That is, pubertal stage was a better predictor of the elevation and slope of the diurnal rhythm than was adolescent age.

This finding of increased basal cortisol levels and a flattening of diurnal rhythms with age/pubertal stage is of potential significance for understanding the development of emotional

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disorders in adolescence (Spear, 2000a, b), especially MDD, which has increased prevalence in late adolescence (Burke et al., 1990, 1991) and is associated with elevated basal cortisol in adults (Chrousos & Gold, 1992). Changes in other hormones, most notably DHEA, are also occurring across this time period (Angold, 2003) and Goodyer et al. (2000, 2003) suggest that cortisol and DHEA may potentially work together to predict the emergence of major depression in late adolescence. To determine whether developmental increases in cortisol across adolescence play a role in the increased incidence of MDD across this same period, longitudinal measurement of hormone levels, pubertal development, and MDD diagnoses are required.

Momentary Emotional State and Within-Person Changes in Cortisol

The central contribution of this study is that it provides clear evidence that moment-to-moment changes in mood states are related to moment-to-moment changes in cortisol levels in adolescents as they go about the course of their everyday lives. Two aspects of negative emotional experience, anger and worry/stress, were positively and independently associated with higher levels of cortisol at the momentary level – at moments when an adolescent was experiencing a higher level of either one of these mood states, his or her cortisol level tended to be higher than expected for that individual at that time of day. Whether adolescents' current mood states alter their levels of cortisol, or whether their hormonal states influences their mood states, cannot be determined conclusively from the current study. The fact that cortisol measurement was lagged 20 minutes after the diary reporting of emotion adds weight to the former interpretation, as does the large body of experimental evidence showing that exposure to stressful situations increases cortisol levels (Kirschbaum & Hellhammer, 1989; Dickerson & Kemeny, 2004). Experimentally manipulated changes in cortisol, however, have also been associated with changes in affective and cognitive state (Lupien & McEwen, 1997). It is likely that mood states and cortisol levels transact dynamically and continuously over time.

While on average this study finds a 5-8% increase in cortisol for every *sd* increase in negative emotion, individual differences in the size of this association were also found to exist, including significant increases in the cortisol response to worry/stress with age. The presence of individual differences in cortisol reactivity to negative emotion in naturalistic settings is not surprising, given the known role of differences in developmental histories, perceptions of stressors, and coping resources for individual differences in cortisol reactivity. Prior research found that the size of the HPA axis response to CRH challenge had a significant genetic component, whereas individual differences in cortisol reactivity to laboratory-based psychosocial stressors did not (Kirschbaum, et al., 1992). Regardless of its origins, variability in cortisol reactivity to negative emotion experienced in daily life is potentially of considerable clinical interest. One hypothesis guiding my work is that “high cortisol responders” to everyday events and emotions will be more likely, over time, to show changes in basal cortisol activity and to develop emotional and physical health problems. Obviously, a test of this hypothesis requires repeated longitudinal measurement of basal cortisol activity and cortisol reactivity to momentary events and emotions in relation to changes in emotional and physical well-being over time. The current normative study helps to lay the conceptual, empirical, and methodological foundations for future longitudinal research of this nature.

An additional state or situation-specific finding of this study was that adolescents’ cortisol levels were higher at moments they were alone, rather than with other people, even after controlling for the effects of time of day and of momentary emotion. Prior studies have found differences in basal cortisol levels to be associated with greater trait loneliness in adolescents (Cacioppo et al., 2000). Never before have the effects within-person changes in loneliness on adolescent cortisol been examined (although more specifically, the current study found effects of *being alone*, rather than *feeling alone*). Prior ESM studies (without cortisol) have found that for adolescents, being alone is the most aversive state of affairs, in terms of their experienced

emotions and cognitive state (Chikszentmihalyi & Larson, 1984). The associations between being alone and higher cortisol were present, however, after controlling for self-reported emotional state. This finding speaks to the possibility that social regulation of cortisol levels may occur independent of the individual's subjective experience of stress/distress, a point which has been shown in young children (see Gunnar & Donzella, 2002) but less so in adolescents.

The within-person association between being alone and cortisol is made more interesting by an associated between-person effect – the association between being alone and cortisol declines significantly with age. In contrast to the prior findings for basal cortisol, this moderator effect was significant for chronological age but *not* pubertal stage. The age-related nature of this finding makes developmental sense given that a major task of early adolescence is greater social engagement, while a task of late adolescence is increasing individuation. Chikszentmihalyi and Larson (1984) found that the effects of being alone on adolescent mood and cognition also declined with age, and using an identical data collection and analytic protocol, no associations between being alone and cortisol levels were found in a sample of adults (Adam, 2005). This illustrates the point that the impact of social contexts on HPA axis activity may vary depending on age/developmental stage, such that what represents a salient stressor or buffer at one age/stage may not be as salient at another.

In summary, this study provides evidence that studying the associations between adolescents' trait and state emotional experiences and their cortisol activity in naturalistic settings is a fruitful approach. Adolescents' everyday situations and emotions are clearly related to moment-to-moment changes in their cortisol levels. Adolescent trait emotional functioning as well as developmental factors are related to individual differences in basal cortisol activity and cortisol reactivity to momentary emotion. In the long term, gaining a better understanding of how the dynamics of adolescent moods and experiences relate to the dynamics of their cortisol

levels in naturalistic settings may help to illuminate the pathways by which everyday emotional experiences get under the skin to influence mental and physical health.

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Table 1. Descriptive Statistics for Trait Emotion and Demographic and Health Control Variables

	<i>Mean</i>	<i>SD</i>	<i>Min</i>	<i>Max</i>
Trait Anger (scale range 0 to 4)	1.49	.76	0	3
Trait Anxiety (scale range 0 to 4)	1.55	.65	0	3
Depressive Symptoms (scale range 0 to 60)	16.04	10.19	3	60
Age (possible range 13 to 19)	15.92	1.55	13	19
Pubertal Stage (scale range 1 to 4)	3.27	.60	1.6	4
Body Mass Index (BMI)	22.16	3.38	15.78	33.38
Tobacco Use (cigarettes per day)	1.75	7.59	0	40
Average Time of Awakening (in hours)	7.12	1.05	5.72	7.12
Length of Waking Day (in hours)	15.84	1.05	13	18
% in 2 Bio Parent Home			62%	
% Caucasian			88%	
Mother has college degree			70%	
Father has college degree			70%	
Family Income < 50 K			21%	
Family Income 50 to 100 K			50%	
Family Income > 100 K			29%	
% Females Premenarchal			8%	
% Females Using Oral Contraceptives			13%	
% Females in Follicular/Periovulatory Phase of Menstrual Cycle			25%	
% Females in Luteal Phase of Menstrual Cycle			21%	
% Females in Menses Phase of Menstrual Cycle			33%	

Table 2. Hierarchical Linear Model of Associations between Adolescent Diurnal (Daytime) Cortisol Rhythms and Developmental, Medical Control and Trait Emotion Variables

Fixed Effect	Coefficient	SE	df	<i>t</i> -value	<i>p</i> -value	Interpretation
Cortisol Intercept (Midday)						
Intercept	-1.987	.068	48	-29.26	.000	$\hat{Y}_{\text{midday}} = .14\mu\text{g/dl}^{\text{a}}$
Pubertal Stage	.210	.082	48	2.57	.014	+23% per scale point ^b
Tobacco Use	.013	.003	48	4.64	.000	+1% per cigarette/day
CESD	-.015	.004	48	-3.69	.001	-1 % per scale point
Time Since Waking						
Intercept	-.119	.006	49	-18.36	.000	-11% per hr
Pubertal Stage	-.022	.009	49	-2.37	.022	-2% per hr per scale pt
Oral Contrac.	.064	.010	49	6.68	.000	+7% per hr if use
Time Since Waking²						
Intercept	.002	.002	51	1.05	.300	n.s.
Day of Testing^c						
Intercept	.044	.047	51	.93	.356	n.s.
Cortisol Awakening Response						
Intercept	.495	.066	49	7.52	.000	+64% if CAR sample
Pubertal Stage	-.256	.092	49	-2.80	.008	-23% per scale point
Trait Anger	.284	.071	49	3.98	.000	+33% per scale point
<hr/>						
Random Effect	Standard Deviation	Variance Component	df	Chi-squared	<i>p</i> -value	
Intercept	.430	.185	33	123.27	.000	

Time of Day	.029	.001	34	59.80	.004
Time of Day ²	.007	.000	36	69.17	.000
Day of Testing	.079	.006	36	59.80	>.50
Awakening Response	.167	.185	33	33.23	>.50

Note. The time since awakening, cortisol awakening response (CAR), and day of testing variables were entered as Level 1 (within-person, repeated measures) variables; the developmental, medical, and trait emotion variables were entered as Level 2 (between-person, individual difference) variables.

^aDue to the logarithmically transformed outcome variable (natural log of cortisol values), the inverse function of that transformation (the exponential function) was applied to return this intercept to its value on its original scale of measurement.

^bSpecial properties of a logarithmic outcome variable allow coefficients predicting that outcome to be interpreted as % change in the outcome per unit change in the independent variable, after the following transformation has been applied to the B coefficient: $B_{\%change} = [\exp(B_{raw})] - 1$. (Neter, Wasserman, Kutner, 1990, p. 1451; Woolridge, 2000).

^cA dummy variable representing day of testing (Day 1=0, Day 2=1) was included to account for any systematic effects of day of testing on cortisol levels.

Table 3. Hierarchical Linear Model of Associations between Momentary Mood and Being Alone and Momentary Cortisol Levels, Controlling for Diurnal (Daytime) Cortisol Rhythms

Fixed Effect	Coefficient	SE	df	<i>t</i> -value	<i>p</i> -value	Interpretation
Cortisol Intercept (Midday)						
Intercept	-2.026	.076	48	-26.55	.000	$\hat{Y}_{\text{midday}} = .13\mu\text{g/dl}^{\text{a}}$
Pubertal Stage	.255	.071	48	3.57	.001	+29% per scale point ^b
Tobacco Use	.013	.002	48	5.35	.000	+1% per cigarette/day
CESD	-.012	.004	48	-3.406	.002	-1% per scale point
Time Since Waking						
Intercept	-.120	.006	49	-18.64	.000	-11% hr since waking
Pubertal Stage	-.019	.009	49	-2.27	.027	-2% per hr per scale pt
Oral Contrac.	.054	.012	49	4.68	.000	+6% per hr if use
Time Since Waking²						
Intercept	.002	.002	51	1.05	.300	n.s.
Day of Testing^c						
Intercept	.055	.051	51	1.07	.287	n.s.
Awakening Response						
Intercept	.496	.062	48	7.98	.000	+64% if CAR sample
BMI	.023	.009	48	2.58	.014	+2% per unit BMI
Pubertal Stage	-.237	.092	48	-2.58	.013	-21% per scale point
Trait Anger	.351	.065	48	5.40	.000	+42% per scale point
State Anger						
Intercept	.075	.027	51	2.74	.009	+8% per sd state anger
State Worry						
Intercept	0.050	.023	50	2.20	.033	+5% per sd state worry
Age	0.041	.014	50	3.06	.004	+4% per year age
Being Alone						
Intercept	.132	.062	50	2.13	.038	+14% if alone
Age	-.084	.030	50	-3.32	.002	-8% per year age

Random Effect	Standard Deviation	Variance Component	<i>df</i>	Chi-squared	<i>p</i> -value
Intercept	.473	.223	29	60.07	.001
Time Since Awakening	.031	.001	30	50.99	.010
Time Since Awakening ²	.008	.000	32	56.25	.005
Day of Testing	.177	.031	32	29.15	>.500
Awakening Response	.178	.032	29	31.09	.361
State Anger	.103	.011	32	30.17	>.500
State Worry	.098	.010	31	37.39	.199
Being Alone	.217	.047	31	49.88	.017

Note. The time since awakening, cortisol awakening response (CAR), day of testing variables, being alone and momentary mood variables were entered as Level 1 (within-person, repeated measures) variables; the developmental, medical control and trait emotion variables were entered as Level 2 (between-person, individual difference) variables.

^aDue to the logarithmically transformed outcome variable (natural log of cortisol values), the inverse function of that transformation (the exponential function) was applied to return this intercept to its value on its original scale of measurement.

^bSpecial properties of a logarithmic outcome variable allow coefficients predicting that outcome to be interpreted as % change in the outcome per unit change in the independent variable, after the following transformation has been applied to the B coefficient: $B_{\%change} = [\exp(B_{raw})] - 1$.

(Neter, Wasserman, Kutner, 1990, p. 1451; Woolridge, 2000).

^cA dummy variable representing day of testing (Day 1=0, Day 2=1) was included to account for any systematic effects of day of testing on cortisol levels.