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Longitudinal Stability and Developmental Properties of Salivary Cortisol Levels and Circadian Rhythms from Childhood to Adolescence

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Abstract

This study aimed to (1) identify a stable, trait-like component to cortisol and its circadian rhythm, and (2) investigate individual differences in developmental trajectories of HPA-axis maturation. Multiple salivary cortisol samples were collected longitudinally across four assessments from age 9 (3rd grade) through age 15 (9th grade) in a community sample of children (N=357). Sophisticated statistical models examined cortisol levels and its rhythm over time; effects of age, puberty and gender were primarily considered. In addition to situation-specific and stable short-term or epoch-specific cortisol components, there is a stable, trait-like component of cortisol levels and circadian rhythm across multiple years covering the transition from childhood into adolescence. Youth had higher cortisol and flatter circadian rhythms as they got older and more physically developed. Girls had higher cortisol, stronger circadian rhythms, and greater developmental influences across adolescence. Distinguishing a stable, trait-like component of cortisol level and its circadian rhythm provides the empirical foundation for investigating putative mechanisms underlying individual differences in HPA functioning. The findings also provide important descriptive information about maturational processes influencing HPA-axis development.

Key Terms

HPA axis; Cortisol; Development; Adolescence; Puberty

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Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is integrally involved in the body's stress response which helps individuals adapt to the changing demands of their environment (Selye, 1976). HPA fluctuations are very dynamic – HPA activity is highly contextually and stress-responsive (Selye, 1976), and cortisol levels fluctuate according to a well characterized circadian rhythm (Knutsson et al., 1997). However, although traits have been identified and studied across multiple biological systems, there have been no long-term longitudinal studies that have investigated whether there is also a stable, trait-like component of the HPA axis. Such identification is a critical prerequisite to investigations of the psychobiological mechanisms underlying HPA stability, change, and long-term risk for stress-related disease outcomes. The present study aims to identify a component of HPA activity that demonstrates long-term stability within individuals, and thus, to determine whether a substantial portion of variability in cortisol reflects an individual's basal levels and, subsequently, diurnal rhythms. Secondly, by focusing on the transition from childhood into adolescence, the study investigates how the HPA-axis matures across this salient developmental epoch.

Distinguishing an individual's trait-like cortisol is very challenging. First, the HPA axis is inherently very responsive, recalibrating to help individuals adapt to the demands of a constantly changing environment. A large body of research has established the associations of chronic stress with alterations in concurrent and, less frequently studied, subsequent HPA functioning (Essex, Klein, Cho, & Kalin, 2002; Miller, Chen, & Zhou, 2007; Tarullo & Gunnar, 2006). Other studies have demonstrated that a variety of negative behavioral and health outcomes are linked with either persistently elevated (Lupien et al., 2006) or decreased cortisol (Miller, et al., 2007). Importantly, there is an implicit assumption in much of the previous work that the various measures of HPA activity used in these studies reflect individuals' basal or trait-like cortisol. However, because previous studies have not distinguished the components of HPA functioning that reflect responses that are situation-specific (e.g., responses to daily events) or stable over short epochs (e.g., responses to major life events) from the component that is trait-like, the meaning of the findings remain unclear. Recently, in ours (Fries, Shirtcliff, & Pollak, 2008; Shirtcliff & Essex, 2008; Shirtcliff, Granger, Booth, & Johnson, 2005) and others' work (Adam, Hawkley, Kudielka, & Cacioppo, 2006; de Weerth, Zijl, & Buitelaar, 2003; Hruschka, Kohrt, & Worthman, 2005; Kirschbaum et al., 1990), a small but significant portion of variability in cortisol has been identified as stable across days; the majority of these investigations do not capture development over time as they are limited to relatively short durations and may mix trait-like and epoch-specific influences on cortisol. To our knowledge, no studies investigate whether there is also a component of cortisol that is longitudinally trait-like. Distinguishing a trait-like component of cortisol from the components reflecting situation-specific and epoch-specific stability would lay the empirical foundation for future studies to investigate the physiologic relevance of each component and clarifying the role of trait-like cortisol levels for understanding long-term risk for stress-related disease outcomes.

Second, circadian rhythmicity also exerts a major influence on cortisol (Knutsson, et al., 1997). The timing of sample collection consistently explains variability in cortisol concentrations (Adam, et al., 2006), and is also important for understanding stress dysregulation (Siever & Davis, 1985). After a response to awakening (Fries, Dettenborn, & Kirschbaum, 2009), cortisol is highest in the morning, declines quickly across the morning hours and continues to decline somewhat across the waking hours. Importantly, distinguishing the trait-like component of cortisol is challenging because rhythmicity is influenced by both intrinsic and extrinsic forces and may have its own unique trait component (Shirtcliff & Essex, 2008). The longitudinal design of this study allows for

visualizing and interpreting the profound developmental change in cortisol's daily rhythm from childhood into adolescence (Gunnar & Vazquez, 2006; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009).

Third, because the vast majority of previous studies have been cross-sectional or short-term longitudinal, they cannot consider developmental trajectories within individuals, raising the potential to confound rather than elucidate changes (Kraemer, Yesavage, Taylor, & Kupfer, 2000). Longitudinal design allows tracking of changes in cortisol levels and its circadian rhythm within the same individual across time – in this case, from childhood (age 9) through the transition into adolescence (age 15). This offers a unique opportunity to examine how age and puberty independently affect cortisol levels and rhythms. Cross-sectional analyses reveal that younger children may have higher cortisol than older children (Matchock, Dorn, & Susman, 2007; Schreiber et al., 2006). However, developmental patterns may shift, such that older adolescents may have higher cortisol than early adolescents (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Rosmalen et al., 2005; Shirtcliff, et al., 2005), possibly linked specifically with pubertal onset (Adam, 2006; Gunnar, et al., 2009; Kiess et al., 1995; Lashansky et al., 1991; Netherton, Goodyer, Tamplin, & Herbert, 2004; Tornhage, 2002). Consistent with this, the few longitudinal studies of adolescents suggest that across one to two years, cortisol increases (Matchock, et al., 2007; Shirtcliff, et al., 2005; Susman, Dorn, Inoff-Germain, Nottelman, & Chrousos, 1997; Walker, Walder, & Reynolds, 2001). Only two longitudinal studies were conducted across longer periods of 4–5 years, one of which did not report mean cortisol levels (Shoal, Giancola, & Kirillova, 2003). The other showed that cortisol levels increased in girls as they transitioned from pre-pubertal to adult-like development (Legro, Lin, Demers, & Lloyd, 2003). A long-term longitudinal design would provide clarity as to whether the developmental patterns described in cross-sectional research are accurate when tracking the same individuals as they mature from childhood to adolescence.

Gender differences are often emergent during adolescence (Zahn-Waxler, Crick, Shirtcliff, & Woods, 2006), and may play an important role in the maturation of the HPA axis. Adolescent girls may have higher cortisol than boys (Schreiber, et al., 2006; Shirtcliff & Essex, 2008; Shirtcliff, et al., 2005; Susman, et al., 1997), and developmental effects on cortisol may be stronger or appear earlier in girls than boys (Netherton, et al., 2004; Tornhage, 2002). Nevertheless, reported gender differences are far from consistent, possibly due to earlier pubertal development in girls (Dahl et al., 1992; Kiess, et al., 1995; Kudielka, et al., 2004; Lashansky, et al., 1991). This renders a longitudinal investigation of HPA development highly important.

The present study aims to address these issues. The primary goal is to investigate the stability in cortisol and its circadian rhythm across ages 9 to 15 (grades 3 to 9), the years covering the transition to adolescence, thereby testing the hypothesis that there is trait-like stability across development. A secondary goal is to investigate developmental influences by examining how HPA functioning changes within individuals across this time period, which spans on average from initiation through completion of pubertal maturation (Shirtcliff, Dahl, & Pollak, 2009). We predict that cortisol levels will increase over time within individuals and that puberty may be a potent marker for HPA-axis development (Dorn, Dahl, Woodward, & Biro, 2006), particularly for girls (Netherton, et al., 2004; Tornhage, 2002). We also determine the importance of medication usage and body mass index (BMI) as statistical controls in relation to longitudinal HPA functioning. A number of studies on HPA axis activity suggest that controlling for medication usage may be worthwhile, but the findings are far from consistent (Granger, Hibel, Fortunato, & Kapelewski, 2009; Hibel, Granger, Cicchetti, & Rogosch, 2007; Hibel, Granger, Kivlighan, & Blair, 2006; Kariyawasam, Zaw, & Handley, 2002; Schreiber, et al., 2006). Likewise, we examine BMI

as a potential control variable as the HPA axis interacts with many metabolic functions (Dallman et al., 2004), and may contribute to overweight and obesity (Dimitriou et al., 2003; Kiess et al., 1995; Rosmalen et al., 2005).

Methods

Participants

Children are participants in a longitudinal study, the Wisconsin Study of Families and Work (WSFW) (Essex, et al., 2002; Hyde, Essex, Clark, & Klein, 2001). Originally, 570 pregnant women and their partners were recruited from prenatal clinics for a study of maternity leave and health outcomes. To be eligible, female participants were required to be over the age of 18, in the second trimester of pregnancy, living with the baby's biological father, and either employed or a full-time homemaker. Of those eligible, 75% agreed. All study procedures were approved according to the University of Wisconsin institutional guidelines; informed consent was obtained from all participants.

Analyses include the 357 families who lived within geographical proximity to the project offices and agreed to participate in saliva collection. There are no significant differences between the 357 participants and the remaining families from the original sample in terms of parental education, marital or ethnic status, or annual family income; parents in the 357 families were slightly older at the time of recruitment: mothers $M = 29.6$ ($SD = 4.2$) versus 28.9 ($SD = 4.6$), $t(568) = -1.97$, $p < .05$; fathers $M = 31.6$ ($SD = 5.2$) versus 30.7 ($SD = 4.8$), $t(548) = -2.17$, $p < .05$. At the time of recruitment, 44% of the 357 mothers and 52% of the fathers had a high school or technical degree or less, while the remainder had at least a college degree; 40% were first-time mothers. Most couples (95%) were married and Caucasian (90%); median annual family income was \$48,000 (range = <\$10,000 to >\$180,000).

Measures

Age across Assessments—Data were collected at grades 3 (M age=9.27, range=8.6–10.3), 5 (M age=11.19, range=10.5–12.3), 7 (M age=13.22, range=11.6–14.2), and 9 (M age=15.50, range=14.8–16.5). At 3rd grade (typically age 9), saliva collection was limited to a subset of 165 children; at 5th (age 11), 7th (age 13), and 9th (age 15) grades, all participating children were asked to collect saliva ($N=297$, $N=306$ and $N=273$ participants, respectively). Age was coded as years-since-school-entry to center maturation on a meaningful event (rather than extrapolate downward to birth), and to capture important experience-based social transitions, thereby taking both chronological age and life experience into account (Shirtcliff & Essex, 2008).

Salivary Cortisol—Within each assessment, children were asked to collect saliva for three consecutive days (weekend and/or weekday) across three target collection times: (1) shortly after waking (before brushing teeth or eating breakfast); (2) between 3:00 PM and 7:00 PM (prior to dinner); and (3) just before going to bed. Target times were selected by the family prior to sample collection to accommodate their schedules and avoid their mealtimes; the same target time was selected across all 3 days. This flexibility was considered an appropriate method of increasing compliance since the afternoon is a quiescent period of HPA activity and this strategy should not impact intra-individual variance estimates. Children were asked to collect saliva on days in which they were healthy. If the child experienced cold, flu, fever or similar health problem, they were asked to re-collect saliva on a different day. Cortisol was assessed in duplicate using a well-established salivary enzymeimmunoassay kit (Salimetrics, State College, PA). Mean intra-assay and inter-assay coefficients of variation (CVs) were 3.8% and 7.4%, respectively. Samples were reanalyzed

if the CV for the duplicate measurements were 20% for samples with values $>.02 \mu\text{g/dL}$ and 30% for samples with values $\leq .02 \mu\text{g/dL}$. To normalize distributions, raw cortisol was log-transformed and extreme values were winsorized.

Puberty—Both mothers and youth completed a self-administered Tanner stage measure based on description and visual inspection of line drawings (Morris & Udry, 1980) and the Pubertal Development Scale (Petersen, Crockett, Richards, & Boxer, 1988). These puberty measures are highly correlated with staging by physical exam ($r_s >.63$), and are useful when the continuum of development is of interest (Shirtcliff, et al., 2009). Only mothers reported puberty at age 9, when over 96% of the children were less than stage II; only youth reported puberty at age 15. At ages 11 and 13, youth- and mother- ratings of Tanner staging were highly reliable with each other for breast/genital ($\alpha=.90$ at age 11 and $\alpha=.89$ at age 13) and pubic hair development ($\alpha=.90$ at age 11 and $\alpha=.93$ at age 13), and were subsequently averaged across informants. The PDS was converted to the Tanner metric, separately for gonadal vs. adrenal hormone-related events (Shirtcliff, et al., 2009). The PDS gonadal score was reliable with Tanner breast/genital stage, $\alpha=.98$; the PDS adrenal score was reliable with Tanner pubic hair stage, $\alpha=.93$. Therefore, these scores were averaged, respectively. Finally, the two axes (gonadal and adrenal) were averaged to form a single puberty measure, $\alpha=.95$.

Body Mass Index (BMI)—Children's height and weight were measured by a researcher at each assessment. BMI was calculated as $(\text{lb}/\text{in}^2)*703$. BMI was converted to z-scored percentiles-for-age-and-sex based on the Centers for Disease Control guidelines (Ogden et al., 2002).

Medication—With each saliva sample, participants reported medication usage (Hibel, et al., 2007; Hibel, et al., 2006; Schreiber, et al., 2006). Classes of drug codes were grouped by presumed mechanism of action on the HPA axis: (1) non-oral steroids (e.g., nasal sprays; inhalers; topical glucocorticoids; 2.1%); (2) Asthma, allergy non-steroidal medications (e.g., antihistamines; leukotriene and beta-adrenergic antagonists; 8.7%); (3) psychotropic medications (e.g., antidepressants; psychostimulants; mood stabilizers; neuroleptics; 4.5%); (4) antibiotics, antifungals, analgesics and NSAIDS (5.9%); (5) Other medications (e.g., vitamins; 3%). Children on oral steroids (e.g., prednisone) were excluded from analyses.

Analytical Strategy

Growth curve techniques allow, in a single analysis, identification of different cortisol components by modeling the inherent auto-correlation or dependency patterns within multiple cortisol measurements. Specifically, within the same three-level hierarchical linear model (HLM) (Collins & Sayer, 2001), the total variance in HPA activity (cortisol levels and circadian rhythms) is parsed into the percentages uniquely (i.e., independently) attributable to situation-specific fluctuations (due to unmeasured contextual forces or momentary experience within any given day) (Level 1), short-term or epoch-specific stability within each assessment and developmental shifts across assessments (Level 2), and – of most interest -- trait-like stability (Level 3). The benefits of growth curves include (a) simultaneous modeling of cortisol levels and circadian rhythms; (b) capturing growth trajectories at the intra-individual difference level; (c) allowing individuals to have unique growth trajectories influenced by their own developmental events (e.g., puberty); and (d) inclusion of subjects with incomplete data without violating missing data assumptions (Schafer & Graham, 2002).

Level 1: Separating out the situation-specific sources of variance in HPA activity—Level 1 examines each single cortisol measure ($N=9144$; maximum of 9 samples/

assessment and 4 assessments; on average participants provided 25 samples). Beyond situation-specific fluctuations, the circadian rhythm is the largest influence. To distinguish situation-specific variability and the circadian rhythm from stable cortisol (both short-term and trait-like), Level 1 includes Time (in hours) Since Waking (TSW) as a random predictor of cortisol levels. The circadian slope is steeper in the morning than in the afternoon (see Figure 1). Therefore, curvature of the slope is modeled with (fixed) quadratic and cubic terms. TSW holds the advantage of centering the slope on the individual's particular biorhythm, but there are also extrinsic influences on the circadian rhythm (Shirtcliff & Essex, 2008). To model the possibility that samples taken later in the day are lower, we include a fixed predictor of 'clock-time' (i.e., Time Since Noon, residualized from TSW to prevent multicollinearity). This controls for variability in collection times and statistically corrects for influences of phase-shifting (i.e., participants waking up later or staying up later as they progress through adolescence). This model does not distinguish one day from another within each assessment because day-to-day fluctuations are minimal (Hruschka, et al., 2005; Shirtcliff & Essex, 2008) and a parsimonious model increases reliability of level and rhythm estimates. Medication usage captures whether the medication was taken at the time of cortisol sampling.

Level 2: Short-term cortisol stability within each assessment and development across assessment—

The growth curve model addresses short-term or epoch-specific stability and development of HPA activity across years by simultaneously modeling a Level 2 hierarchy that captures cortisol measures within each assessment (N=1041; maximum of 4 assessments per person). Both cortisol levels and rhythms (distinguished in the Level 1 model) are outcomes of interest at each assessment using a slopes-as-outcomes approach. Significant variance in cortisol levels distinguishes epoch-specific HPA stability. To capture the trajectory of HPA maturation across assessments, age-since-school-entry is a fixed predictor of cortisol level and its rhythm. A fixed quadratic term of age is included to test if growth across adolescence is nonlinear (Schreiber, et al., 2006). Next, puberty is examined alone (without age in the model) as a marker of development. Finally, age and puberty are examined together to obtain estimates of the independent contributions of these factors. BMI or medication usage is entered as potential control variables at this level of analysis.

Level 3: Stable, trait-like cortisol across development—Level 3 distinguished the component of primary interest - a single estimate of cortisol level and its circadian rhythm for each individual that is trait-like across days and years (N=357). Significant variability indicates that there is a unique component of cortisol that is stable across development, beyond the influence of situation-specific fluctuations or circadian variation (Level 1), and beyond epoch-specific influences and developmental shifts (Level 2). We also examine whether gender influences trait-like cortisol level, slope, and developmental trajectories.

$$\begin{aligned}
 \text{[Level 1]:Cortisol} &= \pi_0 + \pi_1(\text{TSW}) + \pi_2(\text{TSW-squared}) + \pi_3(\text{TSW-cubic}) + \pi_4(\text{time since noon}) + \varepsilon \\
 \text{[Level 2]:} & \pi_0 = \beta_{00}(\text{levels}) + \beta_{01}(\text{age}) + \beta_{02}(\text{age-squared}) + R_0 \\
 & \pi_1 = \beta_{10}(\text{slope}) + \beta_{11}(\text{age}) + R_1 \\
 \text{[Level 3]:} & \beta_{00}(\text{levels}) = \gamma_{000} + \gamma_{001}(\text{gender}) + U_{00} \\
 & \beta_{10}(\text{slope}) = \gamma_{010} + \gamma_{011}(\text{gender}) + U_{10} \\
 & \beta_{11}(\text{age}) = \gamma_{110} + \gamma_{111}(\text{gender}) + U_{11}
 \end{aligned}$$

Results

Level 1: Separating out the situation-specific sources of variance in HPA activity

Cortisol is highest upon awakening, $\beta=1.25$, $t(356)=135.23$, $p<.0001$, and declines across the day, $\beta=-.02$, $t(356)=-23.20$, $p<.0001$. The circadian decline demonstrates a quadratic, $\beta=.0012$, $t(9127)=10.37$, $p<.0001$, and cubic, $\beta=-.00003$, $t(9127)=-6.49$, $p<.0001$, function; the slope is steepest across the morning hours (see Figure 1). ‘Clock-time’ exerts an additional effect such that, after taking TSW into account, samples taken later in the day are lower, $\beta=-.005$, $t(9127)=-7.43$, $p<.0001$. After taking the circadian rhythm into account, state- or situation-specific influences comprise 51.9% of variability in cortisol levels.

Level 2: Short-term cortisol stability within each assessment and development across assessment

Consistent with earlier studies, cortisol levels demonstrate substantial epoch-specific stability, comprising 34.9% of total cortisol variability, $\chi^2(681)=2133.93$, $p<.0001$, independent of stability across years. The circadian rhythm also demonstrates substantial stability within each assessment period, $\chi^2(681)=1451.21$, $p<.0001$, comprising 28.0% of total variability in the circadian rhythm. Cortisol becomes lower as individuals get older, $\beta=-.006$, $t(1037)=-2.25$, $p=.025$, but this is modified by quadratic-age, $\beta=.0003$, $t(1037)=2.07$, $p=.04$. The slope becomes flatter as children age, $\beta=.0004$, $t(356)=6.35$, $p<.0001$. Figure 2 illustrates that morning cortisol declines as the child matures from age 9 to age 11, but the decline thereafter is not as substantial. Because the slope is flatter, afternoon and evening levels of cortisol are higher in 15 year olds than they had been when they were 9 year olds.

There is no initial effect of puberty on cortisol levels, $p=.49$, but a suppressed effect emerges after controlling for age and age-squared. Cortisol levels are higher as individuals advance through puberty, $\beta=.004$, $t(1037)=3.50$, $p<.001$. The slope becomes flatter as individuals progress through puberty, $\beta=.001$, $t(1039)=8.66$, $p<.0001$; however, this effect becomes non-significant, $p=.16$, after accounting for age.

Level 3: Stable, trait-like cortisol across development

After accounting for the sources of variance in HPA activity in Levels 1 and 2, cortisol that is stable across days and years (i.e., trait-like) comprises 13.2% of total cortisol level variability, $\chi^2(356)=620.3$, $p<.0001$. The circadian rhythm shows a very high degree of trait-like stability, $\chi^2(356)=523.01$, $p<.0001$, comprising 72.0% of total variability in the circadian rhythm.

Gender Differences

Gender impacts nearly every component of HPA-axis activity. Girls have higher cortisol, $\beta=.037$, $t(355)=2.37$, $p<.018$, steeper slopes, $\beta=-.0025$, $t(355)=3.91$, $p<.0001$, and more curvature to their rhythm than boys, $\beta=.00011$, $t(9131)=2.99$, $p<.003$. The decline in waking cortisol across development is less pronounced in girls than boys, $\beta=-.0096$, $t(1038)=2.04$, $p<.04$, and the quadratic age-effect shows greater curvature in girls than boys, $\beta=.0008$, $t(1038)=2.43$, $p<.015$ (see Figure 3). When puberty alone is examined, gender moderates the effect of puberty on the slope, $\beta=.0002$, $t(1039)=1.95$, $p=.05$. The circadian rhythm becomes flatter as children advance through puberty, especially girls. This persists after controlling for age, $\beta=.001$, $t(1038)=2.1$, $p=.036$.

Statistical Control Variables

Across assessment periods, children with higher BMI have lower morning cortisol, $\beta = -.004$, $t(1037) = 2.88$, $p < .004$, and flatter slopes, $\beta = .0004$, $t(1038) = 3.02$, $p < .003$. Gender moderates the effect of BMI on the circadian rhythm such that girls with higher BMI have the flattest slopes, $\beta = .0003$, $t(1038) = 2.44$, $p < .015$. None of the medication categories impact cortisol levels, $p > .06$.

Discussion

This study identifies a stable, trait-like component of cortisol, and provides insight into important developmental influences in HPA functioning from childhood across the transition into adolescence. The findings highlight that 13% of variance in cortisol levels is stable across the six years spanning this salient developmental period. The circadian rhythm is remarkably trait-like, with 72% of its variance exhibiting trait-like stability. This long-term stability in cortisol levels and circadian rhythm is distinct from components reflecting situation-specific influences on the HPA axis (52% of the variance in cortisol levels after accounting for the circadian rhythm) and short-term or epoch-specific stability (35% of the variance in cortisol levels; 28% of the variance in circadian rhythm). By following children longitudinally from age 9 to age 15, we offer a unique perspective about the developmental trends of HPA axis maturation. Furthermore, we gain important descriptive information about how the HPA maturation is influenced by puberty and gender.

The findings provide a strong test of trait-like stability by extending the investigation of cortisol output across multiple years of development. Approximately 13% of the variability in cortisol is reflected in an individual's basal levels; that is, it is stable across time and operates like a trait. This trait-like estimate of cortisol's stability is distinct from short-term, epoch-specific stability estimates identified in previous studies (Adam, et al., 2006; de Weerth, et al., 2003; Fries, et al., 2008; Hruschka, et al., 2005; Kirschbaum, et al., 1990; Shirtcliff & Essex, 2008; Shirtcliff, et al., 2005). Short-term stability has been associated with stress exposure (Fries, et al., 2008), developmental events (de Weerth, et al., 2003), mental health symptoms (Shirtcliff & Essex, 2008; Shirtcliff, et al., 2005), and emotion and behavior problems (Adam, 2006; Hruschka, et al., 2005). However, because previous studies do not distinguish components reflecting epoch-specific vs. long-term stability, the etiology and functional significance of these distinct components remain unclear. Indeed, epoch-specific stability within each assessment period is substantial (35%). Importantly, several of these previous investigations reveal longitudinal prediction of the short-term trait to physical development, aggression and mental health symptoms (de Weerth, et al., 2003; Legro, et al., 2003; Shirtcliff & Essex, 2008; Shirtcliff, et al., 2005; Shoal, et al., 2003). The component of cortisol levels and rhythm which is truly stable across days and years may be underlying these robust biobehavioral associations.

Statistically distinguishing this stability from the components of cortisol reflecting situation-specific responses or epoch-specific stability provides the empirical foundation for future studies to investigate the physiologic relevance of each component, and most importantly, the individual and contextual factors (e.g., genetic markers, early stress exposure, brain structure, and temperament) uniquely associated with trait-like cortisol. For example, given the empirical evidence for stable, trait-like cortisol in the present investigation, we are going on to show that early life stress helps establish this trait-like cortisol (Essex et al., 2011). Furthermore, because the present investigation distinguishes epoch-specific cortisol variance, we are going on to show that epoch-specific cortisol covaries with mental health symptoms at each assessment and that this coupling is strongest in children exposed to early life stress (Essex, et al., 2011). Relatedly, distinguishing such remarkably high trait-like variance has set the stage for our preliminary findings that the circadian rhythm has genetic

influences distinct from those on cortisol levels (Van Hulle, Shirtcliff, Lemery-Chalfant, & Goldsmith, under review). Asking these mechanistic questions hinges on knowing the underlying pattern of HPA development and having empirical evidence for a trait-like cortisol and diurnal rhythm component as the current investigation illustrates.

In addition to distinguishing a HPA trait, the longitudinal design affords the opportunity to investigate how the HPA axis develops across the full range of pubertal maturation. Fifteen year olds have lower morning cortisol and flatter circadian rhythms than when they were 9 years old, though cortisol is lowest when youth are 11 years old. Age also impacts the slope; by the evening, fifteen year olds (especially girls) have the highest cortisol. These findings may appear contradictory to other work which found developmental increases in cortisol across adolescence (Matchock, et al., 2007; Schreiber, et al., 2006). However, closer inspection suggests it is consistent given that our work likewise illustrates a “U-shaped” curve (Schreiber, et al., 2006), with cortisol levels decreasing from ages 9 to 11, and then increasing during early adolescence. Given the flattening of the diurnal rhythm, afternoon cortisol levels are notably higher as children advance through adolescence, which is consistent with prior work (Matchock, et al., 2007; Schreiber, et al., 2006). This finding also supports literature that suggests important neurobiological changes take place as individuals approach and enter puberty; in fact, this normative increase in cortisol in early adolescence may be related to increased environmental sensitivity and, for some individuals, increased susceptibility for psychopathology (Dahl & Gunnar, 2009; Gunnar et al., 2009). Puberty exerts an additional independent effect on increased cortisol levels during adolescence (Dorn, Dahl, Woodward, & Biro, 2006), further illustrating that age and puberty are inter-related developmental processes. Conversely, only age exerts an independent effect on the flattening of the circadian slope after age and puberty are both examined, suggesting that this maturational effect is driven primarily by age-related processes. Chronological age captures more than just the passage of time, particularly in youth (Steinberg et al., 2006; Wohlwill, 1973).

The present findings are also consistent with research showing that girls have higher cortisol than boys (Rosmalen, et al., 2005; Schreiber, et al., 2006; Shirtcliff & Essex, 2008; Susman, et al., 1997). Further, girls have steeper circadian rhythms with more curvature to their slopes at each assessment, indicating a robust and reliable effect. It is tempting to postulate that developmental effects are stronger and apparent earlier because girls advance through puberty sooner than boys, an idea consistent with previous research (Netherton, et al., 2004; Tornhage, 2002). However, by considering the independent and joint influences of age and puberty, the findings illustrate that gender differences are primarily due to age-related processes. When considered together with previous evidence of gender differences in the emergence of depression (Hankin et al., 1998) and other health behaviors and disorders (Zahn-Waxler, Shirtcliff, & Marceau, 2008) during the adolescent transition, the findings set the stage for future studies designed specifically to investigate the functional significance of gender differences in trait-like cortisol. This underscores the importance of gender differences in developmental research involving HPA axis activity.

Finally, the study also considered potential effects of statistical control variables on HPA development. Previous studies have shown that the HPA axis interacts with many metabolic functions that may contribute to being overweight and obese (Dallman et al., 2004). We find BMI is associated with lower cortisol and flatter rhythms which is not consistent with prior studies (Kiess, et al., 1995; Rosmalen, et al., 2005). We remain cautious until there is longitudinal replication of these findings. Similarly, previous findings are inconsistent regarding medication effects and strategies for statistically controlling medications, though there may be utility in grouping according to the mechanism of action on the HPA axis (Granger, et al., 2009), as done here. Our null findings may differ from other literature for

two reasons. First, our fine-grained approach examines medication usage at the moment of sample collection rather than categorizing children as on/off medications regardless of whether they are actually taking the medication on that day. Second, in accordance with sample instructions, relatively few samples are collected while children in this cohort are on medications (fewer than 7% of any particular type). Different patterns of medication usage may be evident across cohorts or development. Regardless, this finding suggests that medications effects on HPA axis activity may be minimal, at least in community samples of school-age children.

A number of limitations should be considered. First, biomarkers were limited to salivary cortisol which only indicates the adrenal level of the HPA axis. Second, we did not have measures of developmental stage beyond age and puberty to ensure that boys and girls were at comparable stages, although the longitudinal pubertal measures assuage some of those issues. Third, it would have been ideal to measure Tanner stage by physical exam, although our prior work illustrates a reliable correlation between physical exam and our measure of puberty (Shirtcliff, et al., 2009). Finally, longitudinal health measures beyond the indirect information provided by medication usage could be beneficial. Future studies should address these issues. Nevertheless, the study provides strong evidence for a trait-like component of cortisol and its circadian rhythm, which sets the stage for future investigations aimed at understanding putative mechanisms underlying the development of an individual's trait-like cortisol functioning.

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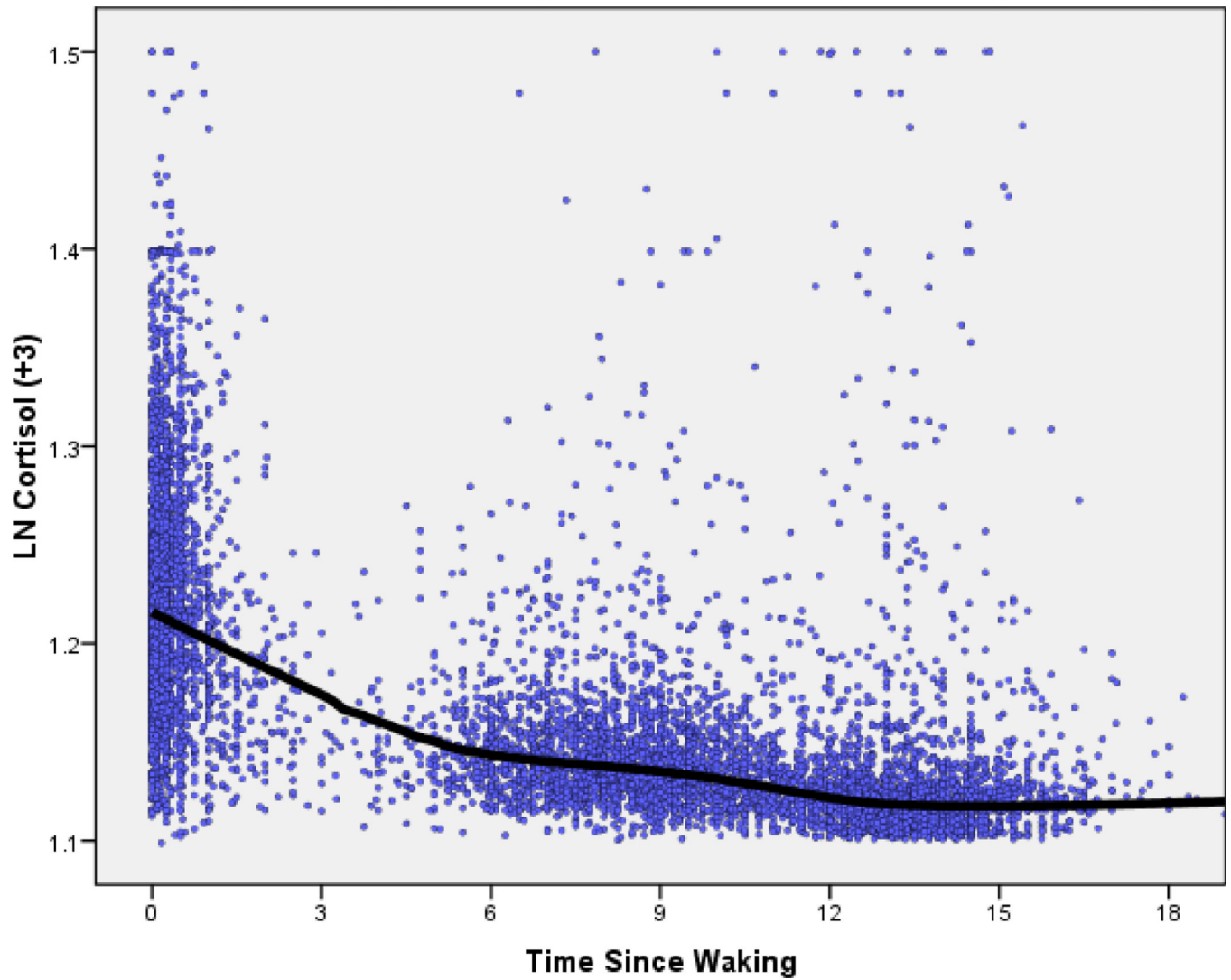


Figure 1. Cortisol levels were highly variable across the day, across years, and across individuals. The diurnal rhythm was steeper in the morning than the afternoon, though levels declined consistently across waking hours.

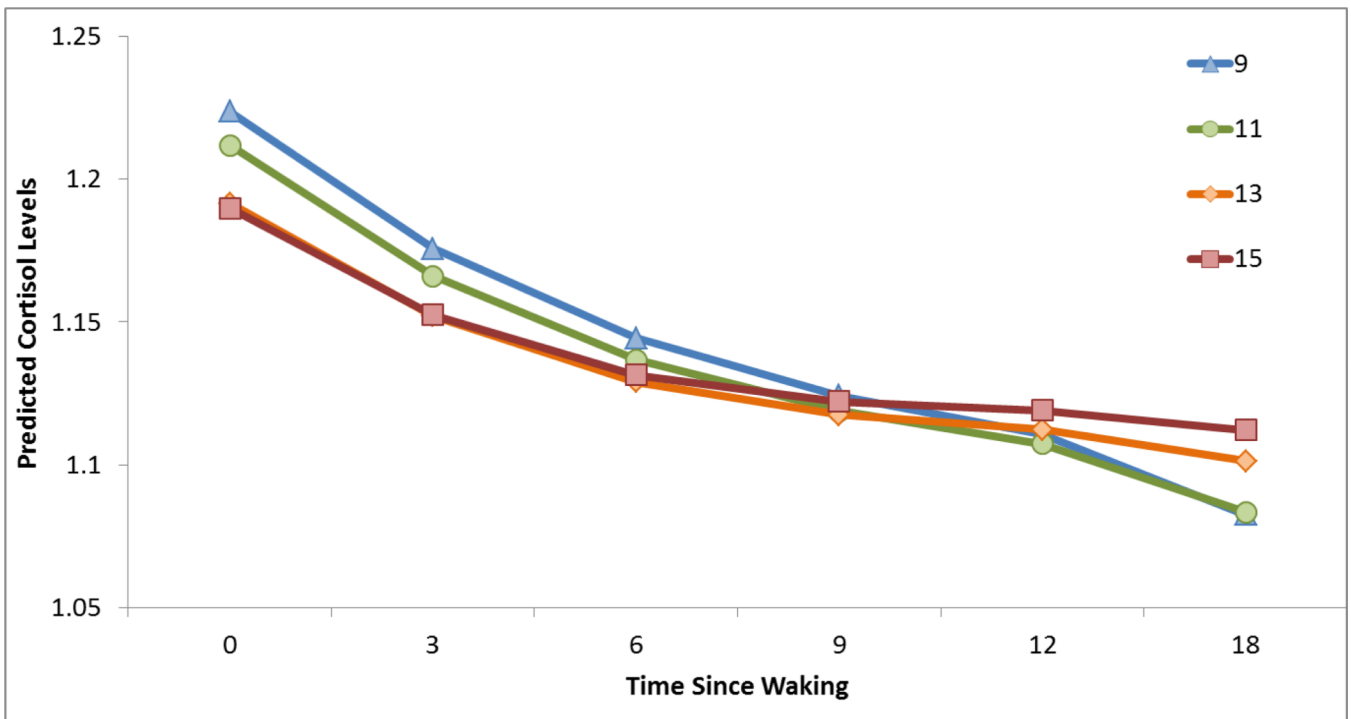


Figure 2. As children got older, their cortisol levels declined and their diurnal rhythm flattened. As 9 year olds, children had the highest cortisol upon awakening and the steepest slopes, but by the time they were 15 year olds, their cortisol levels were significantly lower and the slopes were flatter. The effect of age was quadratic, such that 13 year olds had the lowest morning cortisol values.

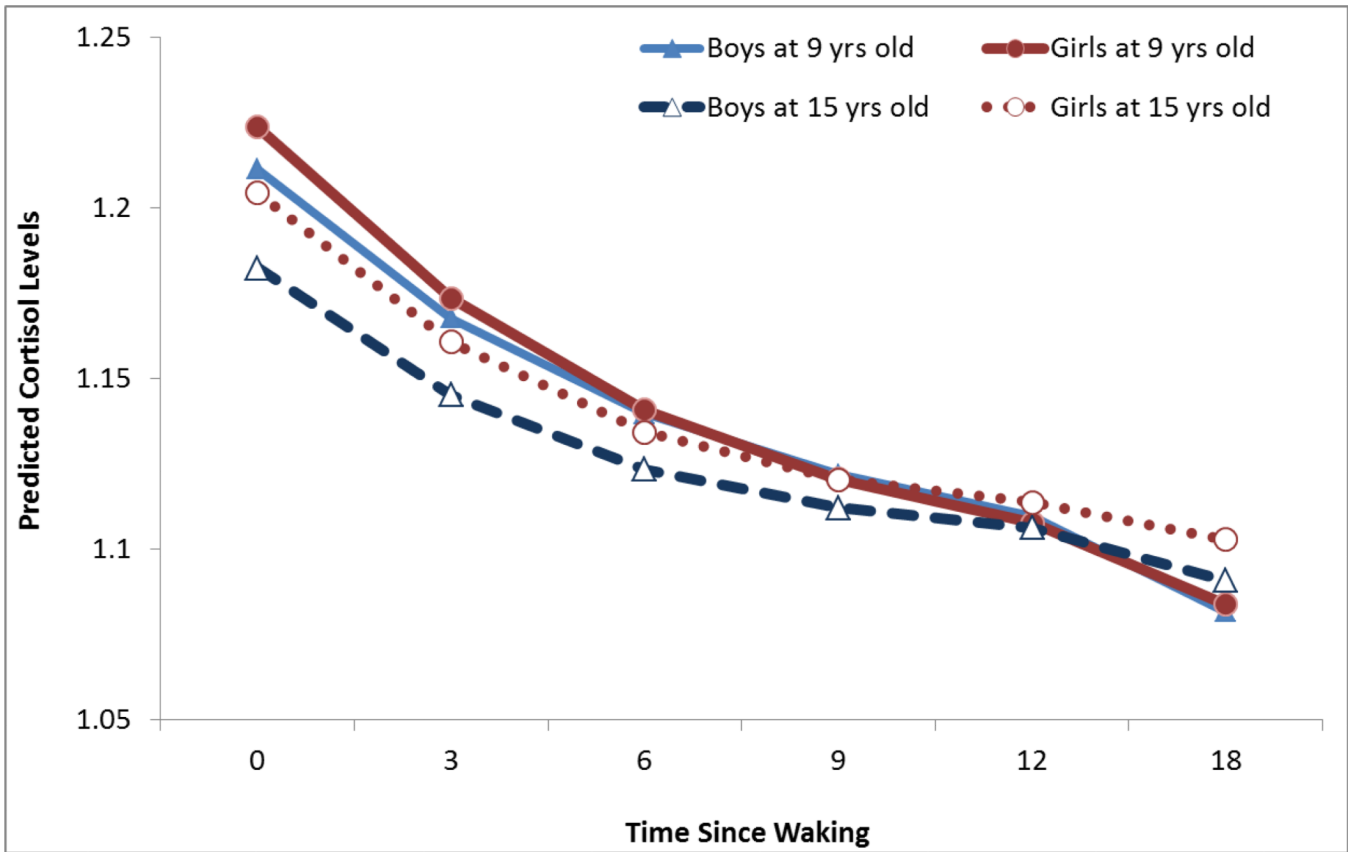


Figure 3. The effect of age on cortisol levels and the diurnal slope was quadratic and moderated by gender. At age 9, for example, girls had the highest cortisol upon awakening and the steepest slopes, but by the time they were 15 year olds, their cortisol levels were significantly lower and the slopes were flatter such that 15 year old girls had the highest cortisol levels in the evening. On the other hand, 9 year old boys had lower cortisol levels and flatter slopes than the 9 year old girls, and by the time they were 15 year olds, boys had the lowest cortisol levels in the morning and also flat slopes so they ended the day having high evening cortisol.