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Within-Person Coupling of Changes in Cortisol, Testosterone, and DHEA Across the Day in Adolescents

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Abstract

We comprehensively examined within-person and between-person associations between cortisol and DHEA and cortisol and testosterone across the day. Data are from a sample of 213 adolescents aged 11–16 (M = 13.7, SD = 1.5 years) from the Northeastern US who were oversampled for psychopathology symptoms. Six repeated measures of hormone levels across 3 days were used to test three specific questions of cortisol-DHEA and cortisol-testosterone associations within individuals (coupling) across the day, and one question of cortisol-DHEA and cortisol-testosterone diurnal slopes were associated between adolescents. Results consistently revealed positive cortisol-DHEA and cortisol-testosterone coupling across the day, often more pronounced in girls relative to boys. Cortisol and DHEA slopes were positively associated, whereas cortisol and testosterone were negatively associated between-adolescents. Findings suggest multiple mechanisms and highlight the multifaceted nature of associations of hormone changes during adolescence and importance of considering both axes for between- and within-person aspects of neuroendocrine development.

Keywords

within-person coupling; cortisol; testosterone; DHEA; diurnal

INTRODUCTION

Hormonal end-products of the Hypothalamic–Pituitary–Adrenal (HPA) and Hypothalamic–Pituitary–Gonadal (HPG) axes share many of the same properties, including similar (though not identical) circadian rhythm profiles characterized by a general decline across the

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daytime hours (e.g., Brown et al., 2008; Essex et al., 2011; Granger et al., 2003; Ice, Katz-Stein, Himes, & Kane, 2004; Mitamura et al., 1999, 2000; Shirtcliff, Zahn-Waxler, Klimes-Dougan, & Slattery, 2007). Furthermore, these two axes are highly interactive, with empirical evidence in adults and animal studies indicating that increased activity in one axis suppresses activity in the other axis (Mastorakos, Pavlatou, & Mizamtsidi, 2006; Rivier & Rivest, 1991; Viau, 2002). The field has begun to acknowledge the benefits of examining multi-system interactions given that various systems within the body work in concert, not in isolation (e.g., Bauer, Quas, & Boyce, 2002; Hastings et al., 2011). Such an approach would ideally be conducted on a within-person level, examining how systems or hormones operate over time *within* an individual. Nonetheless, most research examines each axis in isolation or examines cross-talk using between-person approaches, which are meaningful but address a fundamentally different research question. Further, less work has been done examining the two axes in adolescents, when both axes undergo substantial development. This gap in the research makes it difficult to ascertain whether hormone systems work together differently during development than during adulthood. Current research illustrates the value of using a within-person approach by showing the level of one hormone may indeed influence the level of another hormone in terms of morning level (Ruttle et al., in press); however, it remains undetermined whether changes over the course of the day influence each other. The present study therefore focuses on shared diurnal rhythmicity of these hormones to investigate how patterns of change in cortisol, DHEA, and testosterone are associated across the day during the important developmental transition of adolescence.

Between- and Within-Person Associations

Early biobehavioral investigations into HPA or HPG functioning emphasized between-individual differences of each hormone with behavior, characterizing each individual, for example, as a person with low or high levels of a given hormone relative to other individuals (Dabbs, Frady, Carr, & Besch, 1987; Kagan, Reznick, & Snidman, 1988; Susman et al., 1987). As this research area burgeoned, however, the importance of dynamic within-person changes was increasingly appreciated (e.g., Dickerson & Kemeny, 2004; Eatough, Shirtcliff, Hanson, & Pollak, 2009; Marceau, Dorn, & Susman, 2012; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003; Susman, Dorn, Inoff-Germain, Nottelman, & Chrousos, 1997) and used to illustrate different underlying mechanisms for within-person hormone change (e.g., Booth, Granger, Mazur, & Kivlighan, 2006; Del Giudice, Ellis, & Shirtcliff, 2011; Van Hulle, Shirtcliff, Lemery-Chalfant, & Goldsmith, 2012). This has led to an increased appreciation that a single measure of cortisol, DHEA or testosterone is influenced by several different factors, such as an individual's basal level (Shirtcliff & Essex, 2008; Shirtcliff, Granger, Booth, & Johnson, 2005; Wirth & Schultheiss, 2007), the circadian rhythm (Brown et al., 2008; Granger et al., 2003; Goodyer, Park, Netherton, & Herbert, 2001; Ice et al., 2004; Kirschbaum & Hellhammer, 1994; Klimes-Dougan et al., 2001), awakening response (Fries, Dettenborn, & Kirschbaum, 2009; Wust, Wolf, Hellhammer, Federenko, & Kirschbaum, 2000), distal environmental factors (Essex, Klein, Cho, & Kalin, 2002; Gunnar, Morison, Chisholm, & Schuder, 2001; Halligan, Herbert, Goodyer, & Murray, 2004; Heim et al., 2002; Tarullo and Gunnar, 2006, see Matthews, 2002 and Repetti, Taylor, & Seeman, 2002 for reviews), or concurrent contextual factors (Booth, Johnson, Granger, Crouter, & McHale, 2003; Dickerson & Kemeny, 2004; Dorn et al.,

2009; Fang et al., 2009). The present study builds from this dynamic viewpoint by considering the hormonal milieu, acknowledging that each hormone likely influences other hormones within-individuals. Thus, we examine how each hormone may be differentially related to one another across HPA and HPG axes.

A multiple neurobiological system approach is increasingly championed in the literature in conceptual models that emphasize regulation often involves counter-regulatory processes across systems and dynamic coordination of regulation (Bauer et al., 2002; Koob & Le Moal, 2008; Lupien et al., 2006). Dysregulated patterns may be better represented across physiological systems rather than through changes within any one given system, shaping the physiological processes as they unfold across development, potentially shaping the course of psychopathology (Hastings et al., 2011; El-Sheikh, Erath, Bukhalt, Granger, & Mize, 2008). This multi-system approach may apply to a wide range of regulatory systems, and the current paper emphasizes that the HPA and HPG axes jointly may be more informative than either axis alone (Mehta, Jones, & Josephs, 2008; Mehta & Josephs, 2010; Montoya, Terburg, Bos, & Van Honk, 2012). How to statistically and methodologically capture such dynamic interplay, however, has received less attention than the conceptual idea of functional cross-axis talk.

When examining hormone changes, researchers can take a between-person approach, comparing levels or changes in hormones across people, or a within-person approach, examining levels or changes in hormones of individuals over time (see Nesselroade and Baltes, 1979; Ram & Nesselroade, 2007; Schwartz & Stone, 1998 for a general discussion of between- versus within-person approaches). Between-person approaches compare the relative level (i.e., basal levels) or change (i.e., diurnal slope) of each hormone to the relative levels or changes of other hormones across different people. For example, “do individuals with a steeper-than-average diurnal cortisol slope also have a steeper-than-average diurnal testosterone slope?” Using a between-person approach, changes in each hormone are assessed separately (i.e., using separate growth curve models), without considering the levels or changes of other hormones (i.e., hormonal milieu or endogenous context), and each individual ends up with a single score reflecting change in a particular hormone. Then, the correlation of the change scores (i.e., cortisol slope and testosterone slope) is tested. There has been some work using a between-person approach suggests that diurnal slopes of different hormones are associated. Mitamura and colleagues found that the diurnal changes among HPG hormones (i.e., leutinizing hormone, follicle-stimulating hormone, testosterone, estradiol) were positively associated for girls and boys (Mitamura et al., 1999, 2000). However, recently others using a between-person approach have found no associations between trajectories of cortisol responsivity and trajectories of diurnal testosterone across adolescence (Susman, Peckins et al., in preparation). In the current study, the between-person approach is useful for understanding how diurnal changes in two hormones are associated across people, but it does not examine how changes of hormones transpire within a person.

Within-person approaches consider the level of a hormone relative to levels of that hormone at other times (i.e., the other repeated measures), one person at a time. For associations, within-person approaches are able to capitalize on the fact that hormones do not function in

isolation from other hormones. A strength of the within-person approach is that when testing associations between two hormones, each hormone serves as a contextual factor for the other. Thus, we are able to consider the level of a hormone to the level of the other hormone simultaneously within each individual, and track how changes in the two hormones converge or differ over time. For example, "Across the day, is testosterone higher when an individual's cortisol is higher?" is the within-person analog of the question investigated above. It is important to consider both between-person and within-person changes across the day because each approach provides different information about how the HPA and HPG axes work together or in opposition. By considering the interpretation of both within- and between-individual cross-talk across the axes, a nuanced understanding may help to form theories about how and why hormone biomarkers function together. Until this issue, no studies were identified that examined within-person changes in multiple hormones across the day.

Four Ways to Examine Changes in Multiple Hormones Across the Day

Theories of change separate within-person changes into intraindividual change, which constitutes a strong shape of change definable by a function such as a growth curve, and intraindividual variability, which describes fluctuations that do not conform to a predictable, shape of change (Nesselroade, 1991; Ram & Gerstorf, 2009). Applied to influences on hormone levels across the day, changes in hormone functioning across the day conceptually include the diurnal pattern or slope governed by the pulsatile release and circadian rhythms (the intra-individual change component) and deviations from diurnal slope, for example, driven by environmental factors experienced during the day (the intraindividual variability component). The diurnal slope and deviations from the diurnal slope each may influence a single measure of a hormone to varying degrees, and potentially the interactive links over time or coupling (Ram & Nesselroade, 2007) of a hormone with another. Thus, in the present study, we conducted a set of analyses which together provide a clearer explanation of how cortisol and DHEA and cortisol and testosterone are coupled within individual adolescents.

First, we ask "Are changes in DHEA and testosterone across the day and changes in cortisol across the day coupled within individuals?" That is, across the day, when one hormone decreases (or increases) more sharply within an individual another hormone may also decrease (or increase) more sharply, so the diurnal changes in two hormones are coupled over the course of the day. This question is the most general, indexing change that includes both diurnal slopes and deviations from diurnal slopes. Another possibility is that the general diurnal decline in DHEA or testosterone may pull cortisol off of its expected diurnal rhythm, contributing to deviations in cortisol from its expected diurnal pattern. So next, we ask "Are *deviations* of cortisol from the diurnal slope associated with changes in DHEA and testosterone across the day?" Coupling in this instance would likely represent dysregulation in the allostatic balance (Sterling & Eyer, 1988) of hormone axes, as a smooth diurnal decline has been shown for each hormone. However, in this case we cannot distinguish whether the diurnal pattern of DHEA or testosterone or deviations of DHEA or testosterone from the diurnal pattern is associated with deviations in cortisol from its diurnal slope. Thus, we also tested whether deviations of cortisol and of DHEA or testosterone from their

respective diurnal slopes are coupled within people: "Are *deviations* of cortisol from the diurnal slope associated with *deviations* of DHEA and testosterone from its diurnal slope across the day?" That is, at moments when one hormone strays from its expected diurnal pattern, another hormone may also stray from its expected diurnal pattern in the same direction (positive coupling) or opposite direction (inverse coupling). These coupled deviations from diurnal patterns likely represent changes in both hormones in response to some environmental influence, and are expected to most closely match findings in studies assessing responsivity to specific stressors.

While seemingly overlapping and potentially convergent, these three models are not identical, and lack of convergence would provide useful insights into the mechanisms driving cross-axis hormone coupling. Finding evidence of coupling in the first model would provide a general indication that cortisol and DHEA and/or cortisol and testosterone change together across the day within individuals. Finding evidence of coupling in the first model but not the second model would suggest that diurnal patterns of hormones are associated within individuals because of the similar secretion patterns, or a general diurnal regulatory process. If coupling parameters are significant in the second analysis (i.e. positive coupling of diurnal cortisol deviations with DHEA change or testosterone change) and non-significant in the third (i.e. uncoupled diurnal deviations), then there is evidence that diurnal DHEA or testosterone patterns are associated with deviations from diurnal cortisol patterns within-individuals—potentially indicating a state of dysregulated hormonal milieu. That is, environmentally induced changes in cortisol could result in altered diurnal decline in DHEA and testosterone, or specifically diurnal changes in DHEA or testosterone could result in deviations in cortisol from its diurnal pattern. Finding evidence of coupling in the first and third models, or all three models, would suggest that there are two different mechanisms of within-person hormone coupling—one that reflects a general diurnal regulatory process and one that reflects common processes potentially in response to environmental stimuli experienced during the day. Given that research has only begun to examine within-individual coupling of hormones, understanding which mechanisms may underlie within-person associations is an important first step in this line of research.

Finally, the fourth way diurnal changes in hormones can be associated is at the between-person level, as described above. Thus, we also ask "do individuals with a steeper-than-average diurnal cortisol slope also have a steeper-than-average diurnal testosterone slope?" assessing whether the diurnal slopes of two hormones were associated between individuals. Together, the between-person approach and within-person approaches considering both intraindividual change and intraindividual variability provide a nuanced picture of how hormone changes are associated across the day. Understanding the way(s) in which hormones are associated across the day may help to fine-tune theories of hormone and behavior development.

Present Study

In the present study, we examined within-person coupling of diurnal changes in HPA and HPG hormones during adolescence when the systems are undergoing development. A decreasing diurnal slope of cortisol (Klimes-Dougan et al., 2001), DHEA (Shirtcliff et al.,

2007) and testosterone (Granger et al., 2003) has already been identified in the data used in the present study. Following these studies, we tested four specific questions (1) “Are changes in cortisol across the day and changes in DHEA and testosterone across the day coupled within individuals?” (2) Are *deviations* of cortisol from the linear diurnal slope associated with changes in DHEA and testosterone across the day?” (3) “Are *deviations* of cortisol from the diurnal slope associated with deviations of DHEA and testosterone from its diurnal slope?”, and (4) “do individuals with a steeper-than-average diurnal cortisol slope also have a steeper-than-average diurnal testosterone slope?” The fourth question is included to determine if results differed depending on whether associations were examined between- versus within-persons.

It was hypothesized that positive associations between cortisol, DHEA, and testosterone within-individuals would be identified, consistent with the emphasis within this issue on the observation of positive coupling of HPA and HPG hormones in adolescence. Examining cortisol in conjunction with testosterone and DHEA during adolescence may clarify the hormonal milieu and action of cortisol, particularly during adolescence when hormones change considerably. However, given the novelty of HPA–HPG axis coupling, it was exploratory as to which of the first three questions would yield significant positive coupling. For the between-person associations of cortisol, DHEA, and testosterone, we expected to find a positive association between cortisol and DHEA slopes (based on positive associations of hormones within the same axis; Mitamura et al., 1999, 2000), but were unable to draw hypotheses about the association between cortisol and testosterone diurnal slopes based on the literature. Due to studies suggesting a developmental shift in HPA-HPG hormone coupling (Ruttle et al., in press) and because of sex differences in endocrine development (e.g., Granger et al., 2003; Nottelmann et al., 1987; Susman, Dorn, & Chrousos, 1991), we also tested whether age and sex moderated within-person associations. Because our sample was recruited to include a preponderance of adolescents with elevated levels of psychopathology symptoms, we also examined if hormone coupling was influenced by concurrent psychopathology symptoms.

METHODS

Participants and Procedures

Data were drawn from the Adolescent Emotions Study (Klimes-Dougan et al., 2001), a sample consisting of 213 families of adolescents and their parents who participated in a longitudinal investigation of the role of emotion in the development of psychopathology. Participants were recruited through announcements in newspapers and flyers in the Washington DC metropolitan area. Adolescents were over-sampled for internalizing and externalizing psychopathology. Approximately 1/3 of the adolescents were in the normal range of problems, 1/3 had sub-clinical problems, and 1/3 had clinical problems. Participants were balanced during recruitment for approximately equal proportions of youth with internalizing, externalizing, and comorbid internalizing and externalizing psychopathology among those with sub-clinical and clinical levels of psychopathology (Klimes-Dougan et al., 2001). Youth ranged in age from 11 to 16 (M = 13.7, SD = 1.5 years), and were about 50% male (N male = 106).

Participation occurred on three separate days. One day consisted of adolescents collecting three saliva samples at home. On a separate day families came in for a laboratory visit where youth participated in a social performance paradigm and on a third day experimenters visited families at home and parent–youth dyads filled out questionnaires and participated in a mother–youth and a father–youth conflict discussion task.

Measures

Hormone Assessment—Three saliva samples were collected at home on 1 day to measure diurnal hormone patterns—average sample collection times were 7:23 am (*SD* = 1 hr 12 min), 11:46 am (*SD* = 56 min), and 4:20 pm (*SD* = 1 hr 43 min). On the day of the laboratory visit, a saliva sample was taken in the morning, before the visit, average time = 7:12 am (*SD* = 1 hr 12 min). Additionally, a baseline sample was collected at the lab visit at approximately 11:16 am (*SD* = 27 min) preceding the lab-based social performance paradigm, and a baseline sample was collected in the late afternoon on the day of the home visit (a separate day), preceding the conflict-discussion paradigm at approximately 4:10 pm (*SD* = 40 min; see Klimes-Dougan et al., 2001 for more detail). The pre-visit, and pre-task baseline saliva samples for the two tasks are combined to approximate a second day's diurnal pattern, as has been done previously in this sample (Granger et al., 2003; Klimes-Dougan et al., 2001; Shirtcliff et al., 2007).

Testosterone, DHEA, and cortisol were assayed from saliva collected through expectorating approximately 5 ml of saliva into a test tube, and supervised by research assistants when in the lab and by parents in the home. Participants did not eat during the 30 min prior to each saliva collection. The saliva was stored in the tubes at −25°C. After being shipped overnight on dry ice to the Pennsylvania State University Behavioral Endocrinology Laboratory, saliva was stored at −86°C until assayed in duplicate. Youth with hormone levels over 2.5 *SD* of the sample mean on any assessment, calculated separately for boys and girls (<4%) were windsorized to 2.5 *SD* values, and then scores were log-transformed. Sample descriptive statistics are presented in Table 1.

Covariates—We used age in years and sex (1 = female, 0 = male) as covariates. We also used severity and directionality of psychopathology symptoms. Internalizing and externalizing behavior problems were measured using the parent and youth report internalizing and externalizing subscales on the Child Behavior Checklist/Youth Self Report (Achenbach, 1991). *T*-scores for mother, father, and youth-reported internalizing and externalizing problems are presented in Table 1 (scores below 60 = in the normal range: between 60 and 63 = sub-clinical; above 63 = clinical levels of problems). Principal component weights were used to create composite internalizing and externalizing scores using all three informants to generate a score where all three reporters converged on the same score. These constructs explained 56% and 64% of the total variance in internalizing and externalizing symptoms, respectively; all factor loadings were >.64. To address the comorbidity of internalizing and externalizing symptoms (r = .64, p <.001), the two multi-informant scores were used to construct measures of symptom *Severity* (average of the standardized scores, reflecting what the two scores have in common) and *Directionality* (half difference of the standardized scores, which reflects what differentiates the two scores;

positive score indicates a preponderance of externalizing vs. internalizing symptoms; Essex et al., 2006).

Data Analysis and Results

Prior to hypothesis testing, we examined whether there was any variance in each hormone attributable to different days using a 3-level mixed effects model. Results showed there was no variance in day for any hormone (*u's* <.0001, *SE*s <.0001, *p's* >.05); therefore, we collapsed across both days as has been done previously in this sample (e.g., Granger et al., 2003; Klimes-Dougan et al., 2001; Shirtcliff et al., 2007). Data analysis and results are presented for each question separately.

(1) Are changes in cortisol across the day and changes in DHEA and testosterone across the day coupled within individuals?—To test the first question, two bivariate models were conducted: (1) DHEA predicting cortisol, (2) testosterone predicting cortisol, and then a trivariate model was conducted, with both DHEA and testosterone simultaneously predicting cortisol. Bivariate models assess coupling between two hormones, whereas in the trivariate model, coupling parameters represent the coupling between two hormones while controlling on the coupling of the focal hormone with the third hormone (i.e., cortisol-DHEA coupling controlling for cortisol-testosterone coupling, and vice versa). Thus, in the trivariate model cortisol-DHEA likely represents coupling of purely adrenal hormones and cortisol-testosterone coupling likely represents coupling between stress and reproductive hormones, as any overlapping variance between DHEA coupling and testosterone coupling due to puberty or gonadal maturation is accounted for. The first bivariate model is described below.

Level 1 was specified as:

$$\text{Cortisol}_{ti}=\beta_{0i}+\beta_{1i}(\text{DHEA}_{ti})+e_{ti} \quad (1)$$

where Cortisol_{ti} is individual *i*'s cortisol level at time *t*, β_{0i} is the intercept term indicating the individual's average predicted cortisol, β_{1i} is the coefficient describing the coupling between changes in cortisol and changes in DHEA, and e_{ti} contains individual *i*'s residual errors. As noted above, this analysis provides a very general understanding of whether changes across the day in each hormone are coupled within individuals. For the trivariate models, testosterone was added as an additional predictor (β_{2i}). Coupling parameters from these models are referred to as *coupled changes*. These changes are comprised of the diurnal slope as well as deviations from the diurnal slope of each hormone, but do not distinguish whether within-person coupling is driven by diurnal slopes, or deviations from diurnal slopes.

After the main effects models were run, covariates were added on level 2, thus testing for between-person differences in intercept levels of cortisol and coupling attributable to age, sex, severity and directionality of psychopathology symptoms.

Level 2 was specified as:

$$\beta_{0i}=\gamma_{00}+\gamma_{01}\,(\text{age})+\gamma_{02}\,(\text{sex})+\gamma_{03}\,(\text{severity})+\gamma_{04}\,(\text{directionality})+u_{0i}$$
$$\beta_{1i}=\gamma_{10}+\gamma_{11}\,(\text{age})+\gamma_{12}\,(\text{sex})+\gamma_{13}\,(\text{severity})+\gamma_{14}\,(\text{directionality})+u_{1i} \quad (2)$$

Results: In the main effects bivariate models, cortisol and DHEA were positively coupled, β_{1i} = .05, *SE* = .003, p <.05, as were cortisol and testosterone, β_{1i} = .02, *SE* = .002, p <.05. In the trivariate model, cortisol and DHEA remained positively coupled, β_{1i} = .02, *SE* = .007, p <.05, whereas testosterone and cortisol were not coupled, β_{2i} = .01, *SE* = .007, p >.05.

Results from the analyses including covariates are presented in left-hand panels of Tables 2–4. There was no longer a main effect of cortisol-DHEA coupling in the bivariate model (Table 2, γ_{10}). Instead, there was a sex by coupling interaction (Table 2, γ_{12}). Interactions between coupling and sex were probed by re-running the model separately for boys and girls; and revealed that there was significant positive coupling for both boys and girls, but coupling was stronger on average for girls than boys. Similarly, there was no longer a main effect of cortisol-testosterone coupling (Tab. 3, γ_{10}) but there was a sex by coupling interaction such that there was significant positive coupling for both boys and girls, but coupling was stronger on average for girls than boys (Tab. 3, γ_{12}). Both coupling by sex interaction effects persisted in the trivariate model (Tab. 4, γ_{12}, γ_{22}).

(2) Are deviations of cortisol from the linear diurnal slope associated with changes in DHEA and testosterone across the day?—As before, two bivariate and one trivariate model were conducted. Level 1 was specified as:

$$\text{Cortisol}_{ti}=\beta_{0i}+\beta_{1i}(\text{DHEA}_{ti})+\beta_{2i}(\text{time}_{ti})+e_{ti} \quad (3)$$

where Cortisol_{ti} is individual *i*'s cortisol level at time *t*, β_{0i} is the intercept term indicating the predicted score of cortisol at each individual's first saliva sample, β_{1i} is the coefficient describing the coupling between changes in cortisol and changes in DHEA, β_{2i} is the coefficient describing linear change in cortisol across the day (i.e. the diurnal slope), and e_{ti} contains individual *i*'s residual errors. For the trivariate models, testosterone was added as an additional predictor (β_{2i}), and the effect of time was β_{3i}. This analysis controls on the diurnal slope of cortisol in order to examine how deviations from the diurnal slope of cortisol are associated with changes in, in this case, DHEA (both diurnal slopes and deviations from the diurnal slope). Coupling parameters from this model are referred to as *cortisol deviations with DHEA change or testosterone change*. As above, the main effects models were run first, and then covariates were added on level 2 as predictors of between-person differences in cortisol levels, age (γ_{01}), sex (γ_{02}), severity (γ_{03}), directionality (γ_{04}), and coupled cortisol deviations with DHEA or testosterone change, age (γ_{11}), sex (γ_{12}), severity (γ_{13}), directionality (γ_{14}).

Results: In the bivariate main effects models, cortisol deviations were positively associated with changes in DHEA, β_{1i} = .02, *SE* = .002, p <.05, however, cortisol deviations were positively associated with changes in testosterone, β_{1i} = .01, *SE* = .002, p <.05. In the trivariate model, the positive coupling of cortisol deviations and DHEA changes persisted,

β_{1i} = .02, *SE* = .006, p <.05, but there was no coupling between cortisol deviations and testosterone changes, β_{2i} = .01, *SE* = .007, p >.05.

Results from the analyses including covariates are presented in center panels of Tables 2–4. There was no longer a main effect of coupling between cortisol deviations and DHEA changes in the bivariate model (Tab. 2, γ_{10}). However, there was a sex by coupling interaction for coupled changes deviations and DHEA changes such that there was significant positive coupling for both boys and girls, but coupling was stronger on average for girls than boys (Tab. 2, γ_{12}). Similarly, there was a sex by coupling interaction in the bivariate model including testosterone such that there was significant positive coupling for both boys and girls, but coupling was stronger on average for girls than boys (Tab. 3, γ_{12}). In the trivariate model, both sex by coupling interactions persisted (Tab. 4, γ_{12}, γ_{22}).

(3) Are deviations of cortisol from the linear diurnal slope associated with deviations of DHEA or testosterone from its diurnal slope?—In order to test the third question of whether the diurnal slope of DHEA or testoster-one and/or the deviations from the DHEA or testosterone diurnal slope may drive deviations in cortisol from its diurnal slope, we started by conducting additional data preparation. Mixed effects models were conducted for DHEA and testosterone where we modeled linear diurnal change across the day:

$$\begin{aligned} &\text{Level 1} \quad \text{DHEA}_{ti} = \beta_{0i} + \beta_{1i}(\text{time}) + e_{ti} \\ &\text{Level 2} \qquad \beta_{0i} = \gamma_{00} + u_{0i} \\ &\qquad\qquad\quad \beta_{1i} = \gamma_{10} + u_{1i} \end{aligned} \tag{4}$$

where $DHEA_{ti}$ is individual i's log-transformed DHEA at collection t, β_{0i} is the intercept term indicating the DHEA level at the first collection, β_{1i} is the parameter estimating the diurnal slope of DHEA, u_{0i} and u_{0i} are individual-specific error terms for the intercept and slope, and e_{ti} is individual i's residual scores. We extracted the empirical Bayes estimate for each individual's DHEA and testosterone slope scores across the six collections. These scores provide the single estimate of each individual's DHEA and testosterone diurnal slope, as estimated in a growth curve model, and are therefore appropriate to use to examine between-person associations of the diurnal slopes of cortisol, DHEA, and testosterone (i.e., for question 4, below). We also extracted the empirical Bayes estimates for each individual's residuals at each collection at each assessment, or the leftover variance in hormones across the day after accounting for the diurnal slope, (e_{ti}) which describe the extent to which the hormone level deviated from each individual's diurnal pattern for DHEA and testosterone to be used in hypothesis testing. Here, each individual's residuals are a set of six scores that capture the variance in DHEA or testosterone, respectively, left to be explained after modeling the diurnal slope. This is analogous to the residual variance in cortisol in question 2.

In order to test whether *deviations* of cortisol from its diurnal slope were associated with deviations of DHEA or testosterone from its diurnal slope, two bivariate models and one trivariate model were conducted, as above. The first model is described below.

Level 1 was specified as:

$$\text{Cortisol}_{ti}=\beta_{0i}+\beta_{1i}(\text{DHEA}_{\text{residual s}ti})+\beta_{2i}(\text{time}_{ti})+e_{ti} \quad (5)$$

where Cortisol_{ti} is individual *i*'s cortisol level at time *t*, β_{0i} is the intercept term indicating the predicted score of cortisol at each individual's first saliva sample, β_{1i} is the coefficient describing the coupling between deviations in cortisol and deviations in DHEA from their diurnal patterns, β_{2i} is the coefficient describing linear change in cortisol across the day, and e_{ti} is individual *i*'s residual errors. For the trivariate models, testosterone was added as an additional predictor (β_{2i}), and the effect of time was β_{3i}. The coupling parameters from this model are referred to as *coupled diurnal deviations*. This analysis clarifies the second question by extracting the diurnal slope of DHEA and examining how deviations from each diurnal pattern (of cortisol and of DHEA) are associated. As above, the main effects models were run first, and then covariates were added on level 2 as predictors of between-person differences in cortisol levels, age (γ_{01}), sex (γ_{02}), severity (γ_{03}), directionality (γ_{04}), and coupled cortisol deviations with DHEA or testosterone change, age (γ_{11}), sex (γ_{12}), severity (γ_{13}), directionality (γ_{14}). Note, a fifth level 2 predictor was also added (γ_{15}), described below (question 4).

Results: In the main effects bivariate models, there was positive coupling of diurnal deviations of cortisol and DHEA, b_{1i} = .06, *SE* = .005, p <.05, but there was no coupling of diurnal deviations of cortisol and testosterone, β_{1i} = .005, *SE* = .15, p <.05. In the trivariate model, the positive cortisol-DHEA coupling of diurnal deviations persisted, β_{1i} = .03, *SE* = .01, p <.05, and there remained no coupling of diurnal deviations of cortisol and testosterone, β_{2i} = .01, *SE* = .01, p >.05.

Results from the analyses including covariates (and the between-person analysis described below for question 4) are presented in right-hand panels of Tables 2–4. In the bivariate model of cortisol-DHEA coupled diurnal deviations, there was evidence of positively coupled diurnal deviations; contrary to the first two questions, this positive coupling did not differ for boys and girls (Tab. 2, γ_{10}). For the bivariate model of cortisol-testosterone coupled deviations there was a sex by coupling interaction; there was significant positive coupling for both boys and girls, but coupling was stronger on average for girls than boys (Tab. 3, γ_{12}). In the trivariate model there was significant positive coupling of cortisol and DHEA diurnal deviations (Tab. 4, γ_{10}), but no coupling of cortisol and testosterone: the sex effect did not persist. However, an age effect emerged such that positive cortisol-testosterone coupling was stronger among younger adolescents than older adolescents (Tab. 4, γ_{21}).

(4) Do individuals with a steeper-than-average diurnal cortisol slope also have a steeper-than-average diurnal testosterone slope?—To most clearly address between-individual processes, we also conducted analyses which would allow us to examine differences and similarities between within-individual and between-individual approaches. Using the same model described for Question 3, the DHEA slope as a Level 2 predictor of the cortisol intercept (γ_{05}) and slope (γ_{15}). Of primary importance, the between-individual effect of the diurnal slope of DHEA on cortisol slope tests the between-individuals analog of the coupling question: whether individuals with steeper DHEA slopes also have steeper

cortisol slopes. The effects of DHEA slope on cortisol levels (γ_{05}) were not of primary interest, but were necessary to clarify that γ_{15} captures the interaction of DHEA slope and cortisol slope. As above, cortisol-DHEA and cortisol-testosterone bivariate models were run first, followed by a trivariate model. Main effects models were first run, followed by models including covariates.

Results: In the main effects bivariate models, there was a positive association between cortisol and DHEA slopes such that individuals with steeper cortisol slopes relative to other adolescents in the sample also had steeper DHEA slopes, relative to the sample, $\gamma_{15} = .04$, $SE = .02$, $p < .05$, but no association between cortisol and testosterone slopes, $\gamma_{15} = -.01$, $SE = .01$, $p > .05$. These effects persisted in the trivariate model, cortisol-DHEA: $\gamma_{15} = .12$, $SE = .02$, $p < .05$; cortisol-testosterone: $\gamma_{25} = .003$, $SE = .01$, $p > .05$.

Results from the analyses including covariates are presented in right-hand panels of Tables 2–4. In the bivariate models, there was a positive association between cortisol and DHEA slopes (Tab. 2, γ_{15}), but no association between cortisol and testosterone slopes (Tab. 3, γ_{15}). However, in the trivariate model the association between cortisol and DHEA slopes persisted and a negative association between cortisol and testosterone slopes emerged such that individuals with steeper cortisol slopes relative to other adolescents in the sample had shallower testosterone slopes, relative to the sample (Tab. 4, γ_{15}, γ_{25}).

Summary: In the bivariate models of cortisol and DHEA, there was consistent evidence of positive coupling across methods. Further, coupled changes and coupling of cortisol deviations with DHEA changes were stronger for girls than boys. There was also a positive between-person association between cortisol and DHEA diurnal slopes. In the bivariate models of cortisol and testosterone, there was also consistent evidence of positive coupling across methods and that the positive coupling was consistently stronger for girls than boys in all models. In the bivariate model there was no between-person association between cortisol and testosterone diurnal slopes.

On the whole, these findings persisted in the trivariate models, with three notable exceptions (Tab. 4 right panel). First, the sex effect did not persist in the trivariate model for coupled diurnal deviations of cortisol and testosterone. Second, an age effect emerged in the trivariate model for coupled diurnal deviation for cortisol and testosterone such that positive coupling of diurnal deviations of cortisol and testosterone were stronger among younger adolescents than older adolescents. Finally, in the trivariate model controlling for the between-person association between cortisol and DHEA diurnal slopes, there was a significant negative between-person association between cortisol and testosterone diurnal slopes.

DISCUSSION

The present study comprehensively investigated the ways in which cortisol and DHEA, and cortisol and testosterone are associated during adolescence. We tested three within-person questions and one between-person question, all aimed to investigate how cortisol and DHEA and cortisol and testosterone are associated across the day. Generally, we found evidence of

positive within-person associations between cortisol and DHEA and cortisol and testosterone, although results sometimes varied with specific models, suggesting multiple mechanisms underlying within-person associations of hormones across the day. Our findings highlight the implications of the specific question for our understanding of how HPA and HPG axes changes are integrated, and suggest that both between- and within- person approaches are necessary for a fuller understanding of neuroendocrine functioning. Across every within-person question we found positive associations of hormones within the HPA axis and across the HPA and HPG axes. Only when controlling on between-person associations within HPA hormones and other covariates (age, sex, and psychopathology) did a negative between-person cross-axis association emerge, highlighting that consideration of both axes are important for both between- and within-person questions of how hormones are associated across development. These observations add to the growing appreciation that multiple neurobiological systems operate to regulate and counter-regulate the body's physiological setpoints by illustrating such dynamic processes unfold across the day and not just in reactivity to an acute challenge.

Four Ways to Examine Changes in Multiple Hormones Across the Day

We tested three specific ways in which cortisol and DHEA, and cortisol and testosterone may be coupled across the day. Results from these three questions together reveal specific information about potential mechanisms underlying within-person coupling. Results from the first question, "Are changes in cortisol across the day and changes in DHEA and testosterone across the day coupled within individuals?" suggest that cortisol and DHEA and cortisol and testosterone travel together across the day, in the same direction. This evidence is bolstered by results from the second question "Are *deviations* of cortisol from the diurnal slope associated with changes in DHEA and testosterone across the day?" which suggests that changes in DHEA and testosterone may pull cortisol from its diurnal slope. Together, results from these two questions suggest that associations of hormones within individuals may be driven by a general regulatory mechanism potentially reflecting the similar secretion patterns, a common circadian driver in the brain through the suprachiasmatic nucleus and hypothalamus, and in part because cortisol and to a lesser extent testosterone are derivatives of DHEA (see Marceau, Ruttle, Shirtcliff, Essex, & Susman, this issue; Viau, 2002).

In addition, results from the third question "Are *deviations* of cortisol from the linear diurnal slope associated with deviations of DHEA and testosterone from its diurnal slope?" suggest that associations of hormones within individuals may likewise be driven by both hormones deviating from the expected diurnal patterns together, likely because of the same environmental forces influencing each hormone. Each of these hormones have been shown to be responsive to the same laboratory-induced stressors, including MRI (Eatough et al., 2009), venipuncture (Marceau et al., 2012), and competition (Bateup, Booth, Shirtcliff, & Granger, 2002; Mazur, Susman, & Edelbrock, 1997), albeit in differing magnitudes. Our findings for coupled diurnal deviations are consistent with these studies and suggest that all three hormones may be recruited to deal with environmental stressors experienced throughout the day. Thus, measures of the diurnal profile of single hormones are influenced by multiple factors, including time, the functioning of other hormones, and the environmental. The consistent results uncovered by the within-person associations here

further suggest that associations between these hormones are also multifaceted, and so coupling is likely driven by multiple mechanisms.

It may be that in some circumstances the multiple mechanisms that may drive coupling could be differently implicated, and thus positive coupling may not be definite in all circumstances. Often, these within-person associations were identified in both boys and girls, but were stronger for girls than boys when examining coupled changes and coupled cortisol deviations with DHEA and testosterone changes. This pattern of findings is consistent with previous research that has found stronger ties between cortisol and DHEA for women relative to men (e.g., Kroboth, Salek, Pittenger, Fabian, & Frye, 1999). However, the sex differences were not as strong or consistent for the coupled diurnal deviations models. These findings may suggest that while salient for boys and girls, the first potential mechanism—the general regulatory mechanism governing diurnal secretion—is more prominent for girls. Perhaps because testosterone has a more prominent role in boys' development it has a more unique pattern from the stress hormones than is observed in girls. We found no systematic differences in how boys versus girls recruit multiple hormones in response to environmental experiences which pull testosterone or DHEA from its diurnal slope, and also pulls cortisol from its diurnal slope (i.e., coupled diurnal deviations), suggesting that sex differences do not necessarily apply to the second proposed mechanism.

Further, the age effect found in the trivariate model for coupled diurnal deviations suggests that as youth progress through adolescence the positive cross-axis coupling declines, consistent with other studies (Matchock, Dorn, & Susman, 2007; Ruttle et al., in press). It may be that in another developmental context, for example adulthood when the HPA and HPG axes are no longer rapidly developing, the mechanisms of association across hormonal axes may differ. That is it would not be beneficial for the normal developmental increase in HPG hormones during adolescence to suppress adolescents' ability to cope with stressors, as it may be in adulthood because the meaning of increases in HPG hormones differs when occurring in adolescence as a needed aspect of development as compared with adulthood, after development is completed. Thus, the pattern of sex differences and the age effect further highlights the probability of multiple mechanisms of within-person associations in hormone changes during adolescence that are differently implicated given different developmental and environmental contexts. More research is needed to replication the sex and age effects noted here and to probe general biological mechanisms that may explain these sex differences.

Thus, the present findings provide strong evidence of multiple ways in which cortisol and DHEA and cortisol and testosterone are associated within individuals. Sex differences in coupled diurnal changes appear to apply specifically to the general diurnal regulatory mechanism, and not to changes potentially related to environmental cues. However, developmental shifts in HPA-HPG cross talk appears to apply specifically to coupled changes likely related to environmental cues. Interestingly, concurrent psychopathology symptoms did not shape any of the patterns of hormone coupling across the day; however, given that this is the first study to examine this effect, replication is necessary.

Between- and Within-Person Associations

Our findings highlight the differences in conclusions drawn using between- and within-person approaches. There was evidence of a positive between-person association of cortisol and DHEA slopes, suggesting individuals with a steeper-than-average cortisol slope are also more likely to have a steeper-than-average DHEA slope. This paralleled the general findings of positive within-person coupling of cortisol and DHEA across the day. For testosterone, in the bivariate model there was no evidence of the hypothesized inverse between-person association between cortisol and testosterone slopes. However, when cortisol-DHEA and cortisol-testosterone coupling were modeled simultaneously in the trivariate model, an inverse between-person association emerged: adolescents with steeper-than-average cortisol slopes were more likely to have shallower-than average testosterone slopes.

This negative association is in opposition to the positive association found within-individuals in the analogous model (general coupled changes) and suggests that although cortisol and testosterone tend to follow the same pattern within-individuals, these individuals may not have the same rank order when comparing the overall slopes of cortisol and testosterone. That is, the between- and within- person approaches answer different questions. By assessing the two approaches simultaneously, we discovered a seemingly paradoxical effect. Individuals with steeper cortisol slopes have shallower testosterone slopes relative to others in the sample when cortisol and testosterone diurnal changes are considered in isolation. However, when cortisol and testosterone changes are examined in the context of the other hormone, cortisol and testosterone follow generally the same patterns of change across the day within-individuals—both in general diurnal declines and in deviations from diurnal declines. Thus, failing to distinguish explicitly within and between person effects may add to the apparent inconsistencies in the literature.

These findings may not actually be paradoxical, however. The reasons why adolescents differ from each other in regard to correlated hormone changes across the day may not be the same as the reasons an individual adolescent's hormones change together over the course of the day. Coupling may be driven by the mechanisms highlighted above, whereas between-person differences in how slopes are associated may be driven by different indices of individual differences in hormone function generally. In support of this interpretation, basal hormone levels can function in an opposing manner to hormone change (Mehta et al., 2008; Ramsay & Lewis, 2003). Furthermore, the stability of hormone levels is different from the diurnal rhythm (Shirtcliff et al., 2012), suggesting distinct underlying mechanisms. Behavioral genetic studies have shown that morning cortisol is heritable, genetic influences decline across the day, and evening levels of cortisol are mostly explained by environmental influences (Bartels, Van den Berg, Sluyter, Boomsma, & de Geus, 2003; Van Hulle et al., 2012). Thus, there may also be differences in the genetic and environmental influences contributing to between- versus within-person associations of hormones over time, and to the different aspects of change in hormones across the day (i.e., intraindividual change and variability). Future research assessing the etiology of between- and within-person associations of cortisol and DHEA or cortisol and testosterone is needed in order to fully understand how and why correlated hormone changes differ within individuals and between individuals.

Limitations and Future Directions

As with all studies, there are limitations in the current study important for interpreting the current findings. First, we used only cross-sectional data, and thus in the future longitudinal data is needed to understand whether and how coupling across the day changes over time. Second, while we had six repeated measures of each hormone, these were drawn from similar times on three days—there was only one complete day (with three assessments). Thus, our findings may in part reflect day-to-day variation in hormone levels at any given collection time, which could affect the measure of diurnal deviations in particular. That is, diurnal deviations could be confounded with daily differences, although day to day influences may be minimal within each axis (Shirtcliff & Essex, 2008). Relatedly, because of our assessment schedule we were unable to incorporate the known cortisol awakening response and other aspects of hormone changes across the day (i.e., changes in the speed of decline across the day). Finally, we did not have measures of the environment on the day of the cortisol collection. In the future, measures of the specific environment could be incorporated into the coupling models in order to test whether specific environmental influences affect the strength of coupling, and may be particularly informative for models of coupled diurnal deviations.

Nonetheless, our findings showed robust positive associations between cortisol and DHEA and cortisol and testosterone across the day in terms of general declines, deviations in cortisol from its slope and general changes in DHEA and testosterone, and in terms of diurnal deviations. For the more general diurnal models there were sex differences such that coupling for girls was stronger than for boys. There was an interesting effect of age where specifically cross-axis coupling of diurnal deviations were stronger for younger adolescents than older adolescents. Finally, at the between-person level there was an inverse association of cortisol slopes and testosterone slopes only when controlling on the association between cortisol slope and DHEA slope as well as within-person coupling. Our findings highlight the multifaceted nature of associations between changes in hormones during adolescence, and suggest that multiple mechanisms may drive coupling, while other mechanisms may drive between-person associations in hormone changes. Thus, we highlight here that the analytic methods used in studies of change have vast implications in the interpretation of findings as well as the pattern of effects found. In the future, research on hormone changes and associations between hormone changes and behavioral development over time will benefit from careful consideration of the theory and question, and from using multiple approaches to test related sets of questions in order to gain a nuanced understanding of the complexities of neuroendocrine development.

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Table 1

Sex-Specific Descriptive Statistics for Study Variables

	Boys (*N* = 106)		Girls (*N* = 107)	
	Mean	***SD***	**Mean**	***SD***
Cortisol (μg/dl)				
Day 1 collection 1 (7:23 am)	.18	.09	.16	.07
Day 1 collection 2 (11:46 am)	.13	.06	.10	.05
Day 1 collection 3 (4:20 pm)	.11	.05	.09	.04
“Day 2” collection 1 (7:12 am)	.17	.08	.16	.07
“Day 2” collection 2 (11:16 pm)	.19	.08	.17	.07
“Day 2” collection 3 (4:10 pm)	.15	.06	.16	.08
Testosterone (ng/dl)				
Day 1 collection 1 (7:23 am)	25.97	19.24	11.86	5.37
Day 1 collection 2 (11:46 am)	24.47	19.47	10.10	5.07
Day 1 collection 3 (4:20 pm)	22.73	18.53	9.48	4.74
“Day 2” collection 1 (7:12 am)	31.41	20.03	11.67	4.97
“Day 2” collection 2 (11:16 pm)	31.52	22.39	11.98	5.06
“Day 2” collection 3 (4:10 pm)	30.09	21.61	11.61	5.10
DHEA (ng/dl)				
Day 1 collection 1 (7:23 am)	63.24	26.54	83.00	41.78
Day 1 collection 2 (11:46 am)	56.27	24.38	69.29	33.19
Day 1 collection 3 (4:20 pm)	51.74	23.19	65.43	31.30
“Day 2” collection 1 (7:12 am)	59.42	24.78	74.95	33.61
“Day 2” collection 2 (11:16 pm)	65.58	26.72	85.07	44.09
“Day 2” collection 3 (4:10 pm)	63.87	25.47	83.84	40.69
Externalizing T-scores				
Mother report	52.71	11.04	52.05	10.29
Father report	51.23	10.18	51.37	8.73
Youth self report	51.56	10.46	53.24	9.40
Internalizing T-scores				
Mother report	52.34	10.59	52.18	10.31
Father report	51.23	11.38	49.67	9.53
Youth self report	49.04	12.15	52.26	10.13
Age	13.65	1.46	13.66	1.60

Note. Day 2 is comprised of two separate days—the first two collections were the early morning baseline collected on the day of the laboratory visit and the pre-task sample from the laboratory visit, and the third collection was the pre-task sample from the home-visit which occurred on a different day.

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Table 2

Bivariate Diurnal Coupling of Cortisol and DHEA

	Coupled diurnal change		Coupled deviations with change		Coupled diurnal deviations	
Parameter	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***
Fixed effects						
Intercept, γ_{00}	1.70*	.009	1.71*	.006	1.68*	.003
Coupling parameter, γ_{10}	.01	.008	−.002	.006	.04*	.009
Diurnal slope, γ_{20}	—	—	−.004*	<.001	−.005*	<.001
Effect of moderators on Cortisol level						
Age, γ_{01}	.002	.002	.001	.001	.001	.001
Sex, γ_{02}	−.07*	.007	−.05*	.005	<.001	.002
Severity, γ_{03}	−.001	.003	<.001	.002	<.001	.001
Directionality, γ_{04}	<.001	.003	−.001	.002	−.001	.001
DHEA diurnal slope, γ_{05}	—	—	—	—	−.87*	.22
Effect of moderators on Coupling						
Age, γ_{11}	<.001	.001	.001	.001	.002	.002
Sex, γ_{12}	.03*	.005	.02*	.005	.006	.006
Severity, γ_{13}	.001	.002	<.001	.002	<.001	.003
Directionality, γ_{14}	−.001	.002	<.001	.002	−.005	.003
Between-person association of slopes						
DHEA diurnal slope, γ_{15}	—	—	—	—	.11*	.03
Random effects						
Cortisol level variance, σ^2_{u0}	.001*	.0002	.0003*	.0001*	.0006*	<.0001
Coupling variance, σ^2_{u1}	.0003*	.0001	<.0001*	<.0001	.0006*	.0002
Diurnal slope variance, σ^2_{u2}	—	—	<.0001*	<.0001	<.0001*	<.0001
Residual, σ^2_{e1}	.0007*	<.0001	.0006*	<.0001	.0005*	<.0001
Fit statistics						
−2LL	−4775.1		−5056.1		−5243.7	
AIC	−4767.1		−5042.1		−5229.7	

Note. Coupling refers the within-person association of cortisol with DHEA. −2LL = log likelihood function. AIC = Akaike information criterion. SE = standard error.

* = $p < .05$.

Table 3

Bivariate Diurnal Coupling of Cortisol and Testosterone

	Coupled diurnal change		Coupled deviations with change		Coupled diurnal deviations	
Parameter	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***
Fixed effects						
Intercept, γ_{00}	1.57*	.01	1.64*	.01	1.68*	.004
Coupling parameter, γ_{10}	−.01	.009	−.02*	.01	.01	.01
Diurnal Slope, γ_{20}	—	—	−.004*	<.001	−.005*	<.001
Effect of moderators on Cortisol Level						
Age, γ_{01}	<.001	.003	<.001	.002	.001	.001
Sex, γ_{02}	.11*	.01	.05*	.01	.003	.002
Severity, γ_{03}	−.001	.004	.001	.002	<.001	.001
Directionality, γ_{04}	−.001	.004	−.004	.002	−.001	.001
Testosterone diurnal slope, γ_{05}	—	—	—	—	.05	.10
Effect of moderators on Coupling						
Age, γ_{11}	<.001	.002	−.001	.001	.001	.003
Sex, γ_{12}	.05*	.007	.02*	.005	.03*	.009
Severity, γ_{13}	<.001	.003	.001	.002	−.001	.004
Directionality, γ_{14}	<.001	.002	−.003	.001	−.005	.004
Between-person association of slopes						
Testosterone diurnal slope, γ_{15}	—	—	—	—	−.02	.01
Random effects						
Cortisol level variance, σ^2_{u0}	.0008*	.0003	.0007*	.0002	.0007*	.0001
Coupling variance, σ^2_{u1}	.0002†	.0001	.0001	<.0001	.0005	.0004
Diurnal slope variance, σ^2_{u2}	—	—	<.0001*	<.0001	<.0001*	<.0001
Residual, σ^2_{e1}	.0009*	<.0001	.0007*	.0003	.0007*	<.0001
Fit statistics						
−2LL	−4516.2		−4878.2		−5034.7	
AIC	−4508.2		−4864.2		−5020.7	

Note. Coupling refers the within-person association of cortisol with testosterone. −2LL = log likelihood function. AIC = Akaike information criterion. SE = standard error.

* = $p < .05$.

Table 4

Trivariate Diurnal Coupling of Cortisol with DHEA and Testosterone

	Coupled diurnal change		Coupled deviations with change		Coupled diurnal deviations	
Parameter	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***
Fixed effects						
Intercept, γ_{00}	1.65*	.008	1.66*	.007	1.68*	.004
DHEA Coupling parameter, γ_{10}	.02*	.006	.01*	.006	.03*	.01
Testosterone Coupling parameter, γ_{10}	.01	.007	.003	.006	.01	.01
Diurnal Slope, γ_{30}	—	—	−.002*	<.001	−.005*	<.001
Effect of moderators on Cortisol Level						
Age, γ_{01}	−.002	.001	−.001	.001	.001	.001
Sex, γ_{02}	.004	.007	.003	.006	<.001	.002
Severity, γ_{03}	<.001	.002	<.001	.002	<.001	.001
Directionality, γ_{04}	−.003	.002	−.002	.002	−.001	.001
DHEA diurnal slope, γ_{05}	—	—	—	—	−1.11*	.23
Testosterone diurnal slope, γ_{06}	—	—	—	—	.20*	.09
Effect of moderators on DHEA Coupling						
Age, γ_{11}	<.001	.001	<.001	.001	.003	.002
Sex, γ_{12}	.02*	.004	.02*	.004	.007	.007
Severity, γ_{13}	<.001	.002	.001	.002	<.001	.003
Directionality, γ_{14}	<.001	.001	−.001	.002	−.005	.003
Effect of moderators on Testosterone Coupling						
Age, γ_{21}	−.002	.001	−.001	.001	−.006*	.003
Sex, γ_{22}	.02*	.004	.02*	.004	.007	.009
Severity, γ_{23}	.001	.002	<.001	.002	−.001	.004
Directionality, γ_{24}	−.002	.002	−.002	.002	−.001	.004
Between-person association of slopes						
DHEA diurnal slope, γ_{15}	—	—	—	—	.13*	.03
Testosterone diurnal slope, γ_{25}	—	—	—	—	−.03*	.01

	Coupled diurnal change		Coupled deviations with change		Coupled diurnal deviations	
Parameter	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***
Random effects						
Cortisol level variance, σ^2_{u0}	.0001*	<.0001	.0002*	.0001	.0006*	.0001
DHEA Coupling variance, σ^2_{u1}	.0003*	.0001	.0002*	.0001	.0006*	.0002
Testosterone Coupling variance, σ^2_{u2}	.0003*	.0001	.0002*	.0001	<.0001	<.0001
Diurnal slope variance, σ^2_{u3}			<.0001*	<.0001*	<.0001*	<.0001
Residual, σ^2_{e1}	.0006*	<.0001	.0005*	<.0001	.0005*	<.0001
Fit statistics						
−2LL	−5137.2		−5262.5		−5223.4	
AIC	−5123.2		−5240.5		−5203.4	

Note. −2LL = log likelihood function. AIC = Akaike information criterion. SE = standard error.

* = p <.05.