Coupling of waking cortisol with DHEA and testosterone across the pubertal transition: Associations with threat-related early life stress

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Abstract

Atypical regulation of the hypothalamic-pituitary-adrenal (HPA) axis is a putative mechanism underlying the association between exposure to early life stress (ELS) and the subsequent development of mental and physical health difficulties. Recent research indicates that puberty is a period of HPA-axis plasticity during which the effects of exposure to ELS on cortisol regulation may change. In particular, increases in the hormones that drive pubertal maturation, including dehydroepiandrosterone (DHEA) and testosterone, may be implicated in pubertal recalibration of cortisol regulation. In the current study, we used a longitudinal design to examine the associations among levels of threat-related ELS and waking cortisol, DHEA, and testosterone across pubertal development in a sample of 178 adolescents. We found that cortisol was positively associated with DHEA and testosterone in both early and late puberty; cortisol and DHEA were more strongly positively coupled in late puberty than in early puberty, an effect that was driven by boys. Increases in DHEA and in testosterone from early to late puberty were associated with increases in cortisol, indicating positive longitudinal coupling of these hormones; however, longitudinal cortisol–DHEA coupling was attenuated in adolescents who were exposed to more severe threat-related ELS prior to puberty. Importantly, these effects held when controlling for threat-related stress occurring during the transition through puberty. These findings advance our understanding of the development of the HPA-axis and its association with environmental risk during the sensitive period of puberty.

Keywords: cortisol; DHEA; testosterone; puberty; adolescence; early life stress

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis mediates physiological responses to stress (Gunnar & Quevedo, 2007), and functioning of the HPA axis has pervasive effects on mental and physical health (Koss & Gunnar, 2018). Indeed, a burgeoning literature implicates atypical cortisol regulation, both diurnally and in response to stress, in the development of psychopathology (e.g., Adam et al., 2017; Colich, Kircanski, Foland-Ross, & Gotlib, 2015; Essex et al., 2011). Further, many studies have linked exposure to early life stress (ELS; with "early" defined as occurring during childhood) with subsequent cortisol dysregulation (e.g., Bernard, Frost, Bennett, & Lindhiem, 2017; Bunea, Szentágotai-Tătar, & Miu, 2017).

There is little question that the associations among cortisol, ELS, and health are complex. For example, the effects of cortisol on health follow an inverted U-shaped curve (Sapolsky, 1997). Because moderate levels of cortisol are critical for adaptive responses to stress, atypically low or "blunted" levels of cortisol may increase risk for poor health; in contrast, chronically high levels of cortisol may indicate the loss of neurobiological resilience (Mcewen, 2019). In addition, the effects of ELS on cortisol regulation may depend on developmental stage. Specifically, there is increasing evidence that puberty is a period of HPA-axis plasticity, and that the effects of ELS on cortisol regulation change with maturation. In two cross-sectional studies, ELS in the form of institutionalization prior to international adoption was associated with blunted cortisol responses to awakening (Quevedo, Johnson, Loman, Lafavor, & Gunnar, 2012) and to social stress (DePasquale, Donzella, & Gunnar, 2019) in children in early puberty; in contrast, the cortisol responses of previously institutionalized children in late puberty were indistinguishable from those of never-adopted children. In another cross-sectional study, we found that the association between ELS and cortisol responses to awakening in adolescents did not dissipate but *differed* in participants as a function of pubertal maturation. Specifically, greater severity of exposure to a variety of life stressors prior to puberty (i.e., parental divorce, maltreatment, community violence) was associated with a blunted cortisol awakening response

in early puberty, but with a heightened cortisol awakening response in later puberty (King et al., 2017).

Changes in the association between ELS and cortisol regulation across puberty may have important implications for the identification of, and intervention with, children at risk for psychopathology. With respect to identification, prior to puberty a pattern of *blunted* cortisol may characterize children who have been exposed to adversity; in contrast, in later puberty, a pattern of *elevated* cortisol may identify children who are at heightened risk for psychopathology. In terms of intervention, treatments offered to remediate the negative effects of early adversity may be more likely to be successful in early puberty than would treatments delivered later in maturation. However, given that the mechanisms underlying pubertal recalibration of cortisol regulation are still unclear (DePasquale et al., 2019; Romeo, 2013), considerable research is needed before such conclusions can be drawn.

Researchers have long known that puberty is characterized by dramatic increases in sex hormones that are responsible for the development of secondary sex characteristics and the attainment of sexual maturation. It is possible that changes in cortisol regulation and its association with ELS during puberty are driven by these increases in sex hormones. Adrenarche is the first major sign of puberty, involving rises in adrenal hormones, including dehydroepiandrosterone (DHEA), beginning at age 6-8 years in girls and approximately one year later in boys (Dorn & Biro, 2011). Increases in DHEA contribute in part to gonadarche, or the re-activation of the hypothalamic-pituitary-gonadal (HPG) axis and attendant rises in testosterone and estradiol (Dorn & Biro, 2011; Marceau, Ruttle, Shirtcliff, Essex, & Susman, 2015). The synthesis and metabolism of cortisol is related to that of DHEA and testosterone, with both cortisol and DHEA produced by the HPA axis and testosterone produced by the HPG axis (Marceau, Ruttle, Shirtcliff, Essex, et al., 2015). Cortisol, DHEA, and testosterone follow a similar diurnal pattern of higher morning levels that decline throughout the course of the day, and show increases in response to acute stress (Marceau et al., 2014), (Hucklebridge, Hussain,

Evans, & Clow, 2005; Matchock, Dorn, & Susman, 2007). The coupling of DHEA with cortisol may be protective. Specifically, DHEA and cortisol appear to have opposing regulatory functions such that DHEA protects against the neurotoxic effects of cortisol (Kamin & Kertes, 2016). In adults, the activation of the HPA axis appears to suppress the HPG axis (Mastorakos, Pavlatou, & Mizamtsidi, 2006); however, emerging research suggests that this is not the case in early adolescence (Marceau et al., 2014).

A small number of studies have examined the normative associations among cortisol, DHEA, and testosterone during adolescence. Findings of cross-sectional studies suggest that there is a positive diurnal coupling of cortisol with DHEA and testosterone in adolescence (Dismukes, Shirtcliff, Hanson, & Pollak, 2015; Johnson et al., 2014; Marceau, Ruttle, Shirtcliff, Hastings, et al., 2015), although this coupling may differ as a function of age and sex. Specifically, Marceau et al. (2014) found that cortisol-*DHEA* coupling in response to stress was stronger in older boys, whereas cortisol-*testosterone* coupling was weaker in older boys. These findings are partially consistent with those of an earlier longitudinal study of adolescents' diurnal hormone production, in which cortisol-DHEA coupling throughout the day become more strongly positive in later adolescence, whereas cortisol-testosterone coupling became negative in later adolescence (Ruttle, Shirtcliff, Armstrong, Klein, & Essex, 2013).

Importantly, previous longitudinal studies have recruited adolescents on the basis of age rather than of pubertal stage. Boys and girls differ significantly in pubertal timing, with girls typically experiencing the onset of puberty 1.5 years earlier than do boys (Negriff & Susman, 2011). Given these sex differences in pubertal timing, age-matched samples of adolescent boys and girls are almost certain to be confounded by sex differences in pubertal stage, making it difficult to isolate the dynamics of cortisol and sex hormones that are specific to the pubertal transition in both boys and girls.

In the current study we used a longitudinal design to assess the association between levels of ELS and waking cortisol, DHEA, and testosterone across the transition from early to

late puberty. We had three objectives in this study. First, we examined the association of cortisol with DHEA and testosterone in early versus late puberty. We hypothesized that cortisol and DHEA are positively associated in both early and late puberty, but that this positive association is stronger in late than in early puberty. In contrast, we hypothesized that cortisol and testosterone are positively associated in early puberty, but that this association is weaker in late puberty. Second, we examined the relation between changes in DHEA and testosterone from early to late puberty and changes in cortisol across this period. We hypothesized that increases in DHEA, but not in testosterone, are associated with increases in cortisol. Third, we examined the effects of ELS in the form of the severity of exposure to threat-related ELS (i.e., domestic and community violence) on the relation between changes in cortisol and changes in DHEA and testosterone from early to late puberty. Based on evidence that different forms of ELS have differential effects on psychobiological development (King, Humphreys, Camacho, & Gotlib, 2019; Lambert, King, Monahan, & McLaughlin, 2017; Sheridan, Peverill, Finn, & McLaughlin, 2017), we focused on threat-related ELS in order to increase the specificity of our hypotheses. Given evidence that exposure to environmental threat (as opposed to deprivation) accelerates biological development (Colich, Rosen, Williams, & McLaughlin, 2019; Del Giudice, Ellis, & Shirtcliff, 2011), we hypothesized that greater severity of threat-related ELS is associated with stronger positive coupling between change in cortisol and change in DHEA, and with weaker positive coupling between change in cortisol and change in testosterone, from early to late puberty.

2. Methods

2.1 Participants

Participants were 214 early adolescents and their parents who were recruited from the community to participate in a longitudinal study of the psychobiological effects of ELS across the transition from early puberty (Time 1 [T1]) to late puberty (Time 2 [T2]); Humphreys, Kircanski, Colich, & Gotlib, 2016; King, Humphreys, Camacho, & Gotlib, 2018). Participants were recruited

from the geographic area surrounding Stanford University through local and media postings. Inclusion criteria at T1 were that the adolescents were between 9 and 13 years of age and proficient in spoken English. Exclusion criteria at T1 included a history of major neurological or medical illnesses, severe learning disabilities that would affect comprehension of study procedures, and, for females, the onset of menses. In addition, participants were selected based on their eligibility to participate in a magnetic resonance imaging (MRI) scan (e.g., no metal implants or braces). Given the focus of the study on pubertal development, boys and girls were matched on self-reported pubertal stage using Tanner staging (see "Pubertal Stage," below). To be included in the current analyses, we required that participants be in early puberty at T1 (Tanner stages 1-3), resulting in the exclusion of 14 participants. We excluded an additional 24 participants who did not provide a waking saliva sample that yielded values for cortisol, DHEA, or testosterone at either T1 or T2. Therefore, the final sample for the current analyses was 178 adolescents (55% female), 171 of whom provided data at T1 and 143 of whom provided data at T2. Descriptive statistics for the final study sample are presented in Table 1. There were no significant differences at T1 between participants who were and who were not included in the final sample with respect to threat-related ELS (described below), family income-to-needs ratio, pubertal stage, or body mass index (BMI). The sample for this study overlaps with that of an earlier study in which diurnal cortisol was examined only at the T1 assessment (King et al., 2017); the cortisol and sex hormone data reported in this study were not reported in the earlier study.

Table 1. Descriptive statistics of the study sample

Measure	Sex	N		Mean (SD)	
		T1	T2	T1	T2
Age	Female	94	75	11.07 (0.95)	12.97 (0.97)
	Male	77	68	11.87 (0.97)	13.82 (0.96)
Pubertal Stage	Female	94	74	2.00 (0.68)	3.41 (0.79)
	Male	77	61	1.85 (0.59)	3.46 (0.92)
BMI	Female	92	62	18.31 (4.04)	20.15 (4.86)
	Male	75	53	19.09 (3.39)	20.85 (4.04)
log(Cortisol) µg/dL	Female	81	71	-1.47 (0.66)	-1.51 (0.58)
	Male	70	65	-1.77 (0.61)	-1.38 (0.61)
log(DHEA) pg/mL	Female	94	73	4.70 (0.92)	4.85 (0.74)
	Male	76	67	4.63 (1.04)	4.95 (0.79)
log(Testosterone) pg/mL	Female	93	75	3.89 (0.54)	4.02 (0.45)
	Male	75	68	3.99 (0.41)	4.74 (0.62)
Threat-related ELS	Female	94	75	2.03 (2.13)	1.13 (1.99)
	Male	77	67	1.97 (1.98)	0.88 (1.44)
Income-to-needs ratio	Female	85	67	1.32 (0.53)	
	Male	71	60	1.33 (0.52)	
Race/ethnicity					
Caucasian	Female	46			
	Male	35			
Biracial	Female	16			
	Male	20			
Asian	Female	15			
	Male	7			
Hispanic	Female	8			
	Male	3			
African American	Female	5			
	Male	5			
Other	Female	4			
	Male	6			
Not reported	Female	0			
	Male	1			

Notes. T1 = baseline assessment in early puberty. T2 = follow-up assessment in late puberty. BMI = body mass index. ELS = early life stress.

2.2 Procedure

The Stanford University Institutional Review Board approved the protocol for this study. In an initial telephone call, research staff provided information about the study to families and screened participants for inclusion/exclusion criteria. Eligible families were then invited to attend a laboratory session during which staff obtained consent from parents and assent from

adolescents. In this session, adolescents reported their pubertal stages, and both parents and adolescents completed interview and questionnaire measures about the child and family. At the end of the session, staff provided families with kits and instructions to collect saliva samples at home for the assessment of waking hormone levels. Families returned the samples to the laboratory at a subsequent visit. These procedures were repeated at a T2 laboratory session that occurred an average of 2 years later (mean[SD]=1.96[0.31] years; range: 1.18-2.88).

2.3 Measures

2.3.1 Pubertal stage. In order to match boys and girls based on pubertal stage at T1, we measured pubertal development using self-report Tanner staging (Marshall & Tanner, 1968; Morris & Udry, 1980). Self-report Tanner staging scores are moderately correlated with physicians' physical examinations of pubertal development (Dorn & Biro, 2011). Participants reported their pubertal stage by selecting how closely their pubic hair and breast/testes resembled an array of schematic drawings on a scale of 1 (prepubertal) to 5 (postpubertal). For the purposes of this study, we used the average of the pubic hair and breast/testes Tanner scores to index overall pubertal stage. Average Tanner scores ranged from 1-3 at T1 and from 1-5 at T2.

2.3.2 Severity of threat-related stress. As previously described (King et al., 2017, 2019), at T1, participants were interviewed about their lifetime exposure to 30 types of stressors using a modified version of the Traumatic Events Screening Inventory for Children (Ribbe, 1996). A panel of three coders, blind to the adolescents' reactions and behaviors during the interview, then rated the objective severity of each type of stressor endorsed on a scale of (0 = non-event or no impact; 4 = extremely severe impact; ICC = 0.99). To quantify the severity of threat-related ELS, we summed the maximum objective severity ratings for the events listed in in Table 2 (see https://github.com/lucysking/els_stress_interview for scoring script). We selected these events to be consistent with Sheridan and McLaughlin's (2014) definition of threat as "the presence of an atypical (i.e., unexpected) experience characterized by actual or threatened

death, injury, sexual violation, or other harm to one's physical integrity" (p. 580). Adolescents were interviewed again at the T2 assessment about exposure to the same 30 types of stressors since the T1 assessment. We computed separate scores for threat-related ELS (i.e., events occurring prior to puberty reported at T1) and threat-related stress during the transition to puberty (i.e., events occurring between the T1 and T2 assessments reported at T2).

Table 2. Endorsement of lifetime threat-related stressors occurring up to early puberty (T1)

Type of ELS	N endorsed	% endorsed
Family verbal conflict	72	41
Bullying	60	34
Community violence	21	12
Community instability	17	10
Domestic violence	12	7
Community verbal conflict	9	5
Emotional abuse	9	5
Physical abuse	8	5
Mugging or robbery	5	3
War or terrorism	5	3
Sexual abuse	3	2
Threats of domestic violence	3	2
Kidnapping	2	1
Threats of physical abuse	2	1
Witness sexual abuse	1	1

Notes. Stressors coded as **"**community instability" included community-level threats (e.g., bomb/active shooter threats at school, hearing gun shots in neighborhood).

2.3.3 Waking cortisol, DHEA, and testosterone. Salivary hormonal assays were conducted for cortisol, DHEA and testosterone. At each time point, participants were asked to provide a saliva sample through passive drool immediately upon awakening (prior to eating breakfast or brushing teeth). Participants recorded collection time and placed the saliva samples in their home freezer after collection. Contemporaneous with the saliva samples, participants reported their use of over-the-counter and prescription medications, including corticosteroids. After participants returned the samples to the laboratory, samples were transferred to a -20°C

freezer in the Psychology Department at Stanford University. The samples were then shipped on dry ice to Salimetrics, LLC (State College, PA), where they were assayed for salivary cortisol, DHEA, and testosterone using a high sensitivity enzyme immunoassays (Cat No. 1-3002 for cortisol; Cat. No. 1-1202 for DHEA; Cat. No. 1-2402 for testosterone). The assay for cortisol used 25 µl of saliva per determination, had a lower limit sensitivity of .0007 µg/dL, and a standard curve range from .012-3.0 µg/dL. The assay for DHEA used 50 µl of saliva per determination, had a lower limit of sensitivity of 5 pg/mL, and a standard curve range from 10.2-1000 pg/mL. The assay for testosterone used 25 µl of saliva per determination, had a lower limit of sensitivity of 1 pg/mL, and a standard curve range from 6.1-600 pg/mL. The average intra- and inter-assay coefficients of variation for cortisol were 4.60% and 6.00%, respectively. The average intra- and inter-assay coefficients variation for DHEA were 5.55% and 8.20%, respectively. The average intra- and inter-assay coefficients variation for testosterone were 4.60% and 9.85%, respectively. We winsorized cortisol, DHEA, and testosterone values that were +/- 3SD from the mean (within time-point and sex), and then log-transformed the values at each time point to correct for positive skew. In addition, we calculated the slopes of cortisol, DHEA, and testosterone from T1 to T2 using the following formula: (hormone T2 - hormone T1) / T2 to T1 interval in years.

2.4 Data analysis

All analyses were conducted in R (R Core Team, 2018). The criterion for significance was set at α=.05. Data are available upon request. Analysis scripts are available at: https://github.com/lucysking/els_cort_dhea. All variables were standardized prior to analysis such that estimates reflect the predicted change in the dependent variable for a 1-SD change in the independent variable.

2.4.1 Objective 1: Examine the association of cortisol with DHEA and testosterone in early versus late puberty. We used multi-level modeling (also known as mixed-effects or hierarchical linear modeling; Woltman, Feldstain, Mackay, & Rocchi, 2012) to examine the

coupling of cortisol with DHEA and testosterone in early and late puberty. We implemented this model using the function “lmer” in the “lme4” package, and we used the package “lmerTest” to calculate degrees of freedom and p-values (Bates, Machler, Bolker, & Walker, 2014; Kuznetsova, Brockhoff, & Christensen, 2016). This model was fit as follows, controlling for the random effect of participant intercepts and dummy-coding time-point (T1=0, T2=1):

$$\text{Equation 1: } \text{cortisol}_{ij} \sim \beta_0 + \beta_1(\text{time-point}_{ij}) + \beta_2(\text{sex hormone}_{ij}) + \beta_3(\text{time-point}_{ij} \times \text{sex hormone})$$

R code: lmer(cortisol ~ sex hormone * time-point + (1|subject ID))

In this context, a significant interaction between time-point and the sex hormone (DHEA or testosterone) indicates that the association between the sex hormone and cortisol at T1 is significantly different from the association between the sex hormone and cortisol at T2.

2.4.2 Objective 2: Examine the relation between changes in DHEA and testosterone from early to late puberty and changes in cortisol across this period. We used ordinary least squares (OLS) linear regression to examine the relation between increases in sex hormones from T1 to T2 (DHEA or testosterone slopes) and increases in cortisol from T1 to T2 (cortisol slope). We implemented this model using the function “lm” in the “stats” package as follows, controlling for cortisol and the sex hormone (DHEA or testosterone) at T1:

$$\text{Equation 2: } \text{cortisol slope}_i \sim \beta_0 + \beta_1(\text{sex hormone slope}_i) + \beta_4(\text{T1 sex hormone}_i) + \beta_5(\text{T1 cortisol})$$

R code: lm(cortisol slope ~ sex hormone slope + T1 cortisol + T1 sex hormone)

In this context, a significant main effect of the sex hormone slope (β_1) indicates that changes in the sex hormone from early to late puberty are associated with changes in cortisol from early to late puberty, above and beyond early puberty levels.

2.4.3 Objective 3: Examine the effects of the severity of threat-related ELS on the relation between longitudinal coupling of cortisol and DHEA and testosterone. Finally, we tested the effects of the severity of threat-related ELS on the coupling of changes in cortisol and

changes in DHEA and testosterone from early to late puberty by adding an interaction between the sex hormone slope and the severity of threat-related ELS to the model above. Specifically, we fit the following model:

Equation 3: cortisol slope$_i$ ~ β_0 + β_1(sex hormone slope$_i$) + β_2(threat severity) + β_3(sex hormone slope$_i$ $\times$ threat severity$_i$) + β_4(T1 sex hormone$_i$) + β_5(T1 cortisol)

R code: lm(cortisol slope ~ sex hormone slope * threat severity + T1 cortisol + T1 sex hormone)

In this context, a significant interaction between the sex hormone slope and the severity of threat-related ELS severity (β_3) indicates that the association between changes in the sex hormone and changes in cortisol depends on the level of threat severity.

To examine the impact of potential covariates, including interval in years between T1 and T2, use of medication, sex, BMI, age at T1, and threat-related stress severity at T2, on the effects of interest, we first conducted formal model fitting (Chambers, 1992) in which we tested whether each of the covariates significantly improved the model fit using likelihood ratio tests (multi-level models) or analyses of variance (OLS regression). Next, we conducted sensitivity analyses including the covariates that improved model fit. In addition, we tested separate models in which we removed observations from participants who were taking any medications at the time of hormone collection.

Finally, given sex differences in the production and function of cortisol, DHEA, and testosterone, we followed previous research investigating hormonal dynamics in adolescence (Simmons et al., 2015) by running the models specified in Equations 1-2 separately for boys and girls.

3. Results

3.1 Sample characteristics

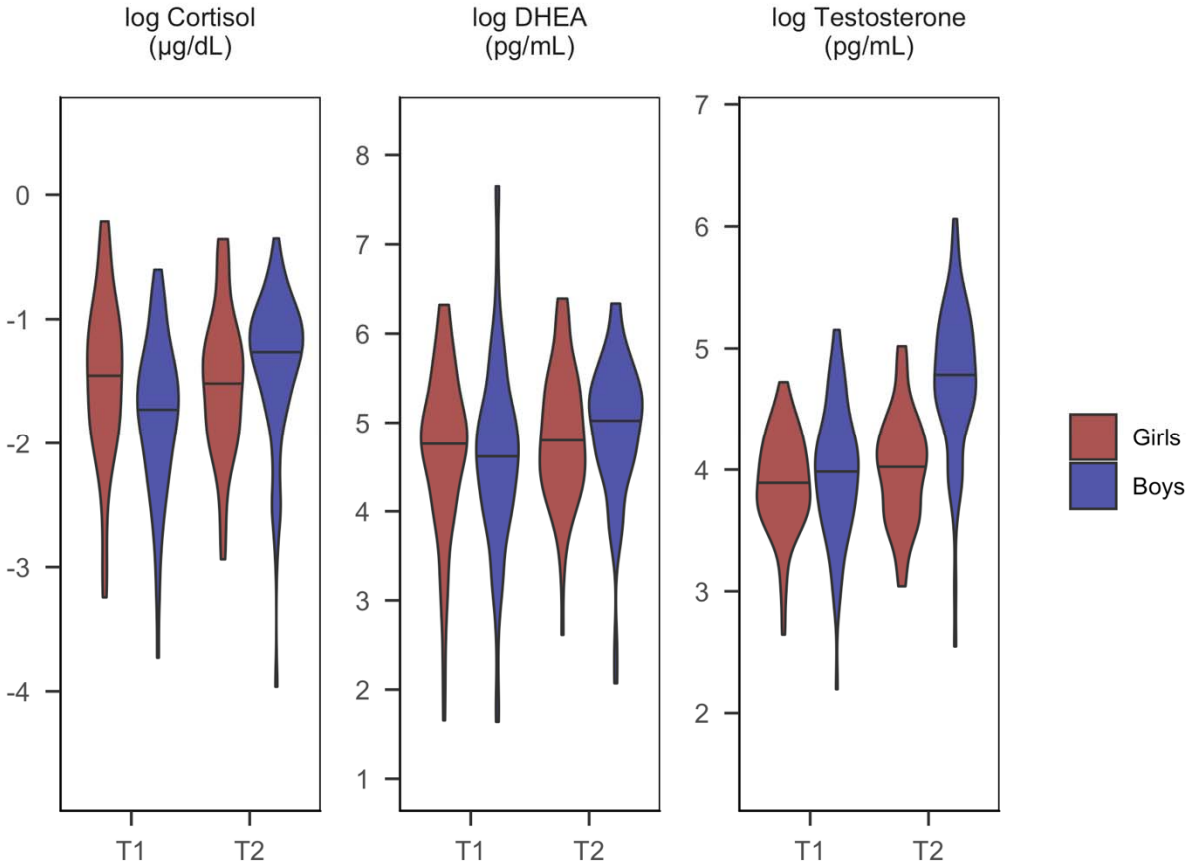
Characteristics of the sample are presented in Table 1. At T1, 15% of the adolescents reported using medications contemporaneous with saliva samples; 4% reported using a

corticosteroid. At T2, 22% of adolescents reported using medications; 6% reported using a corticosteroid. None of the girls in the study reported using hormonal birth control at either time point. Severity of threat-related ELS at T1 was not correlated significantly with adolescent age, pubertal stage, BMI, or years between the T1 and T2 assessments; however, severity of threat-related ELS was correlated with severity of threat-related stress during the transition through puberty ($r(132)=.40$, $p<.001$). As expected based on the design of the study, self-reported pubertal stage increased significantly from T1 to T2 ($t(130)=22.09$, $p<.001$), as did BMI and levels of cortisol, DHEA, and testosterone (**BMI:** $t(107)=6.62$, $p<.001$, **cortisol:** $t(115)=2.59$, $p=.011$; **DHEA:** $t(133)=3.99$, $p<.001$; **testosterone:** $t(133)=8.31$, $p<.001$). DHEA was not correlated with pubertal stage at T1 ($r(168)=.11$, $p=.144$) but was positively correlated with pubertal stage at T2 ($r(127)=.38$, $p<.001$). Similarly, testosterone was weakly correlated with pubertal stage at T1 ($r(166)=.19$, $p=.015$) and positively correlated with pubertal stage at T2 ($r(129)=.37$, $p<.001$). Cortisol was not correlated with pubertal stage at T1 at either time-point (**T1:** $r(149)=.01$, $p=.904$; **T2:** $r(123)=.16$, $p=.076$).

Boys and girls did not differ significantly in severity of threat-related ELS or threat-related stress during the transition through puberty, in the length of the interval between T1 and T2, in levels of pubertal stage, BMI, or DHEA at either time-point, or in increases in these measures from T1 to T2. At T1, boys and girls did not differ significantly in levels of testosterone (β=0.09, SE=0.07, $t(270.06)=1.38$, $p=.167$, 95% CI[-0.04, 0.22]); however, at T2, boys had significantly higher levels of testosterone than did girls (β=0.60, SE=0.07, $t(287.30)=8.60$, $p<.001$, 95% CI[0.46, 0.73]). Although both boys and girls had significant increases in testosterone from T1 to T2, the increase was larger for boys (β=0.62, SE=0.05, $t(145.98)=11.41$, $p<.001$, 95% CI[0.52, 0.73]) than for girls (β=0.12, SE=0.05, $t(146.43)=2.32$, $p<.021$, 95% CI[0.02, 0.22]). Girls had significantly higher levels of cortisol at T1 than did boys (β=0.23, SE=0.08, $t(276.67)=2.83$, $p=.005$, 95% CI[0.07, 0.38]). Further, boys exhibited an increase in cortisol from T1 to T2 (β=0.31, SE=0.08, $t(138.03)=4.09$, $p<.001$, 95% CI[0.16, 0.46]), whereas girls did not (β=-0.03,

SE=0.07, *t*(149.51)=-0.36, *p*=.720, 95% CI[-0.17, 0.12]). Violin plots of the distributions of cortisol, DHEA, and testosterone values at each time-point for each sex are presented in Figure 1.

Figure 1. Sex differences in cortisol and pubertal hormone levels in early and late puberty.



Notes. T1 = baseline assessment in early puberty. T2 = assessment approximately 24 months later in late puberty.

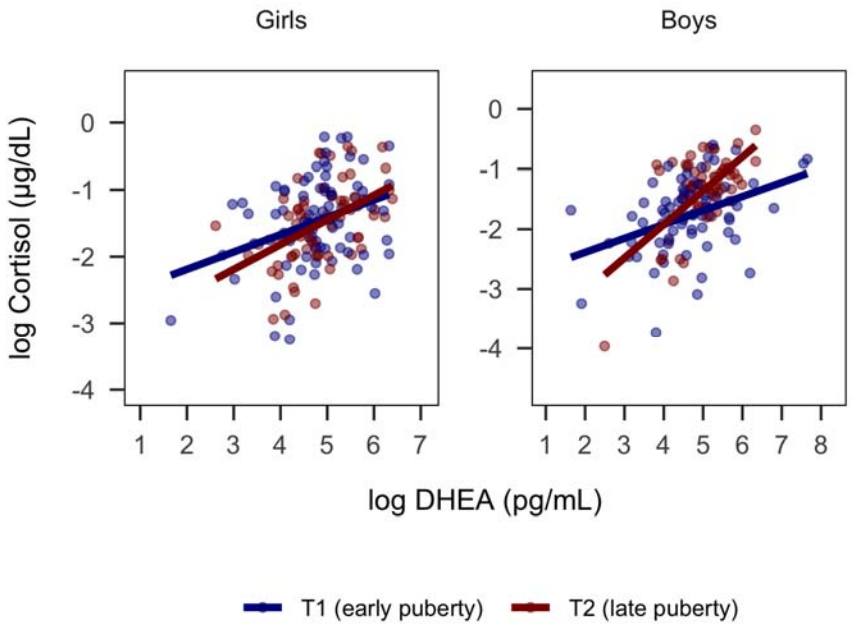
3.2 Objective 1: Associations between cortisol and pubertal hormones in early versus late puberty

3.2.1 Association between cortisol and DHEA in early versus late puberty. Results of a multi-level model indicated that DHEA interacted with time-point to explain cortisol (β=0.25, SE=0.11, *t*(240.78)=2.27, *p*=.024, 95% CI[0.03, 0.46]). Simple effects analyses indicated that

DHEA was positively associated with cortisol at T1 (β=0.38, SE=0.07, *t*(281.03)=5.65, *p*<.001, 95% CI[0.24, 0.51]), and that the positive association between DHEA and cortisol was stronger at T2 than at T1 (β=0.63, SE=0.09, *t*(278.46)=6.77, *p*<.001, 95% CI[0.46, 0.81]). Results of formal model fitting indicated that the main effects of interval between T1 and T2, use of medication, sex, and child age at T1 did not improve model fit; however, BMI at T1 and time of saliva sample collection marginally improved model fit. Results were highly similar when controlling for BMI at T1 and time of collection, and in analyses excluding hormone values that were collected contemporaneous with the use of corticosteroids.

In a separate model conducted within boys only, the interaction between DHEA and time point was larger in effect size than it was in the full model that included both boys and girls (β=0.41, SE=0.16, *t*(109.62)=2.61, *p*=.010, 95% CI[0.10, 0.75]). Simple effect analyses indicated that, in boys, DHEA was positively associated with cortisol at T1 (β=0.37, SE=0.09, *t*(128.83)=4.36, *p*<.001, 95% CI[0.22, 0.53]), and that the positive association between DHEA and cortisol was stronger at T2 than at T1 (β=0.79, SE=0.14, *t*(126.13)=5.74, *p*<.001, 95% CI[0.53, 1.05]). In contrast, in a separate model conducted within girls only, the interaction between DHEA and time-point was not significant (β=0.13, SE=0.16, *t*(123.25)=0.84, *p*=.403, 95% CI[-0.17, 0.44]); DHEA and cortisol were similarly positively associated at T1 and T2. The simple associations between DHEA and cortisol at T1 vs. T2 for each sex are presented in Figure 2.

Figure 2. DHEA and cortisol are positively associated in early and late puberty.



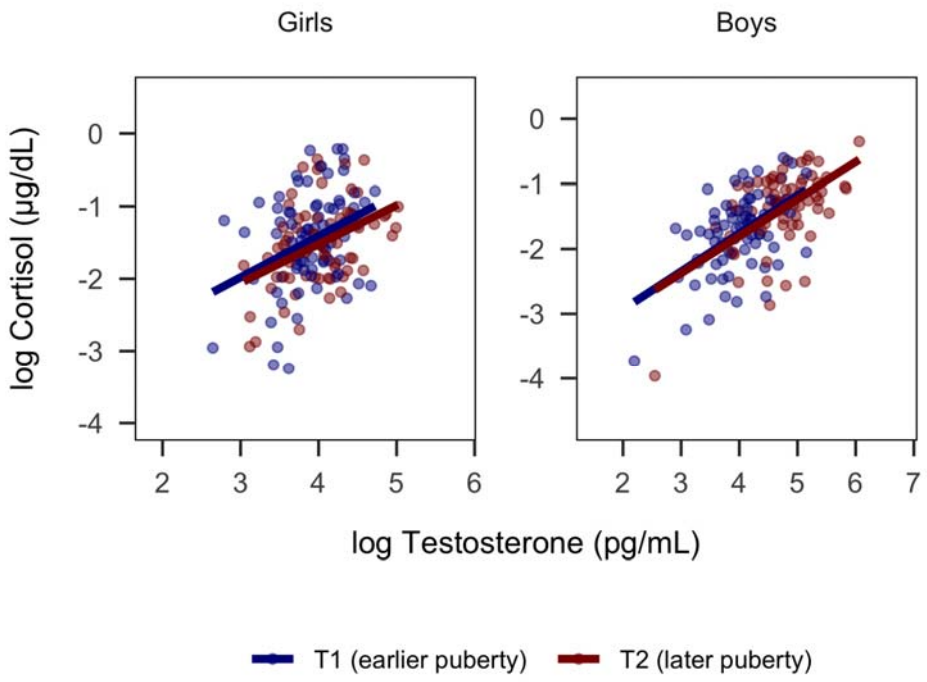
Notes. In girls, DHEA and cortisol were similarly positively associated in early and late puberty. In boys, DHEA and cortisol were more strongly positively associated in late than in early puberty.

3.2.2 Associations between cortisol and testosterone in early versus late puberty.

Results of a multi-level model indicated that testosterone did not interact with time-point to explain cortisol (β=-0.05, SE=0.11, *t*(236.23)=-0.44, *p*=.664, 95% CI[-0.27, 0.17]). Instead, testosterone and cortisol were positively associated at both T1 and T2 (T1: β=0.51, SE=0.09, *t*(281.44)=5.62, *p*<.001, 95% CI[0.33, 0.68]; T2: β=0.46, SE=0.07, *t*(272.35)=6.41, *p*<.001, 95% CI[0.31, 0.59]). Results of formal model fitting indicated that the main effects of interval between T1 and T2, use of medication, BMI, and child age at T1 did not improve model fit; however, time of collection and sex significantly improved model fit. Results were highly similar in sensitivity analyses controlling for time of collection and sex, in analyses excluding hormone values that were collected contemporaneous with the use of corticosteroids, and in models conducted separately in boys and girls. The simple associations between testosterone and cortisol at T1

and T2 for each sex are presented in Figure 3.

Figure 3. Testosterone and cortisol are positively associated in early and late puberty.



Notes. Testosterone and cortisol were similarly positively associated in both early and late puberty in both boys and girls.

3.3 Objective 2: Relation between changes in sex hormones from early to late puberty and changes in cortisol across this period

3.3.1 Longitudinal coupling of cortisol and DHEA. Results of a linear regression model indicated that the slope of DHEA from T1 to T2 was positively associated with the slope of cortisol from T1 to T2 (β=0.53, SE=0.09, $t(109)=5.95$, $p<.001$, 95% CI[0.35, 0.70]). The slope of DHEA from T1 to T2 explained 14% of the variance in the slope of cortisol from T1 to T2, above and beyond levels of cortisol and DHEA at T1. Results of formal model fitting indicated that age at T1 marginally improved model fit. Results were highly similar when age was included in the model and in analyses excluding values collected contemporaneous with the use of corticosteroids. The slope of DHEA was positively associated with the slope of cortisol in both

boys and girls, although the size of the effect was somewhat larger in boys (**boys**: β=0.65, SE=0.16, *t*(51)=4.06, *p*<.001, 95% CI[0.33, 0.98]; **girls**: β=0.46, SE=0.12, *t*(54)=4.01, *p*<.001, 95% CI[0.23, 0.70]).

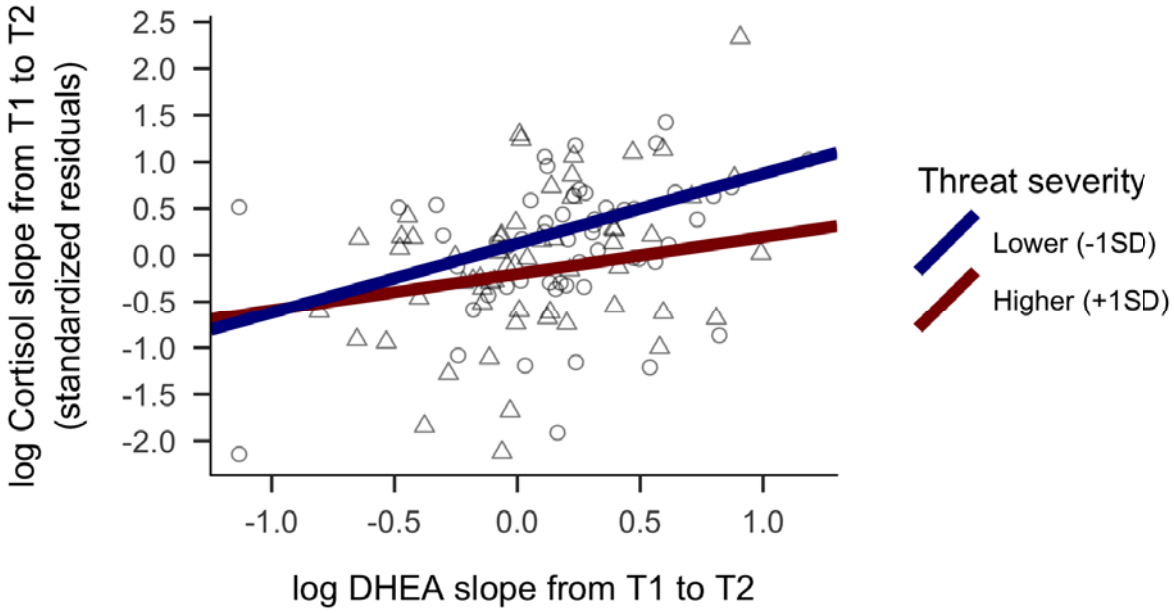
3.3.2 Longitudinal coupling of cortisol and testosterone. Results of a linear regression model indicated that the slope of DHEA from T1 to T2 was positively associated with the slope of cortisol from T1 to T2 (β=0.24, SE=0.07, *t*(110)=3.22, *p*=.002, 95% CI[0.09, 0.38]). The slope of testosterone from T1 to T2 explained 5% of the variance in the slope of cortisol from T1 to T2, above and beyond cortisol and testosterone at T1. Results of formal model fitting indicated that none of the potential covariates improved model fit; results were highly similar when excluding hormone values collected contemporaneous with the use of corticosteroids. The effect of the slope of testosterone on the slope of cortisol was highly similar in boys and girls (**boys**: β=0.28, SE=0.12, *t*(52)=2.44, *p*=.018, 95% CI[0.05, 0.51]; **girls**: β=0.25, SE=0.11, *t*(54)=2.25, *p*=.029, 95% CI[0.03, 0.47]).

3.4 Objective 3: Association of the severity of threat-related ELS with longitudinal coupling of cortisol and sex hormones across the pubertal transition.

3.4.1 Severity of threat-related ELS and the longitudinal coupling of cortisol and DHEA. The severity of threat-related ELS interacted with the slope of DHEA from T1 to T2 to explain the slope of cortisol from T1 to T2 (β=-0.18, SE=0.06, *t*(107)=-2.84, *p*=.005, 95% CI[-0.30, -0.05]). The interaction between threat severity and the slope of DHEA explained an additional 5% of the variance in the slope of cortisol, above and beyond the main effects of the slope of DHEA and levels of cortisol and DHEA at T1. Simple effects analyses at +/- 1SD of the mean of threat-related ELS indicated that changes in DHEA from T1 to T2 were strongly positively associated with change in cortisol from T1 to T2 in adolescents who had been exposed to lower levels of threat-related ELS (β=0.93, SE=0.17, *t*(107)=5.60, *p*<.001, 95% CI[0.60, 1.27]), but were not significantly associated in adolescents exposed to higher levels of threat-related ELS (β=0.21, SE=0.14, *t*(107)=1.52, *p*=.131, 95% CI[-0.06, 0.49]). Results of

formal model fitting indicated that none of the potential covariates significantly improved model fit. In a separate model excluding adolescents who reported using corticosteroids at T1 or T2, the interaction between DHEA and threat severity remained significant. In models conducted with boys and girls separately, the estimates for the interactions between DHEA and threat-related ELS were similar (boys: β=-0.15, SE=0.09, 95% CI[-0.32, 0.03]; girls: β=-0.22, SE=0.10, 95% CI[-0.42, -0.03]), although the effect was smaller in boys than in girls. The simple associations between the slopes of DHEA and cortisol at lower and higher levels of threat severity are presented in Figure 4.

Figure 4. Association between change in DHEA and change in cortisol from early (T1) to late puberty (T2) depends on level of threat-related ELS severity.

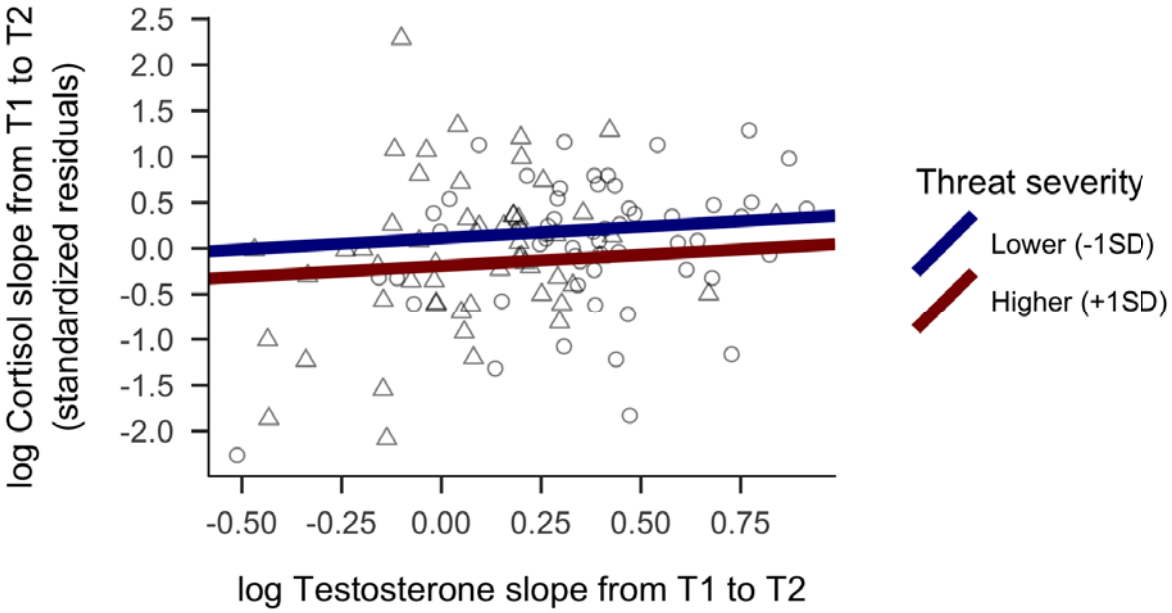


Notes. Triangles = girls; circles = boys.

3.4.2 Severity of threat-related ELS and the longitudinal coupling of cortisol and testosterone. Results of a linear regression model indicated that severity of threat-related ELS did not interact with the slope of testosterone from T1 to T2 to explain the slope of cortisol from T1 to T2 (β=0.00, SE=0.07, *t*(108)=-0.05, *p*=.957, 95% CI[-0.15, 0.14]). Results of formal model fitting indicated that none of the potential covariates significantly improved model fit. Results

were highly similar in analyses excluding hormone values that were collected contemporaneous with the use of corticosteroids, and in models conducted separately in boys and girls. The simple associations between the slopes of testosterone and cortisol at lower and higher levels of threat severity are presented in Figure 5.

Figure 5. Threat-related ELS severity does not moderate the association between change in testosterone and change in cortisol from early (T1) to late puberty (T2).



Notes. Triangles = girls; circles = boys.

4. Discussion

The goal of this study was to advance our understanding of the development of the HPA-axis across the transition through puberty. Specifically, we investigated the associations among levels and changes in sex hormones from early to late puberty with levels and changes in cortisol across this period. Given prior evidence implicating both exposure to ELS and cortisol dysregulation in the development of mental and physical health difficulties (Koss & Gunnar, 2018), we also examined whether the severity of exposure to threat-related ELS prior to puberty moderated the longitudinal coupling of sex hormones with cortisol. Importantly, although we focused on *waking* levels of cortisol and sex hormones, several of our findings are consistent

with those of previous studies examining the coupling of cortisol and sex hormones across the day and in response to stress.

Findings from this study help to elucidate basic developmental processes concerning changes in and relations among cortisol, testosterone, and DHEA during puberty. In contrast to previous studies, we recruited and matched boys and girls based on pubertal stage rather than on age. This design ensured that our findings were not confounded by sex differences in pubertal timing (Negriff & Susman, 2011) and allowed us to isolate the dynamics of cortisol and sex hormones that are specific to the pubertal transition. We found that cortisol and DHEA were positively associated in both early and late puberty, and, further, that the positive association between cortisol and DHEA was stronger in late than in early puberty, an effect that was driven by boys. We found that cortisol and testosterone were also positively associated in both early and late puberty; however, contrary to our hypothesis, the association between cortisol and testosterone did not become weaker in late puberty but remained the same as it was in early puberty.

Previous investigations of the normative associations among cortisol and sex hormones during adolescence have focused largely on how coupling changes with age, rather than on changes across the transition through puberty. Certainly, in these investigations older age was likely partially colinear with greater pubertal maturation. Indeed, consistent with our findings, previous studies found that cortisol-DHEA coupling, both in response to stress and diurnally, was stronger in older than in younger adolescents (Marceau et al., 2014; Ruttle et al., 2013). In fact, Marceau et al. (2014) found that the cross-sectional positive association between age and cortisol-DHEA coupling was driven by boys. Our findings with respect to cortisol-testosterone coupling, however, are not entirely consistent with those of previous studies. Although we replicated findings that cortisol and testosterone are positively coupled (Dismukes, Shirtcliff, et al., 2015; Marceau, Ruttle, Shirtcliff, Hastings, et al., 2015; Marceau et al., 2014), other investigators have found that cortisol-testosterone coupling is weaker (Marceau et al., 2014) or

even negative (Ruttle et al., 2013) in older compared to younger adolescents. Given the younger mean age of the current sample relative to previously examined samples, and the fact that the largest increases in levels of DHEA precede the largest increases in levels of testosterone (Dorn, Dahl, & Woodward, 2006), altered cortisol-testosterone coupling may occur later in pubertal development. Future research should explore this possibility.

Positive coupling of cortisol and sex hormones during puberty is likely due to the integrated synthesis and metabolism of cortisol and sex hormones by the HPA and HPG axes (Marceau, Ruttle, Shirtcliff, Essex, et al., 2015). A shift toward stronger positive cortisol-DHEA coupling in late puberty in boys suggests that puberty is a period of HPA-axis plasticity when recalibration occurs. To date, the mechanisms underlying pubertal plasticity of the HPA axis have not been elucidated. Our finding that changes in DHEA and testosterone from early to late puberty are associated with changes in cortisol across this period suggests that increases in the hormones that are responsible for sexual maturation are related to the plasticity of cortisol regulation during puberty.

The few studies that have examined the impact of life stress on the coupling of cortisol with sex hormones during adolescence have reported equivocal findings (Dismukes, Johnson, Vitacco, Iturri, & Shirtcliff, 2015; Dismukes, Shirtcliff, et al., 2015; Ruttle et al., 2013). In the sole previous longitudinal study, adolescents with low scores on a composite measure of threat- and deprivation-related ELS exhibited linear increases in the strength of diurnal cortisol-DHEA coupling across early adolescence; in contrast, adolescents with high scores on this measure exhibited initial increases in the strength of coupling, which then plateaued (Ruttle et al., 2013). In a cross-sectional study of the diurnal coupling of cortisol with DHEA and testosterone in adolescence, Marceau, Ruttle, Shirtcliff, Hastings, et al. (2015) found that deviations in DHEA and testosterone from the linear diurnal slope were associated with deviations in cortisol from this slope, suggesting that within-individual associations between cortisol and sex hormones are caused by these hormones jointly deviating from typical patterns due to similar responses to the

same environmental influences. In this context, we found that the severity of threat-related ELS prior to puberty was associated with individual differences in the coupling of cortisol and DHEA across the pubertal transition. However, contrary to our hypothesis and the formulation that environmental threat would accelerate development and result in stronger positive coupling of changes in DHEA and cortisol, we found that higher severity of exposure to threat-related ELS was associated with *attenuated* coupling of changes in DHEA and changes in cortisol in both boys and girls. It is possible, therefore, that typical environmental input (e.g., minor daily stressors) results in concordant deviations in cortisol and DHEA, whereas environmental input that is characterized by severe threat leads to cortisol-DHEA discordance. We did not find that threat-related ELS influenced the longitudinal coupling of cortisol and testosterone, suggesting that, in this sample, threat-related ELS affected coordination of hormone production within the HPA axis rather than across the HPA and HPG axes. Finally, we found that the impact of threat-related ELS on longitudinal cortisol-DHEA coupling held when controlling for exposure to threat-related life stress that occurred during the transition through puberty. Previous studies examining pubertal recalibration of cortisol regulation have focused on previously institutionalized children who experienced dramatic shifts in the quality of the environment from early to late childhood and subsequently showed normalization of cortisol regulation during puberty (DePasquale et al., 2019; Quevedo et al., 2012). Among children raised in their families of origin, however, the quality of the environment is likely to remain similar across childhood and adolescence (Dunn et al., 2011); indeed, in our sample, the severity of threat-related ELS was correlated with the severity of threat-related stress occurring during the transition through puberty. Thus, for most children, puberty is likely to be a period during which the effects of exposure to severe ELS on HPA-axis functioning emerge or intensify, rather than remediate.

We should note three primary limitations of this study. First, although this study was longitudinal, we assessed adolescents at only two time-points; therefore, we were unable to model *trajectories* of the coupling of cortisol and sex hormones during puberty, which may not

be linear. Second, while our study design allowed us to examine whether changes in sex hormones predicted changes in cortisol from early to late puberty above and beyond levels of these hormones in early puberty, we cannot draw causal conclusions concerning whether changes in sex hormones *drive* changes in cortisol. Instead, changes in cortisol during puberty may drive changes in sex hormones, although this explanation is less likely given that our design focused on a period in which sex hormones, but not cortisol, increased substantially. Third, we assessed only waking levels of hormones. We selected waking levels in order to minimize the influence of typically unmeasured variables (e.g., minor daily stress); nevertheless, we were unable to examine changes in the dynamics of the coupling of cortisol and sex hormones across the day from early to late puberty. Given the limited number of previous investigations of the coupling of cortisol and sex hormones during adolescence, we compared our findings to those of previous studies that have measured hormones throughout the day or in response to stress; future investigations should examine the relations among these different measures of hormonal coupling during puberty.

The implications of HPA-axis dysregulation for mental and physical health and the effects of life stress on HPA-axis regulation have been studied for decades (Koss & Gunnar, 2018). The vast majority of previous studies, however, have focused on cortisol production in isolation, and have not examined the coordination of cortisol with other related hormones. This gap in the literature has hampered our knowledge of the development of the HPA axis, and, ultimately, of the relation of HPA-axis functioning with long-term mental and physical health outcomes. Findings of the current study indicate that the coordination of HPA-axis hormones changes during puberty and that exposure to threat-related ELS influences the effects of pubertal increases in sex hormones on changes in cortisol. These findings elucidate mechanisms underlying the pubertal recalibration of the HPA axis and have implications for the identification and treatment of adolescents experiencing adverse effects of exposure to ELS.

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The authors report no conflicts of interest.

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