

Differential stability and covariation of diurnal and stress-reactive HPA axis functions in adolescents with and without maltreatment risk

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ARTICLE INFO

Keywords:

Diurnal cortisol
Cortisol reactivity
HPA-axis
Trier Social Stress Task
Adversity
Puberty
Recalibration

ABSTRACT

The hypothalamic–pituitary–adrenal (HPA) axis is a key component of the neuroendocrine system that produces cortisol to regulate both sleep–wake cycles and stress physiology. Prior research shows that early maltreatment disrupts both the diurnal and stress-reactive functions of the HPA axis. However, previous studies—primarily conducted in healthy populations—have reported inconsistent associations between these two functions, and it remains unclear whether their association is moderated by maltreatment history and/or pubertal development. The clinical or at-risk status of the sample may be particularly relevant, as persistently disrupted diurnal cortisol regulation may more reliably predict disrupted stress reactivity than a well-regulated diurnal system that can flexibly adapt to daily demands. The present study addressed this gap by examining the association between diurnal and stress-regulatory functions of the HPA axis in 13-year-old adolescents with and without a history of maltreatment risk (referred to as high- and low-risk, respectively). Our analyses revealed that diurnal cortisol levels were significantly more stable in the high-risk group than in the low-risk group and that maltreatment risk moderated the association between diurnal morning-to-evening cortisol slope and stress reactivity (but not recovery or intercept). Specifically, a flatter—and therefore less optimal or more dysregulated—diurnal slope was coupled with blunted stress reactivity during the Trier Social Stress Task for youth in the high-risk group but not in the low-risk group. Exploratory analyses further showed that, in the high-risk group only, pubertal development moderated this effect, such that more mature adolescents exhibited the strongest decoupling of the diurnal slope and stress reactivity. Together, these findings highlight maltreatment history and pubertal development as key factors for understanding how diurnal cortisol parameters and stress reactivity become dynamically linked—and potentially reorganized—across adolescence.

1. Introduction

The Hypothalamic–Pituitary–Adrenal (HPA) axis is a complex neuroendocrine mechanism that orchestrates a cascade of hormonal responses and feedback loops to regulate sleep–wake cycles and stress physiology (Sapolsky et al., 2000). HPA axis function is disrupted by both prenatal exposure to maternal stress hormones and low quality of postnatal care received by children at risk for maltreatment (Howland et al., 2017). HPA axis dysregulation has been characterized by blunted diurnal cortisol production, lower overall cortisol secretion, and reduced

reactivity and blunted recovery following an acute stressor in children and adolescents (Koss and Gunnar, 2018). These alterations, in turn, have been linked to lifetime complications in physical health and poor mental health outcomes (Adam et al., 2017; Bernard et al., 2015b; Frost et al., 2018). Emerging evidence also suggests potential HPA-axis recalibration during pubertal development following early adversity when sources of dysregulation (e.g., neglect, abuse) are mitigated, and caregiving environments become more stable, sensitive, and responsive (Gunnar et al., 2019; Gunnar and Howland, 2022; King et al., 2017; Quevedo et al., 2012).

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<https://doi.org/10.1016/j.psyneuen.2026.107854>

Received 14 January 2026; Received in revised form 5 April 2026; Accepted 6 April 2026

Available online 7 April 2026

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The HPA axis has two main, relatively orthogonal functions: diurnal cortisol production and stress reactivity. The diurnal cortisol function maintains a circadian rhythm that supports sleep-wake cycles and energy metabolism throughout the day. In contrast, the stress reactivity and recovery functions of the HPA axis mount an endocrine response to an acute stressor and help the body recover from the stressful experience (Abercrombie et al., 2023). Although these two functions rely on overlapping neural and endocrine mechanisms (Androulakis, 2021; Nader et al., 2010), empirical evidence in humans remains limited regarding the relation between diurnal cortisol parameters and stress reactivity and recovery. To empirically address this question, the present study examined the coupling of the diurnal and stress-reactivity functions of the HPA axis in adolescents with prior involvement with Child Protective Services (CPS) versus community adolescents.

1.1. Diurnal cortisol regulation: normative vs. disrupted patterns

Diurnal cortisol regulation plays an important role in energy metabolism and the regulation of sleep-wake cycles across species (Gunnar and Vazquez, 2001; Nader et al., 2010). Diurnal cortisol follows a distinctive normative pattern, with levels beginning to rise before awakening and reaching their zenith approximately 30–45 min after waking. This is followed by a sharp decline and a gradual decrease in cortisol production throughout the day, reaching a near-zero concentration at bedtime. The change in morning-to-evening cortisol concentration, known as the diurnal slope, emerges by 3–6 months after birth and mirrors the adult pattern by 2 years of age (Gunnar and Donzella, 2002). The diurnal pattern is causally shaped by the quality of care that infants receive (Bernard et al., 2015a; Hostinar et al., 2015; Koss and Gunnar, 2018; McLaughlin et al., 2015), with prolonged or recurrent stressors such as extreme poverty (Doom et al., 2014), institutional care (Koss et al., 2014), and child maltreatment (Bruce et al., 2009) leading to a blunted or flattened diurnal slope (Bernard et al., 2010; Bernard et al., 2015a, 2015b; Susman, 2006). Blunted diurnal slopes reflect a diminished daily cortisol decline, largely driven by reduced morning cortisol levels (Adam et al., 2017; Gunnar and Vazquez, 2001) that are associated with negative outcomes in a general rather than a specific way across a multitude of developmental outcomes. These outcomes include poor immune system and cardiovascular functioning, metabolic health, limbic system development, externalizing and internalizing problems, antisocial behavior, and fatigue in youth exposed to adversity (Adam et al., 2017; Gunnar and Vazquez, 2001; Susman, 2006).

1.2. Stress reactivity and recovery: normative vs. perturbed patterns

The stress reactivity and recovery function of the HPA axis plays an important role in acute stress regulation. After exposure to an acute stressor, cortisol levels begin to increase, superseding the diurnal concentration levels (stress reactivity phase), and then gradually return to baseline once the stressor is resolved (stress recovery phase) (Allen et al., 2017; Linden et al., 1997; Sapolsky et al., 2000). This cortisol response is one of the biological mechanisms that sustains the body's 'fight or flight' reaction (Sapolsky et al., 2000) that can be activated by both physical and psychological threats in humans (and other primates), particularly when threats are uncontrollable or involving social evaluation (Dickerson and Kemeny, 2004). Following an acute threat, the release of high levels of cortisol is an evolutionarily adaptive mechanism that supports survival (e.g., vasoconstriction, increased cardiac output, immunosuppression), yet excessive, prolonged, and recurrent utilization of the stress reactivity system or its failure to recover following a stressor can culminate in deleterious outcomes (McEwen, 2018).

Dysregulation in the stress reactivity and recovery systems has been primarily studied in correlational studies, and predominantly manifests as blunted cortisol patterns (Bunea, Szentágotai-Tátrai, and Miu, 2017). Although our understanding of the underlying causal mechanisms remains limited, one hypothesis is that such hypocortisolism reflects a

downregulation following prior hyperactivation of the HPA axis (Fries et al., 2005). One experimental study found causal associations between early extreme experiences of neglect and institutional rearing during the first two years of life and blunted cortisol reactivity at age 13 (McLaughlin et al., 2015). These results suggest that early caregiving adversity causes a blunted cortisol reactivity pattern, unlike the typical increase in cortisol levels during stressful experiences. Such blunted stress reactivity to an acute stressor can manifest as both a failure to mount a cortisol response and a failure or delay in returning to baseline cortisol levels following a previous stressor (Carroll et al., 2017). Taken together, the blunted cortisol patterns across both the diurnal and the stress reactivity and recovery functions of the HPA axis may be indicative of a cortisol regulation system that is unable to adapt to the diurnal metabolic needs of the organism, prepare the body for physiological "fight or flight," and support its recovery (Miller et al., 2007).

1.3. Association between diurnal cortisol and stress reactivity patterns

A blunted diurnal slope is assumed to imply blunted cortisol reactivity and recovery within the stress response system to acute stressors (Gunnar and Vazquez, 2001); however, relatively few studies have examined how these two indices relate to one another. A recent meta-analysis synthesized this modest literature on the associations between diurnal cortisol parameters among healthy adults and children, including the diurnal slope, and cortisol reactivity and recovery measured in experimental designs (Wesarg-Menzel et al., 2024). The authors found a negligible association between these endocrine markers. The only notable finding was that a steeper diurnal slope was associated with better stress recovery, but only when using a cortisol reactivity parameter that was not independent of total cortisol output (C_{min} , the minimal cortisol concentration across all measurement time points). Other cortisol parameters of stress reactivity or recovery (e.g., percentage change or area under the curve) were not significantly associated with the diurnal slope (Wesarg-Menzel et al., 2024). The authors concluded that "evidence that diurnal cortisol parameters would relate to acute cortisol stress reactivity and recovery is rather weak" (Wesarg-Menzel et al., 2024, p. 10).

One potential explanation for this null effect is the low stability of diurnal cortisol indices, in particular the diurnal slope (Ross et al., 2014). Cortisol reactivity and recovery parameters are often measured in childhood and adolescence using the Trier Social Stress Test (TSST-C), a social evaluative stressor paradigm (Buske-Kirschbaum et al., 1997). Stress reactivity profiles measured with the TSST-C appear to be moderately stable (test-retest stability r s between .30 and .68; Hankin et al., 2015; Susman et al., 1997), consistent with the idea that differences in cortisol reactivity in laboratory stressors reflect trait-like characteristics (Hankin et al., 2015). However, the picture is different for diurnal cortisol parameters: although there is variability in the literature, these parameters generally show low stability (Norton et al., 2023). Some have suggested that 8–10 consecutive days of data collection are needed to achieve stable diurnal slope parameters usable for between-subject research, a high burden for participants and researchers that most studies do not follow (Kuhlman et al., 2019; Segerstrom et al., 2014). Ross and colleagues (2014) painted an even bleaker picture of the diurnal slope's stability. The authors concluded that diurnal cortisol parameters reflected day-to-day fluctuations and behaved more in a state-like rather than a trait-like manner. Therefore, they proposed that research on diurnal cortisol parameters should focus on within-person changes and short-term fluctuations, given the difficulty of obtaining a stable estimate of diurnal cortisol activity (Ross et al., 2014). The lack of stability of the diurnal slope measured over days would logically lead to unreliable associations between cortisol reactivity and recovery, as the recent meta-analysis concluded (Wesarg-Menzel et al., 2024).

However, reliable findings in the literature indicate stable, trait-like properties of diurnal cortisol parameters, including a consistent association between early adversity and a blunted diurnal slope, and that

blunted diurnal cortisol regulation predicts negative health outcomes (Adam et al., 2017). Some have suggested that diurnal cortisol patterns may be “stably dysregulated” (e.g., consistently blunted) among certain vulnerable populations, such as maltreated youth (Ross et al., 2014). If the HPA axis becomes “stably dysregulated” in maltreated youth, then we should expect stronger associations between diurnal slope parameters and cortisol reactivity and recovery among clinical or high-risk subgroups than among healthy populations, for whom more variability (and lower stability) of diurnal cortisol parameters may be indicative of adaptive flexibility in stress hormone regulation to respond to daily demands, rather than persistent dysregulation.

1.4. Adolescence as a sensitive period for stress recalibration

Growing evidence suggests that adolescence is a sensitive period during which the stress response system can recalibrate following early adversity (Gunnar et al., 2019; Gunnar and Howland, 2022; King et al., 2017; Quevedo et al., 2012). Much of this work has focused on previously institutionalized youth adopted into high-resource families, and less is known about how recalibration unfolds in never-institutionalized but adversity-exposed youth. Across studies, previously institutionalized youth at more advanced pubertal stages tend to exhibit more normative, reactive cortisol responses to acute stressors than their less mature peers, often approximating levels observed in never-institutionalized youth. A recent longitudinal study of pubertal recalibration found that cortisol reactivity increased across puberty (post-Tanner stage 3), but only among maltreated youth with low parental depressive symptoms, suggesting recovery of normative HPA-axis responsivity in supportive caregiving contexts (Negri et al., 2025). The stress recalibration theory, therefore, posits that within supportive, caregiving environments, adolescents may capitalize on this period of heightened plasticity to normalize stress-reactive functioning despite earlier disruptions (DePasquale, Herzberg, and Gunnar, 2021). Accordingly, pubertal stage may represent a key developmental moderator of HPA axis functioning and may shape associations among the diurnal and stress-reactive indices of cortisol regulation. In particular, whereas ‘stably dysregulated’ diurnal cortisol profiles may lead to stronger associations with cortisol reactivity in youth with a history of maltreatment (given the reduced variability associated with a blunted profile across both indices), pubertal recalibration of the stress regulation system may promote normalization of cortisol patterns. Consequently, associations between diurnal and reactive cortisol indices may come to resemble those observed in non-maltreated populations, namely, weaker or absent associations, as reported in the meta-analysis by Wesarg-Menzel and colleagues (2024).

1.5. The present study

The present study examined the moderating role of maltreatment risk on the associations among three indices of cortisol regulation—diurnal slope, cortisol reactivity, and cortisol recovery—in two groups of adolescents: one with a history of maltreatment risk and CPS involvement during infancy and another without CPS involvement. To enhance index reliability, we used multiple-indicator latent change score analyses to model the diurnal slope—a modeling approach that separates true variance from error variance in diurnal cortisol data, thereby reducing measurement noise associated with day-to-day fluctuations and sampling variability. By examining these biomarker associations in a group of adolescents exposed to caregiving-related adversities where a higher presence of ‘stable dysregulation’ is hypothesized, and in a comparison group of non-maltreated youth with low risk for dysregulation, our goal was also to test whether diurnal slope and stress reactivity associations are indeed more pronounced in a high-risk sample than in a healthy sample. Finally, as an exploratory aim, we examined whether pubertal status moderated these associations.

1.6. Hypotheses

We hypothesized that the cortisol diurnal slope would be significantly associated with both cortisol reactivity and cortisol recovery, such that a less steep (i.e., blunted or flattened) diurnal slope would correspond to reduced reactivity (a more negative slope) and reduced recovery (a less negative slope). We further expected that these associations would be more pronounced in the CPS-involved group than in the low-risk community sample.

2. Methods

2.1. Participants

The analysis sample included 151 adolescents: 102 with a history of CPS involvement (referred to as the “high-risk group”, female $n = 48$) and 49 without CPS involvement (the “low-risk group”; female, $n = 22$). Families became involved with CPS due to factors such as homelessness, possible abuse, parental substance abuse, maltreatment of other children in the family, domestic violence, or physical or educational neglect. Adolescents in the high-risk group were originally part of a larger randomized clinical trial (RCT) evaluating the efficacy of the Attachment and Biobehavioral Catch-up (ABC) parenting intervention. Families were referred by CPS during infancy as part of a foster care diversion program and were randomized to receive ABC ($n = 50$) or a control intervention, Developmental Education for Families (DEF; $n = 52$). After the intervention, families were invited to participate in a longitudinal follow-up study assessing ABC’s long-term effects, with research visits occurring in early childhood, middle childhood, and early adolescence. The low-risk group was recruited during the middle childhood wave through community outreach at local schools and community centers. Informed consent was obtained from parents, and written assent was provided by the adolescents. The study was approved by the University of Delaware Institutional Review Board. See Table 1 for detailed demographics.

2.2. Procedures

2.2.1. Trier social stress test for children (TSST-C)

The TSST-C is a widely used laboratory procedure to assess stress reactivity, based on a social-evaluative stressor (Buske-Kirschbaum et al., 1997). The detailed study procedure is available in Section 1 of the [Supplementary Materials](#).

2.3. Measures

2.3.1. Cortisol reactivity and recovery

The TSST-C was administered to all participants in the late morning or early afternoon, after a series of neuroimaging scans, with the protocol starting between 11:00 am and 3:35 pm. Most participants began the procedure before 2:30 pm, with only three starting after 2:30 pm. Participants were instructed not to eat or drink for 30 min prior to the start of the procedures. Over the course of the TSST-C protocol, adolescents provided six saliva samples via passive drool. Saliva samples were stored at -20°C after the procedure. See Fig. 1 for the sample collection timeline and rows 3 and 4 of Section 3, [Supplementary Figure 1](#) for the sampling time distribution of all TSST-C samples.

2.3.2. Diurnal cortisol

Participants were given an at-home saliva collection kit and instructed to collect saliva samples in the morning, afternoon, and at bedtime for three consecutive days. For the morning sample, adolescents were instructed to take it immediately upon waking, before brushing their teeth; for the afternoon sample, at 12 pm, before lunch; and for the evening sample, before going to bed. Considering that, on school days, afternoon sample collection was often delayed and frequently

Table 1
Demographic characteristics of the study sample.

Variable	High-risk group	Low-risk group	Test Statistic
Sex	Females = 48 (47.06%) Males = 54 (52.94%)	Females = 22 (44.9%) Males = 27 (55.1%)	$\chi^2 = .006$, $p = .94$
Race	African American = 73 (71.57%) Asian American = 1 (0.98%) Biracial = 12 (11.76%) Caucasian = 5 (4.9%) Other = 10 (9.8%)	African American = 22 (44.9%) Asian American = 0 (0%) Biracial = 13 (26.53%) Caucasian = 9 (18.37%) Other = 5 (10.2%)	$\chi^2 = 15.54$, $p = .008$
Ethnicity	Did not answer = 1 (0.98%) Hispanic = 17 (16.67%) Non-Hispanic = 84 (82.35%)	Did not answer = 0 (0%) Hispanic = 10 (20.41%) Non-Hispanic = 39 (79.59%)	$\chi^2 = .095$, $p = .758$
Parental education	Did not answer = 1 (0.98%) No high-school diploma = 21 (20.59%) GED = 18 (17.65%) High-school diploma = 37 (36.27%) Some college = 22 (21.57%) College degree = 1 (0.98%) Postgraduate degree = 0 (%)	Did not answer = 0 (0%) No high-school diploma = 1 (2.04%) GED = 3 (6.12%) High-school diploma = 11 (22.45%) Some college = 20 (40.82%) College degree = 8 (16.33%) Postgraduate degree = 5 (%)	$\chi^2 = 40.727$, $p < .001$
Age (SD) - diurnal cortisol sampling	Did not answer = 3 (%) M = 13.422 (0.4) years Min = 12.986 Max = 14.586	Did not answer = 1 (%) M = 13.33 years SD = 0.32 Min = 12.926 Max = 14.123	$t(104) = -1.181$, $p = .24$
Age (SD) - TSST-C sampling	M = 13.411 (0.36) years Min = 13.022 Max = 14.356	M = 13.316 (0.29) years Min = 12.956 Max = 14.096	$t(146) = -1.576$, $p = .117$
Mean (SD) PDS score	M = 3.419 (0.86) Min = 1 Max = 5 Missing: N = 9	M = 3.543 (0.94) Min = 2 Max = 5 Missing: N = 3	$t(137) = 0.775$, $p = .439$ $\chi^2 = 0.064$, $p = .800$

Note. SD - standard deviation; TSST-C - Trier Social Stress Task for children; PDS - Pubertal Development Scale.

contaminated by food, we decided to model only the morning and evening samples to reduce noise in the data. Participants were asked to write on the prelabeled vials the time, date, and, for the morning samples, how many minutes after waking up the sample was collected, and to immediately place the samples in their freezer. In addition, participants completed a daily diary detailing the date and time samples were taken, medications taken that day, illness, sleep quality and duration, and whether they had any food or drink prior to sample collection. Completed samples were returned to the laboratory by having participants return them during their in-person lab visit, by a staff member picking them up from local participants' homes, or by mailing them back with an icepack in an insulated envelope for distant participants. Immediately after the samples were returned to the lab, they were stored at -20°C in a freezer before being assayed. See [Section 3, Supplementary Figure 1](#), rows 1–2 for diurnal cortisol sampling time distribution.

2.3.3. Maltreatment risk

A binary maltreatment risk variable served as a moderator in our analyses. All participants with a history of CPS involvement were categorized as “high-risk,” and all those without a history of CPS involvement were categorized as “low-risk” for maltreatment.

2.3.4. Pubertal development scale

The Pubertal Development Scale (PDS; [Petersen et al., 1988](#)) is a self-report measure of pubertal development. It includes items assessing growth in height, body hair, and skin changes, with additional sex-specific items (e.g., breast development and menarche for girls, voice changes and facial hair for boys). Each item is rated on a 4-point scale that reflects the degree of physical maturation, ranging from no development to complete development. A composite score on a 5-point scale was computed by averaging across items, with prepubertal stage coded as 1, early pubertal as 2, midpubertal as 3, late pubertal as 4, and postpubertal as 5.

2.4. Cortisol data preparation

Cortisol data were prepared for analysis using methods outlined in previous studies ([Bernard et al., 2015a](#); [Garnett et al., 2020](#)). Briefly, the following exclusion criteria were used during data cleaning: 1) cortisol concentration values equal or larger than $2.0\text{ }\mu\text{g/dL}$ (considered biologically implausible); 2) cortisol concentration values three standard deviations above the mean; 3) cortisol concentration values with an intra-assay CV value above 10%; 4) concentration values below the

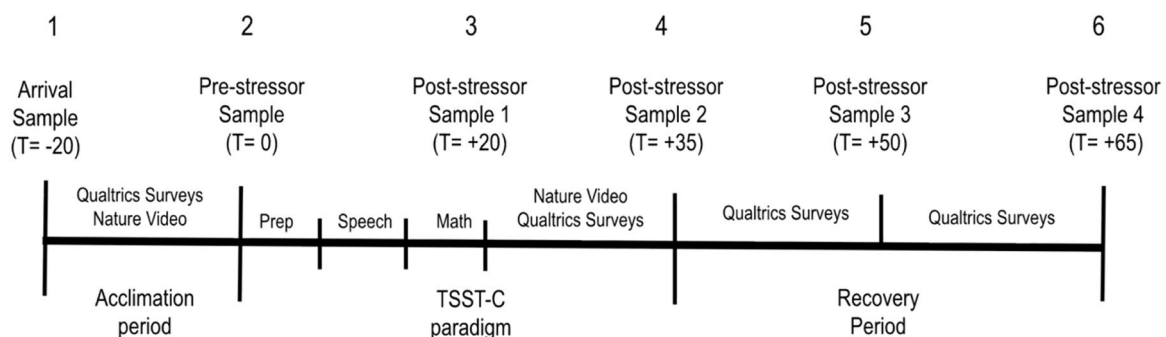


Fig. 1. Procedure outline for the Trier Social Stress Test (TSST-C).

Table 2

Descriptive statistics for diurnal and TSST-C cortisol measures.

		Raw cortisol concentration values (µg/ dL)			Log10-transformed cortisol concentration values (µg/ dL)		
Variable	N	Mean	SD	Min/ Max	Mean	SD	Min/Max
Diurnal Cortisol							
Day 1 wake-up	101	0.236	.206	0.004 / 0.849	-0.819	.453	-2.398 / -0.071
Day 1 bedtime	95	0.129	.128	0.004 / 0.628	-1.092	.442	-2.398 / -0.202
Day 2 wake-up	97	0.219	.180	0.014 / 0.788	-0.815	.397	-1.854 / -0.103
Day 2 bedtime	96	0.120	.128	0.012 / 0.555	-1.120	.413	-1.921 / -0.256
Day 3 wake-up	94	0.196	.161	0.02 / 0.745	-0.862	.391	-1.699 / -0.128
Day 3 bedtime	95	0.090	.085	0.008 / 0.352	-1.218	.398	-2.097 / -0.453
Trier Social Stress Paradigm							
Arrival	146	0.141	.103	0.007 / 0.841	-0.936	.271	-2.155 / -0.075
Pre- Stressor	147	0.129	.096	0.006 / 0.88	-0.973	.269	-2.222 / -0.056
Post- Stressor 1	145	0.166	.128	0.032 / 1.103	-0.866	.265	-1.495 / 0.043
Post- Stressor 2	145	0.229	.217	0.043 / 1.631	-0.769	.320	-1.367 / 0.212
Post- Stressor 3	144	0.198	.187	0.035 / 1.207	-0.823	.306	-1.456 / 0.082
Post- Stressor 4	144	0.165	.152	0.031 / 1.078	-0.886	.280	-1.509 / 0.033

Table 3

Missing cortisol data descriptive statistics.

Diurnal Cortisol (N = 103)		Trier Social Stress Task (N = 148)	
Sample	Number of missing samples (%)	Sample	Number of missing samples (%)
Day 1 wake-up	2 (1.94)	Arrival	2 (1.35)
Day 1 bedtime	8 (7.77)	Pre-Stressor	1 (0.68)
Day 2 wake-up	6 (5.83)	Post-Stressor 1	3 (2.03)
Day 2 bedtime	7 (6.8)	Post-Stressor 2	3 (2.03)
Day 3 wake-up	9 (8.74)	Post-Stressor 3	4 (2.7)
Day 3 bedtime	8 (7.77)	Post-Stressor 4	4 (2.7)

detection limit (1 value) were replaced with the 0.004 µg/dL, the lower-level sensitivity of the assay. See Table 2 for cortisol descriptive statistics, and Table 3 for details on missing data.

2.4.1. Diurnal cortisol data

Participants were asked to collect up to 3 morning and 3 evening saliva samples at home, yielding a maximum of 906 potential samples (6 samples per participant * 151 participants). Out of this total, 48 (31.79%) participants did not provide any samples (288 missing samples) or were omitted due to insufficient saliva volume (21 samples). Outlier exclusion removed a total of 19 samples. Furthermore, 4 samples from 3 participants had cortisol concentrations below the detectable limit, prompting their replacement with 0.004 µg/dL. Therefore, the final sample included 578 diurnal cortisol samples across 103

participants. To address the positively skewed distribution of cortisol concentration values, a log10 transformation was applied.

2.4.2. TSST-C cortisol data

Each participant was asked to provide 6 saliva samples during the research visit, yielding a maximum of 906 samples. Of this total, we did not use the samples collected upon arrival, as they served only as a control (151 samples). Of the 151 participants, 3 (1.99%) did not complete the TSST-C protocol and therefore did not provide saliva samples (15 samples missing), resulting in a final TSST-C sample size of 148. Ten samples were missing due to insufficient saliva volume or premature termination of the TSST-C before the end of the protocol, and 5 samples were excluded as outliers because their intra-assay CV exceeded 10%. The final TSST-C sample comprised 725 samples. The data cleaning procedure and exclusion criteria for the TSST-C samples were the same as for the diurnal data, except that cortisol concentration values 3 SD above the mean were not removed.

2.4.3. Saliva assaying procedure

Description of the assaying procedure is available in Section 2 of the Supplementary Materials.

2.5. Data analytical strategy

We used R (Version 1.2.1335; R Core Team, 2025) for data cleaning, visualization, and analysis.

Diurnal cortisol change was analyzed using multiple-indicator latent change score (MI-LCS) modeling for morning and evening cortisol across three days (Bernard et al., 2015a; Kievit et al., 2018), and nonlinear change across five repeated TSST-C laboratory assessments was examined using latent growth curve modeling. Missing data were handled using full-information maximum likelihood (FIML) estimation, which uses all available data under the assumption that data are missing at random (MAR). MI-LCS and latent growth curve modeling offer greater reliability than traditional approaches (e.g., repeated-measures ANOVA) by accounting for measurement error and modeling latent constructs (King et al., 2006; McArdle, 2009). These methods estimate individual variability through random slopes and intercepts, address nested data structures, and are robust to missing or unevenly spaced data. Additionally, latent growth curve modeling accommodates non-linear trajectories, capturing complex patterns of change over time.

Latent factor scores, representing diurnal slope (delta), TSST-C cortisol intercept, reactivity, and recovery, were extracted using the *lavPredict* function from the *lavaan* package in R (Rosseel, 2012) and subsequently used as observed variables in regression models. In all models, sex and age were included as covariates of no interest.

Model fit was evaluated using absolute fit indices, including Chi-Square difference testing (non-significant *p*-values suggest no improvement in fit), CFI and TLI ($\geq .95$ = good fit), RMSEA ($\leq .06$ = good fit), and SRMR ($\leq .08$ = good fit).

2.5.1. Diurnal cortisol

Wakeup and bedtime log10-transformed cortisol concentration values were plotted to visually inspect within- and between-person change patterns (see Section 3, Supplementary Figure 2). Then the latent factors were defined as wake-up and bedtime cortisol values, each comprising the three log10-transformed cortisol values derived from three morning and three evening samples (for descriptive statistics, see Table 2). The latent change score model denotes the cortisol variation from wake-up to bedtime, with a more negative latent change score indicating a steeper decline in cortisol concentration throughout the day.

2.5.2. Trier social stress task

Based on Miller et al. (2013), participants were categorized as “responders” if 115% increase in cortisol concentration was observed

across any of the Post-Stressor samples (3 through 6), compared to the Pre-Stressor sample. The protocol was successfully administered and triggered a cortisol stress response, with 72.6% of participants categorized as responders.

We expected that the change in log10-transformed cortisol concentrations throughout the TSST-C would show reactivity and recovery phases. The “Arrival” sample was not modeled as it served only to assess acclimation to the laboratory space. The “Pre-Stressor” and the 4 Post-Stressor sample concentrations were visualized in a spaghetti plot (see Section 3, Supplementary Figure 3), which confirmed the reactivity and recovery phases of change, with the Post-Stressor 2 sample representing the knot that separates the reactivity and recovery slopes.

2.5.3. Association between diurnal slope and reactivity and recovery on the TSST-C

Using an SEM framework, we combined the multiple-indicator latent change score model and the TSST-C growth curve model with the best fit into a single model. Specifically, we modeled three regression paths: the latent change score for the diurnal cortisol was regressed on the TSST-C intercept, reactivity, and recovery slopes (see Fig. 2 for a path diagram). Age and sex were included as covariates in the model.

2.5.4. The moderating role of maltreatment risk and pubertal status

Using the extracted latent estimates, we conducted two sets of moderation analyses. The first set of models examined whether risk status and diurnal slope predicted TSST-C intercept, reactivity, and recovery. In each model, predictors included diurnal cortisol slope (delta), risk status, and their interaction (delta*risk).

In a second set of analyses, we tested whether associations between diurnal cortisol slope and TSST-C intercept, reactivity, and recovery varied as a function of both risk status and pubertal development. These models included main effects of diurnal slope (delta), risk status, and pubertal category; all two-way interactions (delta*risk, delta*pubertal status, risk*pubertal status); and the three-way interaction (delta*risk*pubertal status). All models controlled for child sex and age at the TSST-C assessment.

3. Results

3.1. Preliminary analyses

3.1.1. Missingness

The percentage and number of missing values are shown in Table 3. As noted above, FIML estimation was used to handle missing data because it provides valid parameter estimates under MCAR and MAR

assumptions, with MAR more plausible in longitudinal contexts (Ji, Chow, Schermerhorn, Jacobson, and Cummings, 2018). To increase confidence that missingness was not related to key socioeconomic factors that could plausibly affect household stability and parental oversight of diurnal cortisol sample collection, we used binary logistic regression to examine whether parental education and family income were associated with missing diurnal cortisol data. The tests indicated that parental education and family income were not significant predictors of the likelihood of diurnal missingness (education: $\chi^2(1, N = 147) = 0.00, p = .978$; family income: $\chi^2(1, N = 100) = 1.61, p = .238$), and the models did not significantly improve fit compared to the null model (education: $\beta = 0.004, SE = 0.14, z = 0.03, p = .978, OR = 1.00$; family income: $\beta = 0.000006, SE = 0.000005, z = 1.18, p = .238, OR = 1.00$).

3.1.2. Intraclass correlation and test of homogeneity of variances

3.1.2.1. Diurnal cortisol. ICC: The stability of diurnal cortisol concentration levels across six sampling occasions (morning and evening, across three days) was assessed using a one-way random-effects intraclass correlation coefficient (ICC) for average measures. Results indicated moderate-to-good within-person reliability in the total sample, $ICC(1, 6) = .71, 95\% CI [.60, .80], F(79, 400) = 3.49, p < .001$.

Within the high-risk group only, the results indicated good within-person reliability, $ICC(1, 6) = .81, 95\% CI [.73, .88], F(58, 290) = 5.35, p < .001$, whereas in the low-risk group the reliability was moderate-to-poor, $ICC(1, 6) = .50, 95\% CI [.08, .77], F(20, 105) = 2.01, p = .013$. The stability of diurnal cortisol measurements differed significantly between the high- and low-risk groups, Fisher's $z (n \text{ high-risk} = 71, n \text{ low-risk} = 32) = 2.605, p = .009$. Specifically, ICCs indicated greater stability in the high-risk group than in the low-risk group.

Homogeneity of variances: A Levene's test was conducted to examine the homogeneity of variance in diurnal slope between the high- and low-risk groups. The variances did not differ significantly between groups, $F(1, N-2) = 0.79, p = .37$, suggesting that the assumption of homogeneity of variance was met. See Fig. 3 for overlapping frequency distribution histograms of diurnal slopes in the high- and low-risk groups.

3.1.2.2. Trier social stress task. ICC: The stability of the TSST-C cortisol concentration levels across 5 sampling occasions (Pre-stressor, Post-stressor 1–4) was assessed using a one-way random-effects intraclass correlation coefficient ($ICC[1,5]$) for average measures. Results indicated excellent within-person reliability across the risk groups, $ICC(1,5) = .95, 95\% CI [.93, .96], F(136, 544) = 18.70, p < .001$.

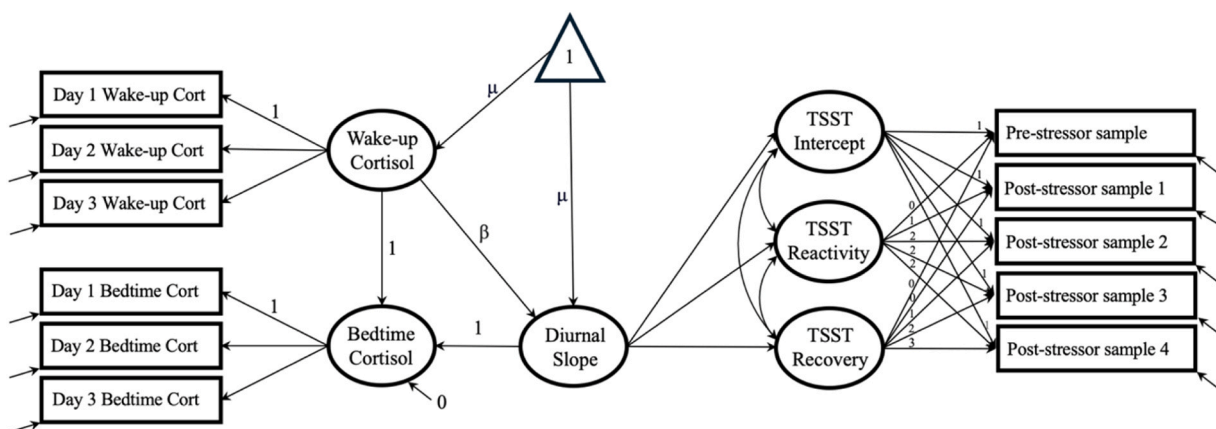


Fig. 2. Path diagram for the combined latent change and growth curve model depicting the association between the latent diurnal cortisol slope and the TSST-C latent intercept, reactivity, and recovery variables. Day 1 Wake-up Cort - Day 3 Wake-up Cort and Day 1 Bedtime Cort - Day 3 Bedtime Cort represent log-transformed wake-up and bedtime cortisol indicators for Days 1 through 3, respectively. Covariates (age and sex) were included in the model, but are not depicted for simplicity.

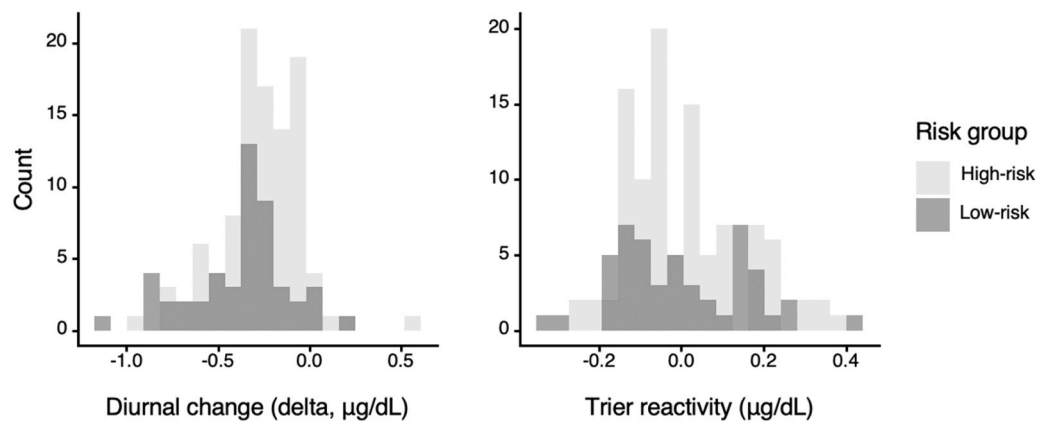


Fig. 3. Overlapping frequency histograms of diurnal slope and TSST-C reactivity by maltreatment risk group.

In the high-risk group, the within-person reliability was excellent ($ICC(1, 5) = .94$, 95% CI [.92,.96], $F(91, 368) = 16.80$, $p < .001$), whereas in the low-risk group it was good ($ICC(1, 5) = .90$, 95% CI [.84,.94], $F(44, 180) = 9.61$, $p < .001$). The stability of TSST-C reactivity measurements did not differ significantly between the high- and low-risk groups, *Fisher's z* (n high-risk = 100, n low-risk = 48) = 1.72, $p = .086$.

Homogeneity of variances: A Levene's test was conducted to assess the variance homogeneity in stress reactivity between the high- and low-risk groups. The variances did not significantly differ between groups, $F(1, 149) = 1.56$, $p = .21$, suggesting that the assumption of homogeneity of variance was met. See Fig. 3 for overlapping frequency distribution histograms of TSST-C reactivity in the high- and low-risk groups.

3.2. Demographic information

As shown in Table 1, there was a significant difference in parental education and racial distribution between the high- and low-risk maltreatment groups, with low-risk parents having significantly higher educational background than the high-risk group, and higher African-American representation in the high-risk than in the low-risk group. In our primary analyses, we did not control for parental education and racial distribution to avoid biasing estimates of risk status, diurnal slope (delta), and interaction effects on TSST-C parameters. Specifically, race and parental education reflect structurally embedded social determinants that may contribute to risk exposure rather than represent independent confounds. Adjusting for them may therefore obscure meaningful variance associated with adversity (Miller and Chapman, 2001).

3.3. Pubertal development scale

As shown in Table 1, there were no significant mean differences in pubertal development between the high- and low-risk groups. Furthermore, no group differences emerged in the extent of missing data.

3.4. Diurnal slope and TSST-C cortisol reactivity and recovery

The combined latent change score and growth curve structural equation model demonstrated excellent fit: $\chi^2(59) = 50.36$, $p = .781$, CFI = 1.00, TLI = 1.01, RMSEA = .000, SRMR = .068. Factor loadings for all latent variables were significant and in the expected direction (see Table 4). The self-feedback parameter from morning to delta was significant ($\beta = -0.663$, $p < .001$), indicating that higher morning cortisol was associated with greater decreases across the day. Diurnal slope significantly associated with TSST-C reactivity ($\beta = -0.106$, $p = .015$), such that individuals with less negative diurnal slope (i.e., flatter slopes) showed lower reactivity. No significant associations were observed

between delta and the TSST-C intercept or recovery slopes. Age and sex were also not significant predictors of any of the latent cortisol variables. See Fig. 4, column A, for a visualization of these effects.

3.5. The moderating role of early maltreatment risk

As shown in Table 5, history of maltreatment risk did not interact with diurnal slope (delta) in predicting TSST-C intercept or recovery, but it did in predicting TSST-C reactivity, such that the association between diurnal slope (delta) and TSST-C reactivity was more negative in the high-risk group than in the low-risk group. Follow-up simple slopes analyses for the significant diurnal slope (delta)*maltreatment risk interaction revealed that the negative effect of delta on TSST-C reactivity was significant for high-risk adolescents ($\beta = -0.26$, $SE = 0.06$, $t(142) = -4.12$, $p < .001$), but non-significant for low-risk adolescents ($\beta = -0.03$, $SE = 0.08$, $t(142) = -0.33$, $p = .74$). This suggests that the association between the diurnal slope and reactivity depends on risk status, and that in the high-risk group only, having a blunted diurnal slope is associated with less reactivity to the TSST-C. See Fig. 4, panel B, for a visualization of the results.

3.6. The moderating role of pubertal development status

Exploratory analyses examined the three-way interaction between diurnal slope (delta), maltreatment risk, and pubertal development stage. No significant three-way interactions emerged when TSST-C intercept or recovery was the outcome; however, a significant 3-way interaction (delta*maltreatment risk*pubertal development stage) was observed for TSST-C reactivity. Follow-up simple slopes analyses revealed that diurnal slope was negatively associated with TSST-C reactivity among high-risk adolescents, particularly at early pubertal maturity ($\beta = -0.44$, $SE = 0.12$, $t(128) = -3.60$, $p < .001$) and at moderate pubertal maturity ($\beta = -0.29$, $SE = 0.07$, $t(128) = -4.30$, $p < .001$), but not among high-risk youth at the late or post-pubertal level ($\beta = -0.12$, $SE = 0.1$, $t(128) = -1.24$, $p = .214$). No significant three-way interaction was observed for low-risk adolescents (all p values $> .36$). See Fig. 4, column C, for a visualization of the results.

4. Discussion

The present study examined whether maltreatment history and/or pubertal development affected associations between diurnal slope and stress responsiveness. Results indicated that both risk status and pubertal development moderated the association between diurnal slope and stress reactivity (but not intercept or recovery), with significant associations emerging only among the high-risk group with a history of CPS involvement during early childhood. Specifically, a flatter diurnal slope was associated with lower stress reactivity during the TSST-C in

Table 4
Measurement model factor loadings for cortisol latent variables.

Latent Variable	Unstandardized Estimate	Standardized Estimate (β) [95% CI]	SE	Est./SE	p-value
Diurnal cortisol					
<i>Morning cortisol</i>					
Day 1 wake-up	1.000	0.675 [0.569, 0.781]	0.054	12.50	< .001
Day 2 wake-up	1.046	0.796 [0.702, 0.890]	0.048	16.60	< .001
Day 3 wake-up	1.033	0.774 [0.680, 0.867]	0.048	16.17	< .001
<i>Evening cortisol</i>					
Day 1 bedtime	1.000	0.685 [0.581, 0.789]	0.053	12.91	< .001
Day 2 bedtime	1.046	0.803 [0.707, 0.900]	0.049	16.30	< .001
Day 3 bedtime	1.033	0.782 [0.693, 0.870]	0.045	17.22	< .001
Diurnal slope (delta = ~ Evening cortisol)	1.000	1.128 [0.893, 1.362]	0.119	9.479	< .001
Trier Social Stress Task (TSST-C)					
<i>TSST-C intercept</i>					
Pre-Stressor	1.000	0.962 [0.910, 1.013]	0.026	36.36	< .001
Post-Stressor 1	1.000	0.937 [0.853, 1.021]	0.043	21.83	< .001
Post-Stressor 2	1.000	0.790 [0.673, 0.907]	0.060	13.24	< .001
Post-Stressor 3	1.000	0.821 [0.705, 0.938]	0.060	13.79	< .001
Post-Stressor 4	1.000	0.899 [0.767, 1.031]	0.067	13.33	< .001
<i>TSST-C reactivity</i>					
Pre-Stressor	0.000	0.000 [0.000, 0.000]	0.000		
Post-Stressor 1	1.000	0.530 [0.450, 0.611]	0.041	12.86	< .001
Post-Stressor 2	2.000	0.895 [0.780, 1.009]	0.058	15.29	< .001
Post-Stressor 3	2.000	0.929 [0.812, 1.047]	0.060	15.50	< .001
Post-Stressor 4	2.000	1.017 [0.871, 1.163]	0.074	13.67	< .001
<i>TSST-C recovery</i>					
Pre-Stressor	0.000	0.000 [0.000, 0.000]	0.000		
Post-Stressor 1	0.000	0.000 [0.000, 0.000]	0.000		
Post-Stressor 2	0.000	0.000 [0.000, 0.000]	0.000		
Post-Stressor 3	1.000	0.289 [0.247, 0.330]	0.021	13.67	< .001
Post-Stressor 4	2.000	0.632 [0.530, 0.733]	0.052	12.22	< .001

the high-risk group. Exploratory analyses further revealed that this association was particularly pronounced among youth at the early- to mid-pubertal stage. As pubertal maturation progressed beyond midpuberty, the association weakened and was non-significant at the late- to post-pubertal stage. No significant moderation was observed among adolescents without a history of early maltreatment. These findings are notable because they help explain why prior research in low-risk samples has often failed to detect meaningful associations between the diurnal and

stress-reactive functions of the HPA axis.

Past work, including a recent meta-analysis (Wesarg-Menzel et al., 2024), has found weak evidence for functional coupling between the diurnal and stress-responsive functions of the HPA axis, despite their shared neurobiological substrates. However, the meta-analysis was largely based on studies using analytical approaches (e.g., total cortisol output, cortisol awakening response, difference score, area under the curve) that did not partition true and error variance in cortisol concentration data, thereby reducing the signal-to-noise ratio and the power to detect meaningful associations. These analytical limitations are compounded by the inherently low stability of diurnal cortisol concentrations, which makes it difficult to isolate true variance in the data (Ross et al., 2014). This challenge may be particularly pronounced in low-risk populations, where a more flexible, adaptive, and “state-like” HPA axis functioning may obscure consistent patterns of coupling. Notably, one previous study with community adolescents did find that a blunted diurnal slope during the day was associated with blunted reactivity and recovery in the TSST (Lucas-Thompson et al., 2018). The partial convergence between the findings of Lucas-Thompson and colleagues (2018) and our own may suggest a meaningful association between diurnal slope and TSST reactivity in laboratory stress paradigms during early adolescence, although the meta-analysis by Wesarg-Menzel and colleagues (2024) did not identify age as a moderating factor.

Youth at risk for early maltreatment often exhibit a dysregulated HPA axis—characterized by blunted diurnal slope and attenuated stress reactivity and recovery—which may confer greater risk for dysregulation across multiple domains of health (Adam et al., 2017; Gunnar and Vazquez, 2001). Because these indices may be more “stably dysregulated” or “trait-like” in high-risk youth, we argued that functional coupling between diurnal and stress-reactive HPA functions would be more detectable in this group. Indeed, our findings supported this hypothesis: the stability (ICC) of diurnal cortisol measures was substantially higher among high-risk youth (ICC = .81) compared with low-risk youth (ICC = .50). This 31-percentage-point difference in within-person variance (0.19 vs. 0.50) indicates that low-risk participants showed considerably greater within-person variability—or less stability—across repeated measures, which may support adaptive flexibility in meeting regulatory demands. Furthermore, analyses of homogeneity of variance suggested that the assumption of equal variances was met, indicating that the high- and low-risk groups had comparable variability in diurnal slope and stress reactivity. This implies that the observed differences are not due to the presence of a wider range of cortisol profiles in the high-risk group; rather, it is likely that the dysregulated profile is more stable in the high-risk group, and the increased within-person variability in cortisol measures among low-risk youth reduces the likelihood of detecting functional coupling in this group. Notably, stability in cortisol reactivity and recovery was high across both groups.

The increased stability of a diurnal slope parameter (delta) likely contributed to our ability to detect a significant moderation effect of risk on the association between diurnal slope and stress reactivity. Specifically, we observed a significant association between diurnal slope and stress reactivity only in the maltreated group. This finding aligns with the established directionality of HPA axis functioning (steeper diurnal slope → higher reactivity; more blunted diurnal slope → lower reactivity), suggesting that meaningful functional coupling is more likely to be detectable in at-risk populations than in low-risk populations. The non-significant effect in the low-risk group may indicate either that 1) there is no meaningful coupling, or 2) the effect is obscured by elevated within-person variability, or 3) that we had less power to detect a significant effect in the low-risk group because our sample size was smaller than in the high-risk group. Empirically testing these possibilities will require a larger sample size to increase statistical power and sufficient sampling days to ensure reliable measurement of diurnal slopes in non-maltreated youth. Future studies are encouraged to further explore these questions.

We extended our moderation analyses based on maltreatment risk

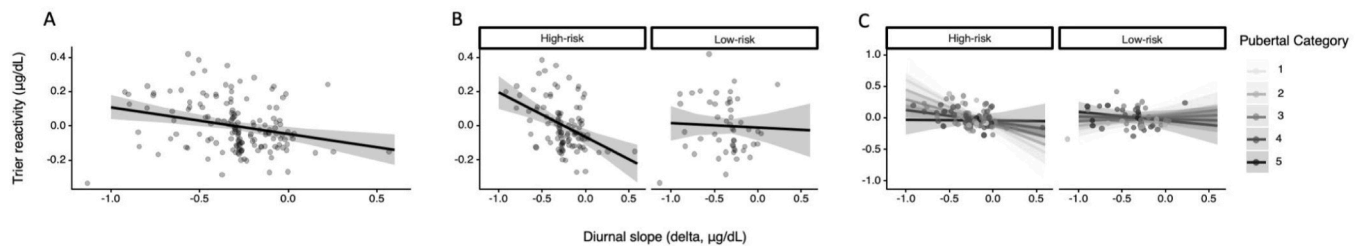


Fig. 4. Visualization of (A) the main effect of diurnal cortisol slope, (B) the diurnal slope*maltreatment risk interaction, and (C) the diurnal slope*maltreatment risk*pubertal development interaction predicting TSST-C cortisol reactivity. **Note.** Panel B illustrates a significant coupling between diurnal slope and stress reactivity in the high-risk group, but not in the low-risk group. Panel C demonstrates that this coupling is moderated by pubertal development among high-risk adolescents, such that advancing pubertal maturation is associated with the release of the diurnal slope–stress reactivity association. No pubertal moderation was observed in the low-risk group.

Table 5

Regression models testing the main and interaction effects of diurnal slope and maltreatment risk on TSST-C cortisol parameters.

Effect	Unstandardized Estimate	Standardized Estimate (β) [95% CI]	SE	Est./SE	p-value
<i>Main effect of diurnal slope (delta) on TSST-C reactivity and recovery</i>					
<i>Diurnal slope (delta) ON</i>					
Morning cortisol	-0.643	-0.556 [-0.732, -0.38]	0.09	-6.194	< .001
<i>TSST-C intercept ON</i>					
Diurnal slope (delta)	0.093	0.127 [-0.108, 0.361]	0.12	1.061	.289
Sex	-0.031	-0.062 [-0.227, 0.1042]	0.085	-0.728	.467
Age	-0.112	-0.151 [-0.314, 0.013]	0.084	-1.8	.072
<i>TSST-C reactivity ON</i>					
Diurnal slope (delta)	-0.106	-0.255 [-0.46, -0.049]	0.105	-2.428	.015
Sex	-0.000	-0.002 [-0.161, 0.158]	0.081	-0.019	.985
Age	0.035	0.082 [-0.076, 0.241]	0.081	1.0162	.31
<i>TSST-C recovery ON</i>					
Diurnal slope (delta)	0.037	0.143 [-0.044, 0.33]	0.095	1.501	.133
Sex	-0.006	-0.033 [-0.166, 0.1]	0.068	-0.49	.624
Age	0.008	0.029 [-0.105, 0.162]	0.068	0.424	.672
<i>Maltreatment risk * diurnal slope interaction effect on TSST-C intercept, reactivity and recovery</i>					
<i>TSST-C intercept ON</i>					
Diurnal slope (delta)	0.031	0.03 [-0.19, 0.25]	0.112	0.277	.782
Risk	0.081	0.16 [-0.2, 0.53]	0.075	1.089	.278
Diurnal slope * Risk	0.135	0.13 [-0.21, 0.48]	0.176	0.768	.444
Sex	-0.031	-0.12 [-0.45, 0.21]	0.041	-0.743	.458
Age	-0.1	-0.14 [-0.31, 0.03]	0.061	-1.649	.101
<i>TSST-C reactivity ON</i>					
Diurnal slope (delta)	-0.261	-0.44 [-0.65, -0.23]	0.063	-4.120	< .001
Risk	0.056	0.11 [-0.46, 0.24]	0.042	1.324	.188
Diurnal slope * Risk	0.236	0.40 [0.07, 0.73]	0.100	2.371	.019
Sex	0.001	0.01 [-0.31, 0.33]	0.023	0.061	.951
Age	0.015	0.04 [-0.12, 0.2]	0.034	0.444	.658
<i>TSST-C recovery ON</i>					
Diurnal slope (delta)	0.054	0.12 [-0.1, 0.34]	0.050	1.068	.287
Risk	0.004	0.02 [-0.35, 0.39]	0.034	0.113	.911
Diurnal slope * Risk	0.007	0.02 [-0.33, 0.36]	0.079	0.087	.931
Sex	-0.011	-0.10 [-0.43, 0.23]	0.027	-0.396	.692
Age	0.011	0.03 [-0.14, 0.20]	0.083	0.405	.686

status with additional exploratory analyses testing the moderating role of pubertal developmental stage. Specifically, we observed a significant negative association among high-risk youth at the early to mid-pubertal stage, which gradually became non-significant during late to post-puberty. This moderation effect suggests that as pubertal maturation progressed beyond midpuberty, the association between the diurnal slope and stress reactivity became less negatively coupled, becoming non-significant at the late- to post-pubertal stage. In the meta-analysis by Wesarg-Menzel and colleagues (2024), age did not moderate the association between diurnal slope and reactivity or recovery. One plausible explanation is that pubertal development—or, more broadly, stages of biological maturation—may be more relevant to the coupling of HPA-axis indices than chronological age.

Our findings related to pubertal stage are based on exploratory analyses involving a three-way interaction and should be interpreted cautiously, given potential power limitations. A speculative

interpretation of these findings is consistent with the stress recalibration hypothesis: adolescents with a history of maltreatment who are in later stages of puberty may show signs of recalibration in stress regulation, exhibiting patterns more similar to low-risk adolescents, who do not demonstrate significant associations between diurnal cortisol slopes and cortisol reactivity. These results may suggest that pubertal maturation is not accompanied by uniform normalization of HPA-axis functioning, but rather by reorganization of the relations between its diurnal and stress-reactive components. More pubertally mature high-risk youth exhibited greater decoupling between diurnal cortisol regulation and stress reactivity, indicating that stress reactivity becomes less constrained by diurnal cortisol regulation and can operate largely independently of it. Importantly, this decoupling may not necessarily reflect recovery or dysregulation per se; rather, it may reflect a developmental release of coupling that creates conditions for recalibration. Future confirmatory studies should examine the role of pubertal status in associations among

cortisol parameters, ideally using larger sample sizes.

Several strengths and limitations of the study should be noted. The sample size was modest, and the two risk groups were unbalanced, with more participants in the high-risk than in the low-risk group. This imbalance may have influenced our ability to detect a significant moderation effect in the high-risk group but not in the low-risk group. Additionally, because the diurnal change analyses modeled change only between wake-up and bedtime time points across three days, this index may be sensitive to small timing or compliance deviations. Moreover, more participants had usable TSST-C data than usable diurnal data, which may have affected the robustness of the diurnal findings. Furthermore, we do not have information about the concurrent experiences of maltreatment or daily stressors experienced in the high-risk group, limiting our ability to separate early maltreatment-associated variability from proximal stress effects. Key strengths include the recruitment of families with a history of CPS involvement, likely representing some of the most vulnerable youth in the U.S. population. Finally, our analytical approach, which separated error from true variance, likely enhanced our ability to detect a meaningful effect.

In summary, this study provides novel evidence that functional coupling between diurnal and stress-reactive HPA axis activity is more detectable among adolescents with a history of maltreatment, particularly at earlier stages of pubertal development. These findings highlight the importance of considering developmental timing and early adversity when examining coordination of stress-regulatory systems. Importantly, the results suggest that in high-risk youth, regulation—or dysregulation—of one aspect of HPA axis function (e.g., diurnal rhythm) may offer predictive insight into another (e.g., acute stress reactivity). This coupling may reflect a more rigid, trait-like pattern of HPA axis activity that confers both risks and opportunities for intervention. More broadly, the findings underscore adolescence as a sensitive period during which neuroendocrine systems can recalibrate in response to environmental input. Understanding these patterns may inform efforts to identify physiological markers of stress-related vulnerability and guide timing for preventive interventions aimed at restoring adaptive stress regulation.

Ethics Approval Statement

The study protocol was approved by the Institutional Review Board of the University of Delaware, and all participants were treated in accordance with established ethical guidelines.

Data and Study Analysis Code Sharing Statement

The data used in this study are available on NDA's website.

Clinical trial registration

<https://clinicaltrials.gov/ct2/show/NCT02093052>

Funding

National Institute of Mental Health R01MH074374; National Institute of Child Health and Human Development (NICHD) F31 HD106764

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.psyneuen.2026.107854](https://doi.org/10.1016/j.psyneuen.2026.107854).

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