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Exploring joint HPA–inflammatory stress response profiles in adolescent girls: Implications for developmental models of neuroendocrine dysregulation

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Abstract

Prior research has struggled to differentiate cortisol stress response patterns reflective of well-regulated versus dysregulated hypothalamic-pituitary-adrenal (HPA) axis function among adolescents. Here, we show how exploring profiles of joint HPA–inflammatory stress responsivity, and linking those profiles to pubertal development and peer stress exposure, may aid such distinction. Adolescent girls (N=157, M_{age}=14.72 years, SD=1.38) at risk for psychopathology completed assessments of salivary cortisol and pro-inflammatory cytokines (i.e., tumor necrosis factor-α, interleukin-1β, and interleukin-6) prior to and following the Trier Social Stress Test. Adolescents, a close friend, and a caregiver completed questionnaire measures of peer stress and pubertal status. Multitrajectory modeling of adolescents' cortisol and cytokine levels revealed three profiles: *Low Cortisol Response–Stably Low Cytokine* (n=75), *High Cortisol Response–*

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Stably Moderate Cytokine (*n*=47), and *Low Cortisol Response–Stably High Cytokine* (*n*=35). Relative to Low Cortisol Response–Stably Low Cytokine, adolescents exhibiting the High Cortisol Response–Stably Moderate Cytokine profile were more advanced in their pubertal development, but presented with similarly low levels of peer stress exposure. Despite showing cortisol responses that were indistinguishable from Low Cortisol Response–Stably Low Cytokine, adolescents exhibiting the Low Cortisol Response–Stably High Cytokine profile were more pubertally advanced, but also more likely to have experienced chronic peer strain (self-report) and relational peer victimization (close friend-report). These findings thus illustrate the potential value of taking a multisystem approach to studying adolescent stress responsivity and underscore the importance of considering developmental and social factors when interpreting cortisol stress response patterns. Ultimately, such work may help inform developmental models of neuroendocrine dysregulation and related risk for psychopathology.

Keywords

cortisol; cytokine; inflammation; adolescent; development; health

Biological stress responsivity has been identified as a key mechanism involved in the development of youth mental and physical health problems (Hibel et al., 2020; Koss & Gunnar, 2018). However, there is ongoing debate about how best to differentiate cortisol stress response patterns indicative of well-regulated hypothalamic-pituitary-adrenal (HPA) axis function from those that potentially signal HPA dysregulation (Shirtcliff et al., 2014; Wadsworth et al., 2019). This lack of clarity has stalled progress toward the development of more nuanced, comprehensive biological stress response models of adolescent physical and mental health (Hostinar et al., 2021). Researchers have cited an over-reliance on single biomarker approaches (e.g., analyzing cortisol only or in isolation; Buss et al., 2019) and a systemic lack of consideration of developmental and social factors (Robert & Lopez-Duran, 2019) as potential contributors to this empirical ambiguity. To address these issues, we examined whether a multisystem (e.g., Bauer et al., 2002; Jones et al., 2020), person-centered approach to modeling biological stress responsivity could facilitate the well-regulated versus dysregulated HPA axis distinction. Using a sample of adolescent girls at risk for psychopathology, we explored the potential existence of joint HPA—inflammatory stress response profiles, specifically, and then aimed to link those profiles to indices of girls' pubertal development and peer stress exposure.

Joint HPA–Inflammatory Stress Response Function

Simultaneous attention to peripheral biological systems that work in concert with the HPA to promote stress adaptation may help distinguish cortisol response patterns that signal well-regulated versus dysregulated HPA function (Buss et al., 2019). This premise has been considered by earlier conceptual models and empirical research investigating patterns of joint HPA-SNS activation (Bauer et al., 2002; Jones et al., 2020). Given the close association between SNS function and inflammatory processes, an examination of joint HPA-inflammatory activation builds on and may perhaps extend this existing literature base. Indeed, models of allostatic load provide insight into how exploring joint HPA and

inflammatory stress responsivity might aid the well-regulated versus dysregulated HPA distinction (McEwen, 2000; Miller et al., 2007). From an immuno-endocrine organization perspective, cortisol is profoundly influential in maintaining immune system homeostasis. Cortisol exerts broad anti-inflammatory effects by inhibiting the action and transcription of many pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) (for a review, see Webster et al., 2002). In the face of a stressor, the initial cortisol response generally serves to down-regulate TNF-α, IL-1β, and IL-6 activity (though there are instances where the HPA axis also upregulates inflammation and stimulates cytokine production; Besedovsky & del Rey, 2000; Shintani et al., 1995; Slavich 2020a, 2020b). Cortisol's broad anti-inflammatory properties promote stress adaptation insofar as they permit an individual to cope with the stressor at hand without the onset of sickness behaviors (e.g., fatigue, withdrawal) that might otherwise hamper coping efforts (Slavich & Irwin, 2014). Therefore, cortisol stress response patterns that signal well-regulated HPA function should be accompanied by low pro-inflammatory cytokine activity.

Under chronic stress conditions, an individual's cortisol response becomes less effective in down-regulating pro-inflammatory cytokine activity (McEwen, 2000; Miller et al., 2007). Specifically, chronic stress-related alterations in HPA function can contribute to (a) reduced cortisol suppression of sympathetic nervous system (SNS) activity (e.g., when unopposed by cortisol, SNS activity is permitted to up-regulate pro-inflammatory cytokine activity; Slavich & Irwin, 2014), and (b) glucocorticoid resistance, whereby immune cells (e.g., monocytes, macrophages) responsible for modulating cytokine activity become less sensitive to cortisol's anti-inflammatory signals (Chen et al., 2015). Therefore, cortisol response patterns that reflect HPA dysregulation should be accompanied by high pro-inflammatory cytokine activity.

Toward a Multisystem Approach

As recommended by Buss and colleagues (2019), the use of person-centered analytic techniques (Bergman & Magnusson, 1997) may enable researchers to explore concurrent cortisol and pro-inflammatory cytokine activity. In doing so, researchers may be able to more realistically approximate and clearly differentiate well-regulated and dysregulated HPA function. By allowing individuals to cluster together on the basis of joint functioning across multiple systems of interest (e.g., HPA axis, immune), these approaches permit examination of how concurrent cortisol and cytokine stress response patterns manifest within individuals. In the current study, we accomplished this by exposing 157 adolescent girls at risk for psychopathology to a laboratory-based social stressor (age-modified Trier Social Stress Test; TSST; for details, see Giletta et al., 2015) and examining salivary cortisol and pro-inflammatory cytokine (i.e., IL-1β, IL-6, TNF-α) levels before and after the TSST. We then utilized multitrajectory modeling (MTM; Nagin et al., 2018) to explore potential subgroups of adolescents based on the extent to which they exhibited distinct patterns of concurrent cortisol, IL-1β, IL-6, and TNF-α activation.

Multitrajectory modeling (MTM; Nagin et al., 2018) is a person-centered analytic technique that offers certain advantages over conventional approaches (e.g., latent profile analysis)

that are germane to our study. First, these latter approaches often utilize *summative* stress-response indices (e.g., area-under-the-curve ground, AUCg), which do not capitalize on the richness of multiple time point stress response data. Additionally, modeling *trajectories* permits examination of specific aspects of the stress response (e.g., baseline, reactivity, recovery) that may further distinguish well-regulated and dysregulated HPA function (Ji et al., 2016). Indeed, a prior analysis of this dataset utilized group-based trajectory modeling (GBTM; Nagin, 2005) of participants' cortisol levels and identified three stress response trajectories (Giletta et al., 2015), with *Hyperresponsive* adolescents (i.e., higher baseline levels, more pronounced reactivity, protracted recovery) exhibiting greater maladjustment (i.e., lifetime suicidal ideation) relative to *Normative* (i.e., lower baseline levels, less pronounced reactivity, efficient recovery) and *Hyporesponsive* (i.e., lower baseline levels, blunted reactivity) adolescents. We extend this research by simultaneously attending to immune system function. It is possible that both the Hyperresponsive and Hyporesponsive trajectories from this prior study reflect HPA dysregulation. If so, each should be accompanied by high cytokine activity (i.e., lack of cortisol suppression, Slavich & Irwin, 2014; glucocorticoid resistance, Chen et al., 2015).

Little is known about optimal means of capturing HPA axis activity in conjunction with pro-inflammatory cytokine activity (Slavich, 2020a). As such, data-driven approaches that capitalize on multiple time point stress response data in an effort to model cortisol trajectories in tandem with IL-1β, IL-6, and TNF-α trajectories may (a) potentially illustrate an inflammatory stress response that co-occurs with the HPA stress response, but also (b) support analysis of key descriptive features of the inflammatory stress response therein (e.g., baseline, reactivity). In a prior analysis of this dataset, which focused solely on pro-inflammatory cytokines and had different goals, confirmatory latent change score models revealed that (a) both higher pre-TSST IL-1β, IL-6, and TNF-α levels (i.e., baseline) and greater IL-1β, IL-6, and TNF-α changes (i.e., reactivity) were critical features of the inflammatory stress response, and that (b) those features were linked to maladjustment. Building upon that research, our data-driven approach to exploring joint HPA–inflammatory stress response profiles permits examination of the extent to which pro-inflammatory cytokine activity in tandem with cortisol can be characterized by variation in both baseline levels as well as reactivity. Importantly, no study to date has explored these potential cross-system patterns of HPA axis and inflammatory activity.

Pubertal Development and Peer Stress Exposure Correlates

If profiles of joint HPA-inflammatory stress responsivity can help distinguish well-regulated and dysregulated HPA function, then theory-driven correlates such as developmental and social factors should characterize the profiles in predictable ways (Robert & Lopez-Duran, 2019). Single bio-marker studies have documented normative increases in cortisol responsivity that occur during the pubertal transition, especially for girls (for a review, see van der Voorn et al., 2017). This developmental shift toward stronger cortisol responsivity is thought to reflect well-regulated HPA function, providing youth physiological support for efficaciously coping with stressors they increasingly encounter over this period (Zimmer-Gembeck & Skinner, 2016). From an immuno-endocrine organization perspective, a *Strong Cortisol Response* reflective of well-regulated HPA function (i.e., increased physiologic

support) should be accompanied by low pro-inflammatory cytokine activity (i.e., cortisol's anti-inflammatory properties), and this *High Cortisol–Low Cytokine* profile should be associated with more mature pubertal status. However, cytokine activation is also stimulated by estrogen levels that increase during puberty (Sacher & Slavich, 2019). These modest elevations in neuroinflammation that are linked to increases in sex-hormone levels also provide an adaptive function for girls in the form of immunologic protection in the face of potential pathogenic threats to reproductive health. Therefore, it is also possible for more mature pubertal status to be associated with a *High Cortisol–Moderate Cytokine* profile.

Single bio-marker studies have also shown lower cortisol production in response to acute stress to be a normative feature of less mature pubertal status (for a review, see Voorn et al., 2017). This weaker cortisol response pattern is thought to reflect well-regulated HPA function, insofar as it protects youth who are less pubertally advanced from the potential neurotoxic effects of cortisol overexposure in the absence of more mature cognitive and emotional capacities for managing stressors (Zimmer-Gembeck & Skinner, 2016). When considering immuno-endocrine organization, a *Weak Cortisol Response* reflective of well-regulated HPA function (i.e., protection from cortisol overexposure) should be accompanied by low pro-inflammatory cytokine activity (i.e., immuno-endocrine homeostatic equilibrium, lower estrogen levels, and related pro-inflammatory cytokine stimulation; Sacher & Slavich, 2019), and this *Low Cortisol–Low Cytokine* profile should be associated with less mature pubertal status.

Joint HPA–inflammatory stress response profiles might also be meaningfully understood through connections with peer stress exposure. Adolescence is associated with a rise in stressful peer experiences and increased sensitivity to such stressors, particularly for girls (Rudolph, 2002; Hankin et al., 2007). Chronic peer stress exposure during adolescence is known to adversely impact the HPA axis (Guerry & Hastings, 2011), but also may impact concurrent immune system function. A prior analysis of this dataset showed that both higher pre-TSST IL-1β, IL-6, and TNF-α levels (i.e., baseline) and greater IL-1β, IL-6, and TNF-α changes (i.e., reactivity) were positively associated with peer victimization. Here, we build on this prior study by exploring joint HPA (i.e., cortisol)–inflammatory (i.e., IL-1β, IL-6, TNF-α) stress response (i.e., baseline, reactivity) profiles and their potential connections with peer stress, in order to help differentiate cortisol response patterns indicative of well-regulated versus dysregulated HPA function.

The single biomarker literature has identified two patterns of cortisol responsivity to social stressors thought to reflect dysregulated HPA function: *Cortisol Hyperresponse* and *Cortisol Hyporesponse* (Koss & Gunnar, 2018; Smyth & Clow, 2020). From an immuno-endocrine perspective, both the *Cortisol Hyperresponse* and the *Cortisol Hyporesponse* reflective of dysregulated HPA function should be accompanied by excessive pro-inflammatory cytokine activity (i.e., glucocorticoid resistance, lack of cortisol suppression). To follow, both *High Cortisol–High Cytokine* and *Low Cortisol–High Cytokine* profiles should be associated with chronic peer stress exposure. Also, given that certain types of peer stress (e.g., social rejection) may be more strongly associated than others (e.g., physical aggression) with biological dysregulation in girls (Kliewer et al., 2019; Slavich et al., 2010), we examined three different forms of peer stress exposure—namely, self-reported chronic peer strain,

close friend-reported relational peer victimization and overt peer victimization—and their potentially divergent connections with our identified profiles.

The Present Study: Aims and Hypotheses

Aim 1: We sought to explore to existence of potential subgroups of adolescent girls with distinct joint HPA (i.e., salivary cortisol)–inflammatory (i.e., pro-inflammatory cytokine) stress response profiles. Based on the developmental literature (Hibel et al., 2020; Slavich & Sacher, 2019; van der Voorn et al., 2017), we expected to identify two subgroups with profiles containing cortisol responses reflective of well-regulated HPA function: *Low Cortisol–Low Cytokine*, *High Cortisol–Low Cytokine*[1]. Based on models of allostatic load and neuroinflammation (McEwen, 2000; Miller et al., 2007; Slavich & Irwin, 2014), we expected to identify two subgroups with profiles containing cortisol responses reflective of HPA dysregulation: *High Cortisol–High Cytokine*, *Low Cortisol–High Cytokine*. **Aim 2:** We sought to examine pubertal development and peer stress (self-reported chronic peer strain, friend-reported relational peer victimization and friend-reported overt peer victimization) correlates of subgroup membership. We expected that the likelihood of membership in the High Cortisol–Low Cytokine subgroup relative to the Low Cortisol–Low Cytokine subgroup would be associated with more mature pubertal development[2]. We also expected that the likelihood of membership in the High Cortisol–High Cytokine and Low Cortisol–High Cytokine subgroups relative to the Low Cortisol–Low Cytokine and High Cortisol–Low Cytokine subgroups would be associated with greater chronic peer stress exposure.

Method

Participants

Participants were 157 adolescent girls between ages 12 and 17 years (M_{age} = 14.72 years, SD = 1.38) drawn from a larger study of girls at risk for psychopathology. They were recruited from inpatient psychiatric units, outpatient clinics, and high schools. Interviewers screened participants using telephone interviews with the adolescent's primary caregiver using items from the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS; Kaufman et al., 1997). Eligibility criteria for the larger study included (a) female sex, (b) between 12 and 16 years old, (c) caregiver report of a history of at least one mental health concern (i.e., a diagnosis or significant symptoms of, or treatment for, mood, adjustment, disruptive behavior, or substance use disorders in the two years prior to the study), and (d) a primary caregiver and close friend who were able to participate. Exclusion criteria included psychosis, intellectual disability, or other developmental disorder. Participants identified as White (64.4%), Black (24.4%), multiple racial background (10.0%), and Hispanic (1.3%). Regarding caregiver (94.9% mothers) educational attainment, 1.3% did not complete high school, 13.8% completed high school, 31.9% had completed a trade degree or some college, 23.1% had a bachelor's degree, and 29.1% had a formal education beyond a bachelor's degree.

[1]We also speculated that a *High Cortisol–Moderate Cytokine* profile might emerge and be related to more mature pubertal status relative to *Low Cortisol–Low Cytokine*.

Procedure

Participants completed a laboratory visit with their primary caregiver and a close, same-aged female friend. Caregivers of the participant and their close friends gave written informed consent, and participants and their close friend gave written assent. Participants, their caregivers, and their close friend individually completed questionnaires. About three hours after arriving to the study visit, participants watched an emotionally neutral film clip intended to promote relaxation and then completed a modified version of the Trier Social Stress Test (TSST-M; for details, see Giletta et al., 2015). For the TSST, participants were instructed to prepare a three-minute audition speech for a fictional reality show about how teens form friendships. They prepared the speech for one minute, after which a male college undergraduate (i.e., confederate), introduced as a judge, entered and instructed the participant to give her speech while facing a video camera and a screen displaying her live image. Participants were told that the judge would be evaluating their audition. The judge maintained a neutral expression and did not provide feedback. To limit diurnal cortisol and cytokine variation, the TSST took place in the afternoon (for details, see saliva sample timing in the Methods section).

Measures

Cortisol.—Saliva samples were collected using SalivaBio Oral Swab (Salimetrics, State College, PA) at four time points during the lab-based procedure: (a) following the film clip/immediately prior to the start of the TSST (Pre-TSST start, +0 min), (b) 20 min after the TSST (Post-TSST start, +25 min), (c) 30 min after the TSST (Post-TSST start, +35 min), and (d) 40 min after the TSST (Post TSST start, +45 min). Saliva samples were stored at −25° C and then shipped on dry ice to the Behavioral Endocrinology Laboratory at Pennsylvania State University (Salimetrics, PA). Samples were assayed for cortisol with a 510-k cleared high-sensitivity enzyme immunoassay with a sensitivity range of 0.007 μg/dl to 1.2 μg/dl. Per the manufacturer, the mean intra-assay and inter-assay coefficients of variation are 7.3% and 5.7%, respectively.

Pro-inflammatory cytokines.—Three pro-inflammatory cytokines (i.e., TNF-α, IL-1β, and IL-6) were assessed using SalivaBio Oral Swab (Salimetrics, State College, PA) at two time points: (1) Pre TSST start, +0 min and (2) Post TSST start, +45 min. This technique has been shown to be valid for assessment of cytokine reactivity (e.g., peak pro-inflammatory cytokine reactivity levels occur between 30 min and 100 min post stressor start; Szabo et al., 2020) that also avoids more invasive procedures like venipuncture (Szabo & Slavish, 2020). Samples were stored at −25° C until analysis and then assayed using a Bio-Plex 200 (Bio-Rad, Hercules, CA) at the UNC Cytokine and Biomarker Analysis Facility. Assays were performed according to recommended guidelines of the manufacturer (R & D Systems, Minneapolis, MN) using high-sensitivity multiplex immunoassay kits, which have a mean minimal detectable dose of 0.29 pg/ml for TNF-α, 0.08 pg/ml for IL-1β, and 0.14 pg/ml for IL-6. Per the manufacturer, the mean intra-assay coefficients of variation are 5.2% for IL-6 and 5.3% for IL-1β and TNF-α, and the mean inter-assay coefficients of variation are 9.6% for IL-6 and TNF-α, and 12.8% for IL-1β.

Pubertal maturation.—Participants and their caregivers completed the Pubertal Development Scale (PDS; Petersen et al., 1988). This measure consists of five Likert-type items (1 = *no development* to 4 = *development seems complete*) that assess aspects of participants' physical development: body hair, skin changes, growth spurt, breast development and menarche (binary item; 1 = *no*, 4 = *yes*). Mean scores computed for self-report (Cronbach's α = .60) and caregiver-report (Cronbach's α = .62) were strongly correlated ($r = .66$, $p < .001$). To incorporate multiple informants and create a more robust index, a cross-rater mean score was computed across self- and caregiver-report items (Cronbach's α = .74) and used in all analyses.

Given the wide age range of the sample, we sought alternate means of examining pubertal maturation effects that might minimize the confounding influence of chronological age. We computed a chronological age-normed pubertal maturation score by standardizing cross-rater PDS mean scores for 12–13 year-olds, 14–15 year-olds, and 16–17 year-olds. This score was examined as a separate independent variable in post-hoc developmental correlate analyses.

Chronic peer strain.—Participants completed the Child Chronic Strain Questionnaire (CCSQ; Rudolph et al., 2001). The chronic peer strain subscale (11 items; e.g., "How often has it been hard for you to make friends?", "How often do you need help and don't have a friend to help you?") was used. Participants reported on a scale from 1 (*not at all*) to 5 (*very much*) for each item. An overall score was computed by averaging peer items (Cronbach's α = .76).

Peer victimization.—Participants' close friends completed the Revised Peer Experiences Questionnaire (RPEQ; Prinstein et al., 2001). The relational (4 items; e.g., "Some teens left your friend out of an activity or conversation that she really wanted to be included in.") and overt (3 items; e.g., "A teen threatened to hurt your friend or beat her up.") peer victimization subscales were used. Friends rated from 1 (*never*) to 5 (*a few times a week*) how often each experience occurred to their friend in the past year. A relational (Cronbach's α = .82) and overt (Cronbach's α = .66) peer victimization score was computed by averaging across respective subscale items.

Covariates

Given that depressed mood can alter girls' stress perceptions and because internalizing problems and obesity are each associated with cortisol and low-grade inflammation (Koss & Gunnar, 2018; Slavish et al., 2015), depressive symptoms and body mass index (BMI) were controlled for in all Aim 2 correlate analyses. Additionally, because chronological age is confounded with pubertal maturation and affects HPA and immune system functioning in ways not directly mediated by reproductive hormones (e.g., size and structure of key regulatory glands; Linton & Dorshkind, 2004), chronological age also was controlled for in all Aim 2 correlate analyses. Following Giletta et al. (2018), an additional secondary set of variables previously linked to HPA function and inflammation (Calhoun et al., 2014; Granger et al., 2009; Slavish et al., 2015) were considered as potential covariates in Aim 2 correlate analyses: saliva sample timing, highest caregiver education level, family-related

stress, recent illness, smoking, same day caffeine consumption, birth control use, medication use, and ethnicity.

Depressive symptoms.—Participants completed a modified version of the Mood and Feelings Questionnaire (MFQ; Costello & Angold, 1988). Participants rated their experience of depressive symptoms in the prior two weeks on a 3-point scale from 0 (*not true*) to 2 (*mostly true*). Given that suicidality was more thoroughly assessed with other measures in the larger study from which the data were obtained, items assessing suicidal ideation ($n = 4$) were omitted from the original 33-item version of the MFQ (Cronbach's $\alpha = .95$). A total depressive symptoms score was used in all analyses ($M = 16.54$, $SD = 12.65$, $Min = 0.00$, $Max = 56.00$).

Body mass index (BMI).—Participants' height and weight were obtained during their study visit. Raw BMI was calculated by dividing participants' weight in kilograms (kg) by their height in meters squared (m^2) ($M = 24.59$, $SD = 6.46$, $Min = 15.90$, $Max = 48.10$).[2]

Saliva sample timing.—A timing variable was computed by subtracting wake time from the initial saliva sample collection time ($M = 6.24$ h, $SD = 1.74$, $Min = 3.67$, $Max = 12.85$). For most participants (98.8%), initial sample collection occurred between 12:00 p.m. and 5:00 p.m. This approach to saliva sample timing accounts for variation in diurnal cortisol and cytokine rhythms, given that wake time is highly variable in adolescents (e.g., Calhoun et al., 2012; 2014; Giletta et al., 2015; Johnson et al., 2019; Owens et al., 2019; Slavich et al., 2020; Young et al., 2021).

Caregiver education.—Participants' caregivers reported their own as well as their partner's highest educational level: (1) Some high school, but did not graduate, (2) High school graduate or GED, (3) AA/Trade Degree, (4) Some undergraduate college, (5) Undergraduate degree/bachelor's, (6) Some graduate school, (7) Master's degree (MA) or law degree (JD), or (8) Doctorate degree (PhD or MD). The higher of the two caregiver educational degrees was used as a proxy for SES ($Median = 5.00$, $Min = 1.00$, $Max = 8.00$).

Family-related stress.—Using the Life Events Checklist (LEC; Johnson & McCutcheon, 1980), participants indicated their exposure (0 = *no*, 1 = *yes*) to a series of negative life events over the previous six months. Based on prior work (e.g., Ehrlich et al., 2016), 16 family-related stress items (e.g., "Your parents separated or got divorced," "Your family had less money for important things you needed - food, electricity, rent, etc.") were summed into a total count score ($M = 2.91$, $SD = 2.85$, $Min = 0.00$, $Max = 14.00$).

Recent illness, drug, and medication use.—Participants and their caregivers indicated whether participants had experienced a recent illness (e.g., coughing, fever, sneezing; $n = 17$). Participants reported if they smoked cigarettes ($n = 16$), used birth control ($n = 25$), or consumed a caffeinated beverage on the day of their visit ($n =$

[2]Analyses conducted using age-specific BMI percentiles calculated following Center for Disease Control (CDC) guidelines yielded results similar to those obtained when raw BMI values were used in analyses. Therefore, the results do not appear to depend on the use of a particular BMI metric.

14). Participants and caregivers also reported on medication use (corticosteroid, $n = 17$; anxiolytic/antidepressant, $n = 80$). Binary indicators (0 = *no*, 1 = *yes*) for each illness, drug, and medication variable were used in analyses.

Overview of Analyses

Data preparation.—Nine cortisol values were > 3 *SD*s from the grand mean: Pre-TSST start, +0 min ($n = 4$), Post-TSST start, +25 min ($n = 2$), Post-TSST start, +35 min ($n = 2$), and Post-TSST start, +45 min ($n = 1$). Seven IL-1β values were > 3 *SD*s from the grand mean: Pre-TSST start, +0 min ($n = 3$), and Post-TSST start, +45 min ($n = 4$). Six IL-6 values were > 3 *SD*s from the grand mean: Pre-TSST start, +0 min ($n = 3$), and Post-TSST start, +45 min ($n = 3$). Six TNF-α values were > 3 *SD*s from the grand mean: Pre-TSST start, +0 min ($n = 4$), and Post-TSST start, +45 min ($n = 2$). Log_{10} and ln transformations were applied to cortisol and cytokine data, respectively, prior to analyses to successfully normalize skew. Following transformation, nine cytokine values remained > 3 *SD* from the mean. These outliers were retained given that the aim of the study was to determine whether potentially meaningful subgroup trajectories might exist at the tail end of the cortisol, IL-1β, IL-6, and TNF-α distribution[3]. Log_{10} (BMI, peer victimization) and square power (pubertal maturation) transformations were applied to skewed covariates and correlates to successfully normalize positive and negative skew, respectively.

Aim 1: Multi-trajectory modeling (MTM; Nagin et al., 2018) was used to explore within-person profiles based on the extent to which participants exhibited similar cortisol, IL-1β, IL-6, and TNF-α stress response trajectories. As described elsewhere (Bendezú & Wadsworth, 2018; Bendezú, Howland et al., 2021), the PROC TRAJ procedure (SAS 9.4; Nagin, 2005) with the MULTGROUPS option employed was used and specified to operate on a censored norm distribution model. Full-Information-Maximum likelihood (FIML) as a method of handling missing data is most suitable when the data are assumed to be missing completely at random (MCAR). Little's (1988) MCAR test, X^2 (564) = 579.20, $p > .250$, supported the use of FIML within the PROC TRAJ procedure. For cortisol (4 time points), a quadratic polynomial function was estimated. For IL-1β, IL-6, and TNF-α (2 time points), a linear polynomial function was estimated[4]. At each step of model specification (e.g., one-group solution, two-group solution, three-group solution), nonsignificant higher order polynomial functions (e.g., quadratic, linear) were removed from each trajectory's equation and the model rerun until a solution containing only significant highest order parameter estimates for each trajectory in each group was obtained. These significant highest order polynomial functions describe the nature of change for each trajectory in each subgroup The log Bayes factor approximation [$2\log_e(B_{10})$] was used at each step as a fit index (e.g., [$2\log_e(B_{10})$] > 10 supports more complex solution; Nagin, 2005). Given our sample size ($N = 157$) and modeling recommendations ($N > 100$; Nagin, 2005), we limited model specification to four groups. Following specification, we evaluated MTM adequacy (i.e., if

[3]Analyses conducted with these outlier data points set to missing (i.e., excluded) or a value equal to 3 *SD* from the grand mean (i.e., winsorized) returned similar results and did not alter study conclusions. Therefore, the findings do not appear to depend on method of handling outliers.

[4]Although at least three time points are needed to define a slope in latent growth curve modeling, MTM constrains slopes to be equal for all adolescents in a given subgroup. As such, a remaining degree of freedom is available to specify a linear slope using only two time points.

MTM accurately identified distinct subgroups) using average posterior probability ($AvePP_j$ > 0.70), odds of correct classification (OCC_j > 5.00), and the ratio of the probability of subgroup assignment to the proportion of adolescents assigned to subgroups ([$Prob_j/Prop_j$] ≈ 1) (Nagin, 2005).

After adequacy evaluation, Wald tests were used to distinguish and label the groups, delineating how intercept and polynomial estimates for cortisol, IL-1β, IL-6, and TNF-α trajectories were comparatively higher or lower across groups (for additional examples, see Bendezú & Wadsworth, 2018; Van Ryzin et al., 2009). To elaborate, a significant Wald test comparing intercept estimates for two groups on a particular biomarker trajectory would indicate that the intercept estimates (i.e., baseline values) were significantly different from one another (i.e., one trajectory defined by an intercept estimate that is relatively higher, one trajectory defined by an intercept estimate that is relatively lower). A significant Wald test comparing highest order polynomial estimates for two groups on a particular biomarker trajectory would indicate that the polynomial estimates (i.e., magnitude of response patterns) were significantly different from one another (i.e., one trajectory defined by a polynomial estimate that is relatively higher, one trajectory defined by a polynomial estimate that is relatively lower). As polynomial estimates describe the nature of change in participants' HPA and inflammatory response to the TSST, a significant Wald test suggests that one polynomial estimate reflected a relatively more pronounced response to the TSST whereas the other reflected a relatively less pronounced response to the TSST.

Aim 2: Multinomial logistic regression (with listwise deletion to handle missing correlate and covariate data) was used to examine associations between subgroup membership and study covariates and correlates. An initial covariate multinomial logistic regression model was run with all secondary covariates (e.g., saliva sample timing, socioeconomic status, family-related stress, recent illness, smoking, same day caffeine consumption, birth control use, medication use, and ethnicity) entered in single step. This initial model helped determine which of our secondary covariates were associated (using a conservative alpha of .10) with subgroup membership and, thus, important to adjust for in subsequent models. Four subsequent correlate multinomial logistic regression models were conducted. First, pubertal maturation was examined as a developmental correlate of subgroup membership. Next, each of three peer stress variables (e.g., chronic peer strain, relational victimization, overt victimization) were independently examined as social correlates of subgroup membership in addition to pubertal maturation. Primary covariates (e.g., depressive symptoms, BMI, and chronological age) as well as those secondary covariates identified in the initial covariate model were included in each of these four models. This approach to model building was motivated by the need to preserve statistical power, as MTM can generate unequal samples across groups, which can limit ability to detect correlate effects (e.g., recommended 10 cases per group per independent variable; Vittinghoff & McCulloch, 2007).

Post-hoc developmental correlate analyses.—Two additional developmental correlate multinomial logistic regression models were conducted. Specifically, each examined one of two developmental factors independently as correlates of subgroup

membership: chronological age independent of pubertal maturation and chronological age-normed pubertal maturation. Primary covariates and secondary covariates identified in the initial model were included in each model. Since chronological age-normed pubertal maturation was computed based on participants' age at the time of the visit, chronological age was not included as a covariate in that model.

Results

Descriptive statistics and bivariate correlations for the primary study variables are presented in Table 1. As has been reported previously (Giletta et al., 2018), variable-centered analyses comparing pre- to post-stressor assay levels showed that, on average for all youths, there was a significant increase in cortisol, a non-significant increase in IL-6, no change in IL-1b, and a non-significant decrease in TNF-a. Cortisol levels were positively correlated across time points. Of the 15 possible correlations among pro-inflammatory cytokine samples, 11 were significant and positive. No significant cortisol–cytokine correlations emerged, further supporting our aim of examining within-person profiles. Chronological age was positively associated with 2 of 10 biological indicators (IL-1β Post-TSST start +45 min and TNF-α Pre-TSST start, +0 min) as well as chronic peer strain and relational victimization. Pubertal maturation was positively associated with 3 of 10 biological indicators (IL-1β Post-TSST start +45 min, TNF-α Pre-TSST start +0 min, and TNF-α Post-TSST start +45 min). Cortisol was not correlated with any measure of peer stress. Chronic peer strain was positively associated with TNF-α levels and relational victimization. Finally, relational and overt peer victimization were positively correlated.

Aim 1.

MTM parameter estimates and adequacy indices are displayed in Table 2. Percentages of participants demonstrating increases in pre-post TSST cortisol and cytokine levels for the full sample and each subgroup are depicted in Table 3. MTM results supported a three-group solution (Figure 1): two- and one-group solution comparison [$2\log_e(B_{10}) = 93.00$], three- and two-group solution comparison [$2\log_e(B_{10}) = 164.40$], and four- and three-group solution comparison [$2\log_e(B_{10}) = -29.28$]. MTM adequacy indices suggested the final model fit the data well. A *Low Cortisol Response–Stably Low Cytokine* profile emerged and was consistent with our expected Low Cortisol-Low Cytokine profile. This subgroup was largest ($n = 75$) and exhibited trajectories characterized by low cortisol baseline levels and significant but less pronounced cortisol reactivity as well as the lowest IL-1β, IL-6, and TNF-α baseline levels in the sample and nonsignificant (i.e., stably low) IL-1β, IL-6, and TNF-α reactivity.

Because the Low Cortisol Response–Stably Low Cytokine subgroup was largest, it served as reference in trajectory distinction analyses that helped to label the remaining subgroups. A *High Cortisol Response–Stably Moderate Cytokine* profile emerged and was partially consistent with our expected High Cortisol–Low Cytokine profile. This subgroup was second largest ($n = 47$) and displayed trajectories characterized by the highest cortisol baseline levels and most pronounced cortisol reactivity in the sample, moderate IL-1β and TNF-α baseline levels and nonsignificant (i.e., stably moderate) IL-1β and TNF-α

reactivity, and low IL-6 baseline levels and nonsignificant (i.e., stably low) IL-6 reactivity[5]. Nonsignificant differences in final time point relative to baseline cortisol levels for the High Cortisol Response–Stably Moderate Cytokine group suggested that cortisol levels returned to baseline at the conclusion of the visit (i.e., efficient recovery). A *Low Cortisol Response–Stably High Cytokine* profile also emerged and was consistent with our expected Low Cortisol–High Cytokine profile. This group was smallest ($n = 35$) and exhibited trajectories characterized by low cortisol baseline levels and less pronounced cortisol reactivity as well as the highest IL-1β, IL-6, and TNF-α baseline levels in the sample and nonsignificant (i.e., stably high) IL-1β, IL-6, and TNF-α reactivity.

Aim 2.

Multinomial logistic regression parameter estimates for the initial covariate model are available as supplementary materials. Consistent with prior studies using this dataset (Giletta et al., 2015; Giletta et al., 2018), no secondary covariates were associated with subgroup membership. As such, no secondary covariates were included in the correlate models.

Multinomial logistic regression parameter estimates for the pubertal maturation correlate model are presented in Table 4. As hypothesized, pubertal maturation was significantly associated with subgroup membership, $X^2(2) = 7.584$, $p = .023$. Specifically, the multinomial log odds of membership in the High Cortisol Response–Stably Moderate Cytokine and Low Cortisol Response–Stably High Cytokine groups (relative to Low Cortisol Response–Stably Low Cytokine) increased with greater pubertal maturation. Pubertal maturation did not differentiate the High Cortisol Response–Stably Moderate Cytokine and Low Cortisol Response–Stably High Cytokine groups. No primary covariates were significantly associated with subgroup membership: BMI, $X^2(2) = 2.404$, $p > .250$; depressive symptoms, $X^2(2) = 0.264$, $p > .250$; age, $X^2(2) = 0.242$, $p > .250$. Pubertal maturation was significantly associated with subgroup membership for each of our three peer stress models, displaying patterns of differentiation similar to those in the puberty model.

Multinomial logistic regression parameter estimates for the chronic peer strain correlate model are presented in Table 5a. As hypothesized, chronic peer strain was significantly associated with subgroup membership, $X^2(2) = 6.143$, $p = .046$. Specifically, the multinomial log odds of membership in the Low Cortisol Response–Stably High Cytokine subgroup (relative to Low Cortisol Response–Stably Low Cytokine) increased with greater chronic peer strain. However, chronic peer strain did not differentiate the High Cortisol Response–Stably Moderate Cytokine and Low Cortisol Response–Stably High Cytokine subgroups, nor did it differentiate the Low Cortisol Response–Stably Low Cytokine and High Cortisol Response–Stably Moderate Cytokine subgroups. No primary covariates were significantly associated with MTM subgroup membership: BMI, $X^2(2) = 3.010$, $p = .222$; depressive symptoms, $X^2(2) = 2.168$, $p > .250$; age, $X^2(2) = 0.424$, $p > .250$.

[5]Although the IL-6 trajectory intercept for the High Cortisol Response–Stably Moderate Cytokine group was not significantly different from that of Low Cortisol Response–Stably Low Cytokine, the “Moderate” labeling convention was based on the overall pattern of cytokine trajectory distinction analyses (i.e., High Cortisol Response–Stably Moderate Cytokine group’s IL-1β and TNF-α intercepts were significantly higher and lower than the Low Cortisol Response–Stably Low Cytokine and Low Cortisol Response–Stably High Cytokine groups, respectively).

Multinomial logistic regression parameter estimates for the relational victimization correlate model are presented in Table 5b. As hypothesized, relational peer victimization was significantly associated with subgroup membership, $X^2(2) = 10.334$, $p = .006$. Specifically, the multinomial log odds of membership in the Low Cortisol Response–Stably High Cytokine group (relative to Low Cortisol Response–Stably Low Cytokine as well as High Cortisol Response–Stably Moderate Cytokine) increased with greater levels of relational peer victimization. Relational peer victimization did not significantly differentiate the Low Cortisol Response–Stably Low Cytokine and High Cortisol Response–Stably Moderate Cytokine subgroups. No primary covariates were significantly associated with subgroup membership: BMI, $X^2(2) = 1.894$, $p > .250$; depressive symptoms, $X^2(2) = 0.666$, $p > .250$; age, $X^2(2) = 0.411$, $p > .250$.

Multinomial logistic regression parameter estimates for the overt victimization model are presented in Table 5c. Contrary to expectation, overt peer victimization was not significantly associated with MTM subgroup membership, $X^2(2) = 3.526$, $p = .171$. No primary covariates were significantly associated with subgroup membership: BMI, $X^2(2) = 2.044$, $p > .250$; depressive symptoms, $X^2(2) = 0.287$, $p > .250$; age, $X^2(2) = 0.199$, $p > .250$.

Post-hoc developmental correlate analyses.

Chronological age independent of pubertal maturation was not significantly associated with MTM subgroup membership, $X^2(2) = 1.612$, $p > .250$. Chronological age-normed pubertal maturation was significantly associated with subgroup membership, $X^2(2) = 6.613$, $p = .037$, demonstrating between subgroup associations similar to that of pubertal maturation when controlling for age. In each model, BMI and depressive symptoms were not significantly associated with subgroup membership.

Discussion

Although patterns of cortisol stress responsivity have long been considered as an index of adolescent stress vulnerability, the nuances of HPA dysregulation have yet to be fully understood (Koss & Gunnar, 2018; Shirtcliff et al., 2009; Smyth & Clow, 2020; Wadsworth et al., 2019). As noted by Shirtcliff and colleagues (2014), framing more and less pronounced cortisol stress responsivity as being inherently “well-regulated” or “dysregulated,” while attractive due to ease of interpretation, may be overly simplistic. If so, ensuing reliance on single biomarker approaches (e.g., analyzing cortisol only or in isolation) may impede our understanding of HPA function (Buss et al., 2019). Instead, more nuanced, multiple biomarker approaches to biological stress responsivity, although more complex, may yield findings that more closely approximate the interrelated nature of stress-sensitive biological systems (e.g., immuno-endocrine organization) and, thus, more accurately differentiate between relatively well-regulated and relatively dysregulated HPA function (Wadsworth et al., 2019). Building on emerging evidence that HPA function may be best understood in tandem with inflammatory processes as well as developmental and social factors (Del Giudice & Gangestad, 2017; Kuhlman et al., 2020), we used a person-centered, multisystem approach to explore joint HPA–inflammatory stress response profiles and

examined profile associations with indices of pubertal maturation and peer stress exposure in adolescent girls at elevated risk for psychopathology.

The profiles identified suggest that the relation between cortisol and pro-inflammatory cytokine activity may be heterogeneous in nature, and that this heterogeneity may be important for distinguishing well-regulated versus dysregulated HPA function and understanding associations with pubertal maturation and peer stress exposure. Adolescents' cortisol and cytokine levels were, for the most part, not associated with their peer stress experiences at the bivariate level, whereas their joint HPA–inflammatory stress response profiles were. Thus, these heterogeneous connections between the HPA axis and inflammation are perhaps best understood in the context of person-centered, multisystem approaches to biological stress response function. Notably, adolescents in each subgroup displayed rather uniform patterns of activity across cytokines (e.g., similarly low or high baseline levels across cytokine variables). Therefore, this study extends prior analyses of this dataset showing uniform IL-1β, IL-6, and TNF-α associations with peer stress (Giletta et al., 2018) by also illustrating for the first time uniform within-person connections with HPA response function.

Although our use of MTM permitted examination of stress response *trajectories* (i.e., baseline, reactivity), our data do not provide evidence of pro-inflammatory cytokine *reactivity* that occurs in tandem with the cortisol response[6]. One possibility may be that the 45 min post TSST saliva collection timeframe may not allow sufficient time for rising cytokine levels to be detected within the 30 min to 100 min window (Szabo et al., 2020). Alternatively, a prior adolescent study of joint HPA–SNS responsivity (to which inflammatory responsivity is closely linked) revealed significant differences in salivary alpha-amylase (sAA) baseline levels *but not* reactivity when modeled in tandem with the cortisol response (Bendezú & Wadsworth, 2018). Therefore, another possibility may be that the lack of peripheral inflammatory and SNS reactivity observed in the present and prior studies is due to the MTM approach used in each. However, it also may be possible that *basal* sAA and cytokine activity are critical descriptive features of immuno-endocrine organization, more so than sAA and cytokine *reactivity* when modeled in a person-centered framework in conjunction with cortisol. Additional research is needed to adjudicate between these possibilities and further investigate this issue, especially given theory and evidence suggesting that potentiated social stress-induced cytokine *reactivity* increases risk for psychopathology (Del Giudice & Gangestad, 2017; Slavich et al., 2019; Slavich, 2020a).

Our findings illustrate the potential value of multisystem approaches in differentiating cortisol responses that signal well-regulated and dysregulated HPA function (Buss et al., 2019). Although adolescents in the Low Cortisol Response (Low Cortisol Response–Stably High Cytokine and Low Cortisol Response-Stably Low Cytokine) subgroups exhibited indistinguishably attenuated cortisol responses, they also exhibited different levels of

[6]As such, we will forego the term *joint HPA-inflammatory stress responsivity* henceforth, in favor of the term *joint HPA-inflammatory activity* with due acknowledgment that our identified profiles did not yield evidence of significant pro-inflammatory cytokine reactivity.

cytokine activity. This may suggest that their respective cortisol responses, though similar quantitatively, are perhaps distinct in a qualitative sense with respect to neuroendocrine function. For Low Cortisol Response–Stably Low Cytokine adolescents, the combination of attenuated cortisol responding paired with low cytokine activity may reflect immuno-endocrine equilibrium (Landau et al., 2021), with HPA–inflammatory countervailing effects helping to maintain immune system homeostasis (Slavich & Irwin, 2014). Conversely, for Low Cortisol Response–Stably High Cytokine adolescents, attenuated cortisol responding in tandem with heightened cytokine activity may indicate weaker cortisol suppression of the SNS and glucocorticoid resistance processes (Chen et al., 2015). If so, our multisystem findings propose that single-indicator identified low cortisol responses may reflect either well-regulated (e.g., *Weak Cortisol Response*) or dysregulated (e.g., *Cortisol Hyporesponse*) HPA function, contingent on pro-inflammatory cytokine activity.

That pubertal status and peer stress exposure were associated with these HPA–inflammatory profiles in a manner consistent with developmental theory and models of allostatic load further supports this proposition. Low Cortisol Response–Stably Low Cytokine adolescents were relatively less advanced in their pubertal development (relative to High Cortisol Response–Stably Moderate Cytokine and Low Cortisol Response–Stably High Cytokine), but also experienced lower levels of peer stress exposure (relative to Low Cortisol–High Cytokine). A *Weak Cortisol Response* to stressors during early puberty protects adolescents' developing brains and bodies against the potential neurotoxic effects of cortisol overexposure (van der Voorn et al., 2017), which may be adaptive in social contexts where threat of harm is low (Flinn et al., 2011; Spear, 2009). Conversely, Low Cortisol Response–Stably High Cytokine adolescents were more pubertally mature (relative to Low Cortisol Response–Stably Low Cytokine), but also experienced greater peer stress exposure (relative to Low Cortisol Response–Stably High Cytokine and High Cortisol Response–Stably Moderate Cytokine). Under conditions of chronic stress, puberty-related elevations in estrogen may contribute to girls' *Cortisol Hyporesponse* and compromise cortisol's anti-inflammatory signaling properties (for details, see Slavich & Sacher, 2019). If so, the Low Cortisol Response–Stably High Cytokine profile extends evidence of *blunted* cortisol responsivity for peer victimized girls in later relative to earlier stages of pubertal maturation (for a review, see Kliewer et al., 2019) by illustrating corresponding elevations in pro-inflammatory cytokine activity.

Our concurrent consideration of HPA and inflammatory processes builds upon prior single bio-marker stress vulnerability research with adolescent samples in ways that may help to clarify weak or inconsistent cortisol–maladjustment linkages (Wadsworth et al., 2019). For example, in a prior single bio-marker (i.e., cortisol only) analysis of this data set (Giletta et al., 2015), the *Hyporesponsive* group exhibited rates of maladjustment that were comparable (i.e., low) or different only at the trend-level (i.e., slightly elevated) to those observed in the *Normative* group. Thus, the current study suggests the possibility that, when studying adolescent samples with variation in developmental and social factors, single-indicator identified low cortisol responses may be unknowingly comprised of *both* a low-risk *Weak Cortisol Response* such as that found in the Low Cortisol Response–Stably Low Cytokine profile *as well as* a high-risk *Cortisol Hyporesponse* such as that found in the Low Cortisol

Response–Stably High Cytokine profile. If so, a multisystem approach such as ours may parse between the two patterns and further clarify connections to risk.

Person-centered exploration of joint HPA–inflammatory stress responsivity also helped distinguish adolescents with more pronounced cortisol response patterns. For High Cortisol Response–Stably Moderate Cytokine adolescents, the combination of heightened cortisol responding paired with more moderate cytokine activity may reflect cortisol's anti-inflammatory properties in the face of threat that support coping and mitigate the onset of sickness behaviors (Slavich & Irwin, 2014). Of note, examining specific aspects of their stress response trajectories revealed that High Cortisol Response–Stably Moderate Cytokine adolescents' cortisol levels returned to baseline at the conclusion of the experiment, signaling *efficient recovery* of marshaled physiologic resources. This post-hoc result highlights the benefits of modeling *trajectories* when interpreting cortisol stress responsivity (Ji et al., 2016). Together, these findings implicate a *Strong Cortisol Response* reflective of well-regulated HPA function in the High Cortisol Response–Stably Moderate Cytokine profile.

The High Cortisol Response–Stably Moderate Cytokine profile is somewhat at odds with our original conceptualization of well-regulated HPA function (e.g., moderate instead of low cytokine levels). Nevertheless, pubertal development and peer stress exposure connected with this profile in ways that advance developmental models of immuno-endocrine organization. Relative to Low Cortisol Response–Stably Low Cytokine, High Cortisol Response–Stably Moderate Cytokine girls were more pubertally advanced and presented with similarly low levels of peer stress exposure. The emergence of a *Strong Cortisol Response* to stress is a hallmark feature of the pubertal transition, especially for girls (for review and meta-analysis, see van der Voorn et al., 2017). Our findings extend this literature by suggesting that the puberty-related shift from *Weak* to *Strong Cortisol Response* also may be accompanied by parallel normative increases in pro-inflammatory cytokine levels. Indeed, estrogen levels that increase during the pubertal transition stimulate cytokine activation (Klein, 2000). These sex-hormone linked modest elevations in neuroinflammation have critical adaptive value, providing girls immunologic protection in the face of potential pathogenic threats to reproductive health. However, perhaps illustrated by Low Cortisol Response–Stably High Cytokine adolescents with similarly advanced pubertal development but also greater relational peer victimization relative to High Cortisol Response–Stably Moderate Cytokine, chronic stress exposure can contribute to more dramatic elevations in cytokine activity via estrogen-related blunting of the HPA response and reduced cortisol anti-inflammatory signaling. These processes are thought to account for sex differences in risk for psychopathology and other inflammation-related health conditions (Slavich & Sacher, 2019).

The lack of evidence with respect to a number of other hypotheses is also noteworthy. First, although our findings partially supported three of our hypothesized profiles, we did not find any evidence of a High Cortisol–High Cytokine profile, one that might have implicated a *Cortisol Hyperresponse* pattern. Future studies utilizing larger sample sizes as well as additional biomarkers (see Limitations and Future Directions) may be needed to identify this profile. Third, while chronic peer strain and relational victimization

differentiated among the profiles, overt victimization did not. Although overt victimization levels were comparable to those seen in community samples (Prinstein et al., 2001), the restricted range of overt victimization may have limited variability to detect effects. However, an alternate explanation may be that certain types of stressors (e.g., social rejection, exclusion; Casper & Card, 2016; Kliewer et al., 2019; Slavich et al., 2010) more profoundly impact HPA and cytokine activity than others. Fourth, though included as primary covariates, girls' depressive symptoms and BMI were not associated with our profiles. Although the association between BMI, depression, and cortisol activity has been consistently documented (e.g., Dockray et al., 2009; Doom et al., 2019; Lewis-de Los Angeles & Liu, 2021), evidence supporting such associations with inflammatory biomarker activity has been inconsistent (for a review, see Del Giudice & Gangestad, 2017). Some research has demonstrated a positive association between BMI, depression, and IL-6, while less to no support has been generated for their association with IL-1β and TNF-α (e.g., Dowlati et al., 2010; Haapakoski et al., 2015; Himmerich et al., 2006; Howren et al., 2009; Köhler et al., 2017; Van Dongen et al., 2015). Future person-centered cytokine reactivity studies examining these various cytokines independently may reveal such associations.

Strengths and Implications

The present study has a number of strengths. First, the administration of an experimental stressor task provided a unique opportunity to simultaneously examine HPA and inflammation stress responses. Such *in vivo* examinations of the HPA axis and immune system can provide important information about how these systems operate in tandem during times of stress. Second, consideration of both HPA and immune function provided an opportunity to explore unique patterns of multisystem responsivity to a social stressor. Third, the community- and clinic-based recruitment strategies used for this study produced a sample of girls with a wide range of interpersonal experiences, which, in turn, increased variation in stress response patterns and allowed for meaningful determination of groups in the MTM analyses (i.e., groups indicating both low and high stress vulnerability). Fourth, the inclusion of self-reported chronic peer stress and friend-report of peer victimization strengthened the validity of findings indicating that the Low Cortisol Response–Stably High Cytokine group was highest in peer stress exposure and exhibited a cortisol response that signaled HPA dysregulation (i.e., *Cortisol Hyporesponse*).

Although cautious interpretation is necessary due to our cross-sectional design, our findings may implicate a developmentally curvilinear association between the HPA and immune system functioning for girls in the context of chronic peer stress exposure. For girls, pubertal maturation may contribute to stronger HPA responsivity (Hibel et al., 2020; van der Voorn et al., 2017) and mild-to-moderate levels of inflammation (Klein, 2000). The HPA axis becomes more stress sensitive as girls advance into adolescence and enter more complex, hierarchical social contexts. Therefore, we may expect that stronger HPA response to social stress is beneficial developmentally insofar as it marshals biological resources for coping with increasing interpersonal demands (e.g., protecting or enhancing social status, Slavich, 2020b). Changes in pro-inflammatory sex hormones (e.g., estrogen) during puberty can also increase inflammation. However, when chronic in nature, peer-related stressors can become overly taxing and lead to pathological increases in inflammation via estrogen-

associated blunting of the HPA, compromised cortisol suppression of biological resource-depleting SNS activity, and glucocorticoid resistance processes (Slavich & Sacher, 2019). Taken together, changes in HPA-inflammation activity attributable to naturally occurring, developmental processes during the adolescent transition may be accelerated or compounded by chronic exposure to peer stress.

Limitations and Future Directions

This study also has several limitations that may help guide future research. First, the results are limited to a sample of girls at risk for psychopathology. Replicating these findings with adolescents from different demographic backgrounds is needed to investigate the generalizability of these findings. Complementary research also is needed to understand whether similar profiles emerge in samples including both adolescent boys and girls. Second, the sample size was relatively small for a person-centered approach. Future research with larger samples may potentially detect additional profiles (e.g., High Cortisol–High Cytokine). Third, our efforts to illustrate joint HPA–inflammatory stress responsivity was limited to cortisol obtained from four saliva samples and cytokines obtained from only two saliva samples. Although cortisol levels generally peak 20 min post-stressor (Kirschbaum & Hellhammer, 1994), some evidence suggests that salivary cytokine emergence may be both more delayed and variable than cortisol (Szabo et al., 2020). Research including additional post-stressor samples may more fully capture cross-system reactivity and recovery patterns. Still further, HPA axis and immune system function is complex and bidirectional, with acute HPA axis activation inhibiting and enhancing different cytokine types (e.g., cortisol exerts transcriptional control on immune cells that modulate the production of specific cytokines), whereas certain cytokine types (e.g., IL-1β, IL-6) also directly stimulate the HPA axis (Besedovsky & del Rey, 2000; Shintani et al., 1995; Slavich 2020a, 2020b). Additional time-points may therefore support examination of *intra-individual coupling* of multi-system biomarkers, which may strengthen inference about directionality (e.g., whether cortisol is modulating specific cytokines and vice versa) beyond that afforded by MTM (e.g., Howland et al., 2020; Marceau et al., 2014, 2015).

Fourth, study-wide mean levels of IL-6 and TNF-α were low and within the range of error of the low/negative control for cytokine assays. Although we excluded cytokine values that were extrapolated beyond the standard range, these low levels may have hindered our ability to detect effects. Nevertheless, our Low Cortisol Response–Stably Low Cytokine group was meaningfully associated with puberty and peer stress correlates in expected ways, perhaps suggesting that our person-centered approach circumvented this potential limitation. Fifth, as is common with group-based modeling of development (Nagin, 2005), covariate effects were estimated after adolescents were classified into subgroups. Future studies may wish to adjust for known covariates of immuno-endocrine function during model specification. Nevertheless, a more parsimonious model such as ours may have been favorable given that no study to date has attempted to explore joint HPA–inflammatory stress responsivity (for further justification, see Landau et al., 2021). Sixth, our assessment of peer stress exposure focused solely on relatively recent (i.e., past year) stressors in the peer domain. Future examinations that attend to additional temporal aspects of stress exposure (e.g.,

chronicity, timing) on joint HPA–inflammatory responsivity may help to further characterize immuno-endocrine organization and clarify cortisol stress response patterns.

While our multisystem approach (i.e., including IL-1β, IL-6, and TNF-α in an effort to clarify heterogeneity in adolescent girls' cortisol responses to stress) is perhaps an improvement over existing single bio-marker approaches (Buss et al., 2019), the inclusion of additional biological stress response indices would likely clarify this heterogeneity even further (Ellis et al., 2017). Of note, such heterogeneity was not found among girls presenting with High Cortisol. It is possible that a *Cortisol Hyperresponse* may be reflected through cross-system profiles that also include SNS indices (e.g., sAA, skin conductance). One recent MTM study demonstrated that some girls with elevated baseline cortisol levels and exaggerated cortisol reactivity also present with elevated sAA baseline levels and exaggerated sAA reactivity (Bendezú & Wadsworth, 2018). Such a profile could present in advantageous ways (e.g., cortisol produced by the SNS-innervated HPA axis helps modulate physically taxing SNS response, down-regulating inflammatory processes; Bauer et al., 2002) or disadvantageous ways (e.g., cross-system hyperarousal linked to hypervigilance and threat monitoring, exacerbating inflammatory processes; Urasche & Blair, 2015) that are different from those characterizing Low Cortisol Response–Stably High Cytokine adolescents (e.g., insufficient cortisol production that fails to modulate the SNS, exacerbating inflammation; Bauer et al., 2002; Heim et al., 2000).

Future multisystem work may also benefit from including positive valence system responses, as emerging research suggests that stress-induced changes in these systems could differentiate stress responses generated by negative valence systems (i.e., those traditionally understood to be primarily implicated in processing threat/stress, such as the HPA axis; e.g., see Bendezú, Calhoun et al., 2021). Additionally, longitudinal research is needed to verify the speculated associations between pubertal development, chronic peer stress, and joint HPA-inflammation responsivity. Reassessment of stress responsivity and peer stressors at pre-, peri-, and post-puberty time points could help clarify the theoretical postulations presented above, as well as help delineate the psychobiological importance of examining cumulative lifetime peer stress exposure relative to peer stress exposure in a particular window of time (i.e., sensitive periods).

Conclusion

In conclusion, the present findings emphasize the importance of using a person-centered, multisystem approach to examining associations between acute stress responses, developmental markers, and peer stress exposure. This approach may explain the variety of different HPA axis reactivity profiles that have been associated with life stress exposure and psychological symptoms in adolescents. Ultimately, this work may lead to the development of new models for understanding the early origins of lifespan mental and physical health problems that are affected by biological stress processes, differences in pubertal maturation, and life stress exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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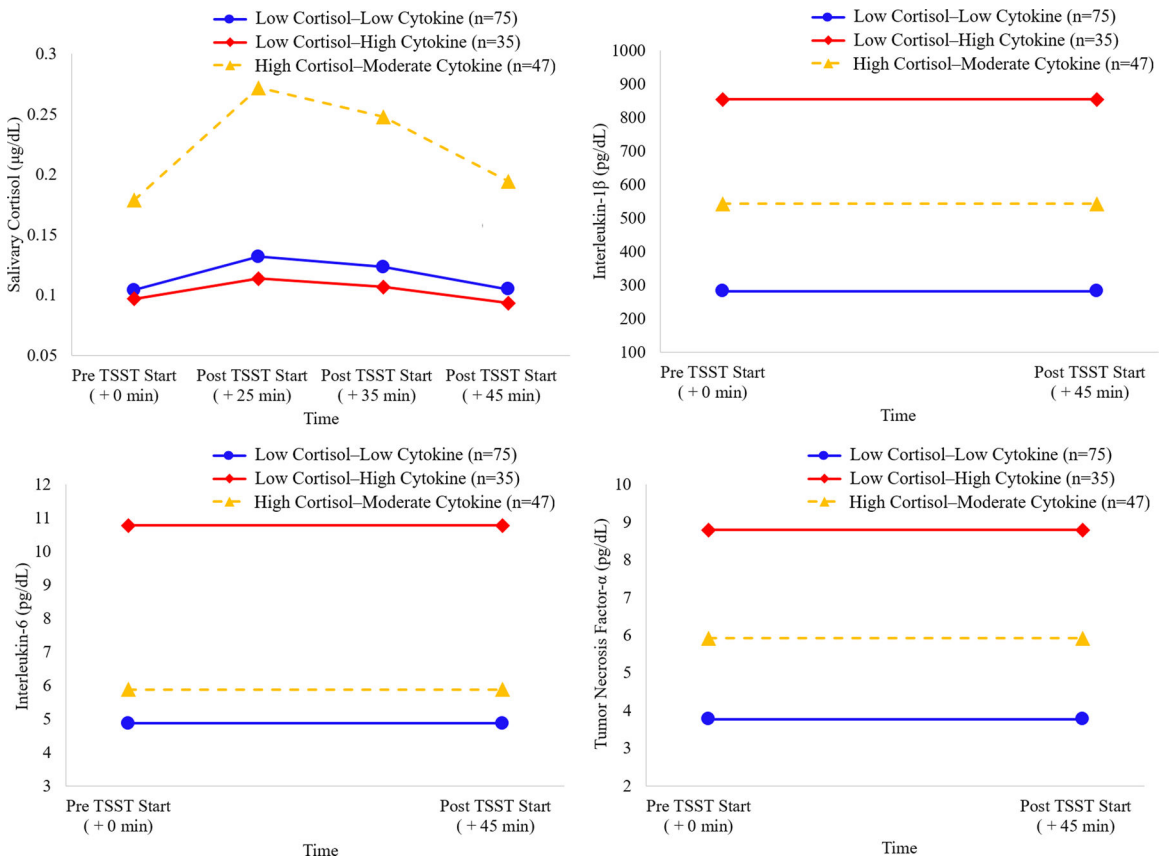

Figure 1.

*C*ortisol and pro-inflammatory cytokine trajectories in response to the Trier Social Stress Test (TSST) for the final three-group solution. Reverse transformed values presented for ease of interpretation and cross study comparison.

Table 1:

Descriptives and Correlations for Main Study Variables

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
1. Cortisol +0 min	—														
2. Cortisol +25 min	.59*	—													
3. Cortisol +35 min	.54*	.93*	—												
4. Cortisol +45 min	.56*	.86*	.94*	—											
5. IL-1β +0 min	.09	.04	−.01	.03	—										
6. IL-1β +45 min	.01	−.03	.01	.08	.67*	—									
7. IL-6 +0 min	.04	−.01	−.04	−.01	.41*	.34*	—								
8. IL-6 +45 min	−.07	−.02	−.01	.04	.17	.40*	.52*	—							
9. TNF-α +0 min	−.07	.01	.03	.03	.34*	.27*	.47*	.09	—						
10. TNF-α +45 min	.05	−.03	.02	.09	.08	.23*	.27*	.19	.68*	—					
11. Chronological age	.06	.14	.13	.19	.11	.27*	.11	.05	.22*	.16	—				
12. Pubertal maturation	−.01	.14	.12	.15	.15	.21*	.08	−.01	.23*	.21*	.58*	—			
13. Chronic peer strain	.07	−.01	.01	.01	−.04	−.07	−.06	−.05	.28*	.32*	.25*	.05	—		
14. Relation victimization	−.14	−.11	−.06	−.03	−.02	.16	−.03	.07	.14	.18	.31*	.19	.14	—	
15. Overt victimization	−.05	−.12	−.12	−.11	−.16	−.08	−.17	−.10	.01	−.11	.10	−.02	.11	.26*	—
M	0.13	0.18	0.16	0.14	584.6	584.4	9.28	9.76	7.06	6.64	14.72	3.41	2.28	1.64	1.23
SD	0.07	0.10	0.08	0.06	538.5	538.4	13.73	11.44	6.64	4.94	1.38	0.43	0.70	0.63	0.44

Note. IL = Interleukin; TNF = tumor necrosis factor. Minutes refer to time since the start of the Trier Social Stress Test.

* $p < .05$.

Table 2:

Parameter Estimates (Standard Errors) and Model Adequacy Indices for the One-Group and Final Three-Group MTM Solution

	Cortisol	Interleukin-1β	Interleukin-6	Tumor Necrosis Factor-α	$AvePP_j$	OCC_j	$Prob_j$	$Prop_j$	*Ratio*
One Group									
Intercept	−0.915* (0.018)	6.132* (0.058)	1.854* (0.054)	1.707* (0.044)					
Linear	0.011* (0.002)								
Quadratic	−0.001* (0.001)								
Three Group									
Low Cortisol Response–Stably Low Cytokine									
Intercept	−0.983* (0.020) A	5.640* (0.097) A	1.582* (0.089) A	1.328* (0.070) A	.933	27.807	.423	.477	0.887
Linear	0.009* (0.002)								
Quadratic	−0.001* (0.001) a								
High Cortisol Response–Stably Moderate Cytokine									
Intercept	−0.748* (0.023) B	6.297* (0.090) B	1.771* (0.096) A	1.778* (0.067) B	.969	63.386	.322	.299	1.077
Linear	0.015* (0.003)								
Quadratic	−0.001* (0.001) b								
Low Cortisol Response–Stably High Cytokine									
Intercept	−1.014* (0.027) A	6.750* (0.115) C	2.378* (0.130) B	2.174* (0.089) C	.928	25.580	.254	.223	1.139
Linear	0.007* (0.003)								
Quadratic	−0.001* (0.001) a								

Note. MTM = Multitrajectory Modeling; $AvePP_j$ = Average posterior probability; OCC_j = Odds of correct classification; $Prob_j$ = Probability of group assignment; $Prop_j$ = Proportion of children assigned to each group; *Ratio* = Ratio of $Prob_j$ to $Prop_j$; Upper- and lower-case superscripts denote significant differences in intercept and polynomial estimates, respectively, within the same biomarker.

*$p < .05$.

Table 3:

Full Sample and Subgroup Percentages of Participants Demonstrating Increases in Pre-Post TSST Cortisol and Cytokine Levels

Group	Cortisol	Interleukin-1β	Interleukin-6	Tumor Necrosis Factor-α
Full Sample				
% showing any pre-post TSST increase	74.7%	51.0%	57.0%	38.6%
% showing a 10% pre-post TSST increase	61.7%	16.8%	47.4%	28.1%
Low Cortisol Response–Stably Low Cytokine				
% showing any pre-post TSST increase	73.6%	48.6%	58.0%	40.4%
% showing a 10% pre-post TSST increase	61.1%	18.9%	46.0%	34.0%
High Cortisol Response–Stably Moderate Cytokine				
% showing any pre-post TSST increase	80.9%	51.1%	54.5%	41.7%
% showing a 10% pre-post TSST increase	72.3%	21.3%	48.5%	27.8%
Low Cortisol Response–Stably High Cytokine				
% showing any pre-post TSST increase	68.6%	55.9%	58.1%	32.3%
% showing a 10% pre-post TSST increase	48.6%	5.9%	48.4%	19.4%

Table 4:

Parameter Estimates from a Pubertal Maturation Correlate Multinomial Logistic Regression Model Predicting Multitrajectory Modeling Group Membership

Reference Group vs. Comparison Group	Covariates and Correlates	X^2 (df) [a]	*B*	*SE*	Odds Ratio	95% CI for Odds Ratio
Low Cortisol Response–Stably Low Cytokine vs. High Cortisol Response–Stably Moderate Cytokine						
	Intercept	0.248 (2)	0.498	2.978		
	Body mass index	2.404 (2)	−2.073	1.820	0.126	0.004, 4.495
	Depressive symptoms	0.264 (2)	−0.008	0.016	0.992	0.962, 1.024
	Chronological age	0.242 (2)	−0.032	0.174	0.969	0.689, 1.362
	Pubertal maturation	7.581 * (2)	0.207 *	0.096	1.231 *	1.019, 1.487
Low Cortisol Response–Stably Low Cytokine vs. Low Cortisol Response–Stably High Cytokine						
	Intercept		1.667	3.365		
	Body mass index		−2.764	2.077	0.063	0.001, 3.694
	Depressive symptoms		0.001	0.017	1.000	0.967, 1.033
	Chronological age		−0.093	0.189	0.911	0.629, 1.321
	Pubertal maturation		0.230 *	0.106	1.259 *	1.023, 1.549
High Cortisol Response–Stably Moderate Cytokine vs. Low Cortisol Response–Stably High Cytokine						
	Intercept		1.168	3.668		
	Body mass index		−0.691	2.260	0.501	0.006, 41.99
	Depressive symptoms		0.007	0.018	1.007	0.972, 1.044
	Chronological age		−0.061	0.203	0.941	0.632, 1.401
	Pubertal maturation		0.023	0.116	1.023	0.815, 1.284

Note. Beta parameter estimates reflect multinomial log-odds of comparison group membership relative to the reference group for each unit increase in the correlate or covariate of interest.

[a] = X^2 estimates were the same for each comparison.

* $p < .05$.

Table 5:

Parameter Estimates for (5a) Chronic Peer Strain, (5b) Relational Peer Victimization, and (5c) Overt Peer Victimization Correlates Added to the Multinomial Logistic Regression Model Predicting Multitrajectory Modeling Group Membership

Reference Group vs. Comparison Group	Correlates	X^2 (df) [a]	*B*	*SE*	Odds Ratio	95% CI for Odds Ratio
Low Cortisol Response–Stably Low Cytokine vs. High Cortisol Response–Stably Moderate Cytokine						
	5a Chronic peer strain	6.143 * (2)	0.411	0.353	1.508	0.756, 3.010
	5b Relational peer victimization	10.33 * (2)	−2.193	1.417	0.112	0.007, 1.795
	5c Overt peer victimization	3.526 (2)	−3.193	1.417	0.037	0.001, 1.447
Low Cortisol Response–Stably Low Cytokine vs. Low Cortisol Response–Stably High Cytokine						
	5a Chronic peer strain		0.892 *	0.370	2.440 *	1.181, 5.039
	5b Relational peer victimization		2.932 *	1.472	18.59 *	1.039, 332.8
	5c Overt peer victimization		−1.173	1.731	0.309	0.010, 9.196
High Cortisol Response–Stably Moderate Cytokine vs. Low Cortisol Response–Stably High Cytokine						
	5a Chronic peer strain		0.481	0.370	1.618	0.784, 3.339
	5b Relational peer victimization		5.115 *	1.670	166.6 *	6.312, 4394.8
	5c Overt peer victimization		2.114	2.167	8.284	0.119, 578.8

Note. Beta parameter estimates reflect multinomial log-odds of comparison group membership relative to the reference group for each unit increase in the correlate of interest. Body mass index, depressive symptoms, age and pubertal maturation were included in all models (see Table 4).

[a] = X^2 estimates were the same for each comparison.

* $p < .05$.