

## RESEARCH ARTICLE OPEN ACCESS

# Psychoneuroimmunological Evidence for Biological Embedding During Early Adolescence

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## ABSTRACT

Adolescence is a period of vulnerability wherein stress can become biologically embedded in ways that impact long-term trajectories of mental and physical health. In particular, stressors are transduced into physiological changes via the hypothalamic–pituitary–adrenal (HPA) axis in ways that can impact both physical and mental health. However, there continues to be uncertainty regarding how to best differentiate and understand well-regulated HPA axis reactivity to stress from dysregulated HPA axis reactivity. One promising approach involves examining multiple biomarkers; indeed, there is evidence that dysregulation of the HPA axis profoundly influences the regulation of the immune system. A cohort of adolescent youth was followed across two ubiquitous stressors—the transition to high school and the COVID-19 pandemic. Nuanced longitudinal associations between HPA axis activity (i.e., cortisol) and immune system activity (i.e., panel of inflammatory markers) were examined. Findings provide evidence of biological specificity wherein HPA axis hyperactivity during the COVID-19 pandemic was associated with elevated levels of an empirically derived inflammatory composite, which may be driven by elevations in tumor necrosis factor- $\alpha$ , interleukin-6, and c-reactive protein. The current work advances the literature on allostatic load and the glucocorticoid-resistance model in youth. By extending our current understanding of how stress influences adolescent well-being, it also has important implications for mental and physical health prevention and intervention efforts.

## 1 | Introduction

Adolescence is a developmental period marked by concurrent changes across biological systems. This positions adolescence as a paradoxical period of both opportunity and vulnerability: while this stage confers opportunities for resilience via the recalibration of neurobiological systems, during adolescence, stress can become biologically embedded in ways that impact long-term trajectories of mental and physical health. In particular, the hypothalamic–pituitary–adrenal (HPA) axis is seminal in

transducing social stressors into physiological changes that can impact health and well-being. Indeed, cortisol dysregulation during adolescence has been linked with an increased risk of numerous physical and mental illnesses across the lifespan (Adam et al. 2014; Vrshek-Schallhorn et al. 2013). However, there continues to be uncertainty regarding how to best differentiate and understand well-regulated HPA axis activity from dysregulated HPA activity: hyper- or hypoactivity at any one point in time may be initially adaptive, though increasingly maladaptive over time and across contexts. Given evidence that biological

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systems are interconnected and that altered development of one biological system can lead to lasting effects across other systems (Buss et al. 2018), one promising approach to developing a more nuanced understanding of HPA axis functioning involves examining levels of multiple biomarkers in conjunction to characterize and differentiate well-regulated HPA axis reactivity to stress from dysregulated HPA axis reactivity. The argument for examining multiple biomarkers is strengthened by the fact that the biological embedding of early life experiences within the HPA axis can induce dysregulation across other systems, most notably the immune system (Berens et al. 2017).

Immune system activation triggers inflammatory responses that are both reactive (in response to existing tissue damage and infection) and proactive (in response to potential threats) (Ackerman et al. 2018). While mechanisms of the immune system are in place to maintain homeostasis, accumulating evidence identifies elevated inflammatory processes as a common mechanism of disease for both mental and physical illnesses such as depression and cardiovascular disease (Furman et al. 2019).

Inflammation is commonly indexed by interleukin (IL) proteins IL-1 $\beta$ , IL-6, and IL-8; tumor necrosis factor-alpha (TNF- $\alpha$ ); and c-reactive protein (CRP). Each of these proteins contributes to systemic inflammation, and theories postulate that these inflammatory proteins are jointly influenced by a shared latent inflammatory process (Cavaillon and Adib-Conquy 2002). Yet, given the many different components of the inflammatory response, these markers can also demonstrate specificity. Clinically, each of these markers stimulates inflammatory and auto-immune processes involved in multiple disease states, including psychiatric illnesses such as depression and schizophrenia (Yuan et al. 2019) and chronic illnesses such as cardiovascular disease, multiple sclerosis, and rheumatoid arthritis (Koptoge et al. 2010).

Cortisol profoundly influences the regulation of the immune system, and disturbances within the HPA axis can lead to immune imbalance. In response to acute stress, glucocorticoids (composed primarily of cortisol in humans) downregulate the expression of inflammatory markers such as IL-1B, IL-6, IL-8, TNF- $\alpha$ , and CRP (Sapolsky 2002). This downregulation is adaptive as it allows an organism to respond to a stressor without promoting inflammatory-driven sickness behaviors (e.g., fever, lethargy) (Myers 2008). However, chronic HPA dysregulation can lead to glucocorticoid resistance, in which immune cells become less sensitive to cortisol's typical anti-inflammatory properties; in other words, cortisol becomes less effective at downregulating the expression of inflammatory proteins (Miller et al. 2002).

Accordingly, dysregulated immune system functioning could result from chronic HPA axis dysregulation in adolescence, and this proposition has received empirical support. For instance, research using animal models has shown that chronically stressed mice exhibit glucocorticoid resistance and corresponding enhanced inflammatory responses (Sheridan et al. 2000; Stark et al. 2001). In humans, there has also been cross-sectional work demonstrating that dexamethasone, a synthetic cortisol analog, is less able to inhibit IL-6 release in chronically stressed adults (Wirtz et al. 2003). Additional work in adults has shown that long-term stress results in functional resistance to glucocorticoid signal transduction in monocytes, leading to elevated levels of

pro-inflammatory markers (Miller et al. 2008). Similar findings have been demonstrated using in vitro methodologies, with the peripheral blood cells of participants with depression showing a reduced ability to respond to glucocorticoids (Carvalho et al. 2008). Importantly, much of the work to date has been conducted with specialty populations (e.g., participants with depression), the use of synthetic analogs of cortisol such as dexamethasone, and/or in vitro methods. Whether these associations are observed among youth and across naturalistic stressors remains unclear. Further, while cross-sectional work has supported an association between HPA axis dysregulation and elevated levels of inflammatory markers in both adolescents and adults (Wedervang-Resell et al. 2020), longitudinal associations have not yet been examined in youth despite recent calls for longitudinal work in this area (Chen et al. 2017).

## 1.1 | Aim

The present study examined deeper biological embedding of HPA axis dysregulation among youth, as evidenced by elevated levels of inflammatory markers. We examined HPA axis output across two successive stressors: the transition to high school and the COVID-19 pandemic. In addition to modeling HPA axis dysregulation separately across these stressors, HPA axis dysregulation was also modeled as a composite score of total cortisol output across both stressors. This approach is in accordance with allostatic load theory, which posits that repeated "hits" from multiple stressors are more likely to result in multisystem dysregulation than dysregulation in the context of a single stressor (McEwen 1998). Inflammatory markers were also assessed during the COVID-19 pandemic, after the collection of cortisol. Inflammation was modeled both as levels of individual inflammatory proteins and as an empirically identified inflammatory composite. We took this approach given that examining associations between HPA axis activity and individual inflammatory markers allows for the identification of protein-specific pathways (thus maximizing specificity), and given that inflammatory composite factor scores are thought to have some statistical advantages over approaches examining single biomarkers such as increased reliability (Moriarity et al. 2020).

## 2 | Materials and Methods

### 2.1 | Participants

Early adolescent youth taking part in a broader initiative, the *UBC Study on Adolescents*, were recruited for study participation. This study was approved by The University of British Columbia's (UBC) Behavioural Research Ethics Board (BREB: #H17-01901). Participants were eligible if they were fluent in English and if they did not have a history of serious head trauma, current substance use disorder, and medical conditions or medications known to affect the HPA axis or immune system. The study team made a substantial effort to recruit youth from diverse neighborhoods by partnering with school boards across the region, using both online and paper advertisements, and offering compensation for transportation to the university to minimize financial barriers to participation. A total of 50 youth provided data for the current study. The sample had a mean age of 12.86 years (range = 11.86–

13.52;  $SD = 0.37$ ) at the baseline assessment, with a relatively equal split for sex assigned at birth (56% male) and gender identity (56% boys); all participants were cisgender. The majority of participants identified as White (60%), followed by Chinese (16%), South Asian (4%), Canadian Indigenous (4%), and additional endorsed racial identities (16%). Household income ranged from \$20,000 to over \$200,000; the median household income was between \$120,000 and \$140,000.

## 2.2 | Measures

### 2.2.1 | Cortisol

During both the high school transition and the COVID-19 pandemic, participants provided eight saliva samples across 2 days using a sampling protocol consistent with current expert consensus guidelines for pediatric populations and prior work in this population (Stalder et al. 2016; Kuhlman et al. 2019). Specifically, saliva was collected immediately upon awakening; 30 min post-awakening; at 3:00 p.m.; and at bedtime. As part of the saliva collection protocol, participants were required to track the exact time of day at which the sample was collected using a daily diaries approach. Cortisol concentrations were measured using chemiluminescence immunoassay with high sensitivity at the Technische Universität Dresden. Samples were assayed in duplicate, and the intra- and interassay coefficients were below 8% (high school transition) and 9% (COVID-19 pandemic). Area under the curve with respect to ground (AUCg) was calculated using trapezoidal integration for both the high school (HS) transition and the COVID-19 pandemic (Pruessner et al. 2003). Consistent with allostatic load theory, these values were then summed to create a *cumulative cortisol index*, representing total cortisol output across both sampling timepoints (McEwen 1998). Additional details are presented in the Supporting Information.

### 2.2.2 | Inflammatory Markers

Passive drool samples were assayed for levels of five inflammatory markers: IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , and CRP. Four cytokines were quantified by a multiplex salivary cytokine assay multi-cytokine array (IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ ). Samples were assayed in duplicate at the Salimetrics SalivaLab (Carlsbad, CA) using a proprietary electrochemiluminescence method developed and validated for saliva by Salimetrics. Levels of CRP were quantified using a high-sensitivity enzyme-linked immunosorbent assay (ELISA). The average intra- and interassay coefficients of variance (CVs) were 3.00% and 3.00%, respectively, for IL-1 $\beta$ ; 3.67% and 6.67% for IL-6; 3.00% and 8.00% for IL-8; 6.00% and 13.00% for TNF- $\alpha$ ; and 3.20% and 2.60% for CRP. See the Supporting Information for additional details.

### 2.2.3 | Covariates

**2.2.3.1 | Demographic and Health Variables.** Key demographic characteristics (youth's sex, gender identity, date of birth) were reported by both youth and their caregivers using a brief questionnaire. In addition, caregivers reported youths' height,

weight, and medication use when youth provided passive drool samples.

**2.2.3.2 | Psychiatric Diagnosis.** Youth completed the Kidie Schedule for Affective Disorders and Schizophrenia for School Aged Children—Present and Lifetime Version for DSM-5 (K-SADS PL DSM-5) to assess for the presence of current and lifetime psychiatric diagnoses (Kaufman et al. 2016). See the Supporting Information for additional details.

**2.2.3.3 | Pubertal Staging.** Pubertal stage was assessed using the self-report Tanner Staging Questionnaire, which was completed by youth (Marshall and Tanner 1968). In line with previous work, we averaged Tanner scores for each participant to create an index of average pubertal development.

**2.2.3.4 | Time of Saliva Sample.** Given that cortisol and inflammatory marker production exhibits diurnal rhythmicity, youth provided the exact time they provided all saliva samples.

## 2.3 | Procedure

At baseline, prior to the transition to high school, caregivers completed a brief demographic questionnaire, and youth completed the K-SADS-PL DSM-5. Youth then provided saliva samples across the first 2 days of high school to assess diurnal cortisol, at which point they also completed the Tanner Staging Questionnaire. In the school system in which this research was conducted, students transition directly from elementary school (Kindergarten through Grade 7) to high school (Grade 8 through Grade 12). While the study initially focused on the transition to high school, following the onset of the COVID-19 pandemic, we included an additional assessment timepoint during the pandemic to test hypotheses related to prospective associations between cortisol and inflammatory markers. Thus, in May–June 2020, approximately 3 months after a state of emergency was declared and when the strictest physical-distancing measures of the first wave of the COVID-19 pandemic were in place, youth once again provided saliva samples across 2 days for the assessment of diurnal cortisol. A local state of emergency was in place throughout the duration of the present study, as were restrictions on social gatherings (e.g., suspension of in-class instruction) and travel restrictions and regulations. Youth were attending virtual school during this second timepoint. The average time between the two cortisol collection timepoints was 287.04 days ( $SD = 7.61$ ). After providing saliva for the purposes of cortisol during the pandemic, youth provided a separate saliva sample using the passive drool method to assess inflammatory proteins. For all sampling timepoints, youth were instructed not to eat, drink, or brush their teeth for 1 h prior to collecting each sample. Finally, at this same timepoint, youth recompleted the Tanner Staging Questionnaire and the K-SADS-PL DSM-5.

## 2.4 | Planned Data Analyses

### 2.4.1 | Risk Score Calculation

In line with current practices, cortisol outliers were winsorized to 2 standard deviations ( $SDs$ ) from the sample mean to adjust

for skew. Post-winsorization, skew statistics were acceptable (transition to high school range =  $-0.54$  to  $0.53$ ; COVID-19 pandemic range =  $-0.72$  to  $0.59$ ). The cortisol AUCg, representing total cortisol output, was then calculated at each timepoint. Variables known to influence HPA axis functioning were assessed as potential covariates for youths' AUCg values at each timepoint using a series of correlation analyses. Tested covariates included sex, age, medication use, pubertal stage at the relevant timepoint, and presence of a psychiatric diagnosis. Participant's self-reported gender identity was not tested as a covariate given that all participants identified as cisgender; thus, this variable was redundant with sex. After controlling for significant covariates by computing the residual variance in cortisol AUCg at each timepoint after accounting for variance explained by significant covariates, a cortisol risk score was developed for each participant by summing cortisol AUCg values across the two timepoints. Using residualized cortisol values allows us to isolate individual differences in cortisol that are not explained by the relevant covariate(s), without adjusting for the covariate(s) influence on outcome measures. In addition to the HS cortisol index and the pandemic cortisol index, this summed score was standardized for use in the main analyses, described below.

#### 2.4.2 | Latent Inflammatory Composite

In line with previous work, an empirically identified composite factor of inflammatory proteins was computed as a proxy measure of systemic inflammation (Moriarity et al. 2020). Following current practices, a log transformation ( $\text{Log}[10 \times \text{value}]$ ) was first applied to raw values of each of the five inflammatory proteins (IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , and CRP) to correct for skew, and to allow for their use in the development of a latent inflammation factor. This transformation resulted in acceptable skewness statistics, in line with assumptions of normality. An exploratory factor analysis (EFA) via parallel analysis was then conducted to determine the number of factors to retain. Parallel analysis was combined with the K1 rule (i.e., Kaiser-Guttman rule), which contends that retained factors should have eigenvalues greater than 1.0 (Kaiser 1960).

Confirmatory factor analysis (CFA) using the *lavaan* package was then used to estimate a latent factor representing systemic inflammation that was composed of each of the aforementioned inflammatory proteins. The model was estimated using maximum likelihood estimation with robust standard errors (MLR). Missing data were handled using full information maximum likelihood (FIML). Model fit was evaluated using the chi-square ( $\chi^2$ ) test, with a nonsignificant chi-square test indicating good model fit. We also used incremental fit indices, including the root mean square error of approximation (RMSEA), the standardized root mean square residual (SRMR), and the comparative fit index (CFI), which is consistent with recommendations put forth by Kline (2016). RMSEA and SRMR values of less than 0.08 and CFI values over 0.90 indicate acceptable model fit (Schermelleh-Engel et al. 2003). Following model estimation, the *predict* function was then used to extract estimated values for the latent factor for each participant.

Prior to conducting analyses, a series of variables known to influence levels of inflammatory proteins were tested as potential covariates for the inflammatory composite, including sex, age, racial identity, medication use, pubertal stage, body mass index (BMI), presence of a psychiatric diagnosis, and time of day the sample was collected (O'Connor et al. 2009). Taking a data-driven approach and based on best-practice recommendations, only significant covariates were retained in the final model by directly including them in the regression to maximize power and prioritize model parsimony.

#### 2.4.3 | Main Analyses

All analyses were conducted in R version 4.2.1. A priori power calculations based on a small effect size of  $f = 0.25$  indicated that 48 participants were required to reach a power of 0.80 (Faul et al. 2007). In line with prior work, diurnal cortisol dynamics were first analyzed using a multilevel piecewise model in which we modeled levels of cortisol at wake, the cortisol awakening response (awakening to 30-min post-awakening), and the daytime cortisol slope (30-min post-awakening to bedtime). By treating time as a Level-1 predictor within a nested structure, HLM slopes are able to incorporate multiple timepoints (e.g., in the case of the DCS, which incorporates three timepoints) (Jopling et al. 2025; King et al. 2017). Following best-practice recommendations, a multistage factor score regression was then used for composite analyses (Moriarity et al. 2021). We first extracted values on a latent factor representing an inflammatory composite (described above). Composite analyses then involved regressing inflammatory factor scores on youths' HPA axis indicators to examine their direct effects on inflammation. Three separate regression analyses were conducted, each including a single predictor (i.e., cumulative cortisol index, HS cortisol index, and pandemic cortisol index). Specificity analyses were then conducted using a series of bivariate correlation analyses examining associations between indices of cortisol regulation and both individual inflammatory proteins and the inflammatory composite.

### 3 | Results

#### 3.1 | Preliminary Analyses

##### 3.1.1 | Diurnal Cortisol Dynamics

Hierarchical linear models indicated that the expected pattern of diurnal cortisol was observed across the first 2 days of the high school transition: participants' level of cortisol was significantly different than zero at waking,  $t$ -ratio (45) = 15.30,  $p < 0.001$ , increased significantly from waking to 30-min post-waking,  $t$ -ratio (45) = 4.70,  $p < 0.001$ , and decreased significantly across the remainder of the day,  $t$ -ratio (45) =  $-15.02$ ,  $p < 0.001$ . During the COVID-19 pandemic, participants' level of cortisol was significantly different than zero at waking,  $t$ -ratio (48) = 18.03,  $p < 0.001$ , increased nonsignificantly from waking to 30-min post-waking,  $t$ -ratio (48) = 0.44,  $p = 0.665$ , and decreased significantly across the remainder of the day,  $t$ -ratio (48) =  $-13.03$ ,  $p < 0.001$ . When controlling for pubertal stage, cortisol AUCg did not differ between the high school transition and the COVID-19 pandemic,  $t = 0.002$ ,  $p = 0.998$ , though there was substantial interindividual

variability at each timepoint (high school transition  $SD = 1851.83$ , COVID-19 pandemic  $SD = 1396.51$ ).

### 3.1.2 | Covariate Analyses

More advanced pubertal status during the high school transition was associated with greater AUCg during the high school transition,  $r = 0.360$ ,  $p = 0.016$ . Similarly, pubertal status at the COVID-19 timepoint was associated with youths' AUCg at the COVID-19 timepoint,  $r = 0.294$ ,  $p = 0.045$ . As such, youth's AUCg was regressed on their pubertal status at the respective timepoint. Residuals resulting from these analyses were then summed and subsequently standardized for use in main analyses.

### 3.1.3 | Inflammatory Composite Development

**3.1.3.1 | EFA.** The parallel analysis indicated a single-factor solution, as only one factor had an eigenvalue greater in the original dataset than the corresponding eigenvalues from the generated datasets (see Figure S1 and Table S1). A one-factor solution was also consistent with the K1 rule. This inflammatory protein composite, composed of IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , and CRP, represents overall levels of systemic inflammation. EFA results can be found in the Supporting Information (Table S2).

**3.1.3.2 | CFA.** The empirically derived factor structure was replicated through CFA,  $\chi^2(5) = 13.86$ ,  $p = 0.017$ , RMSEA = 0.188, SRMR = 0.058, and CFI = 0.938.<sup>1</sup> In addition, protein loadings provided evidence of unidimensionality given that all standardized loadings exceeded 0.40 (see Table S2). We also considered the coefficient omega, which is an indicator of composite reliability under a one-factor measurement model. This analysis indicated that the proportion of variance in the five-protein composite score explained by the latent factor is 79% ( $\phi = 0.792$ ). None of the aforementioned potential covariates were associated with individual differences in the inflammatory composite,  $ps \geq 0.162$ .

## 3.2 | Main Analyses

### 3.2.1 | Cortisol and Inflammatory Composite

Three regression analyses were conducted to examine the association of the cumulative cortisol index, HS cortisol index, and pandemic cortisol index with the inflammatory composite. In contrast to expectations, youths' cumulative cortisol index was not associated with youths' levels of systemic inflammation ( $\beta = 0.15$ ,  $p = 0.324$ ). Instead, we observed specificity in the associations between the HPA axis and the immune system, such that the inflammatory composite was predicted by cortisol production during the COVID-19 pandemic ( $\beta = 0.295$ ,  $p = 0.040$ ), but not by cortisol levels during the high school transition ( $\beta = -0.011$ ,  $p = 0.942$ ). Specifically, greater cortisol levels during the pandemic were associated with elevated scores on the inflammatory composite. Associations are illustrated in Figure 1.<sup>2</sup> Full results are presented in Table S3.

To examine the association of HPA axis activity with specific inflammatory markers, we conducted a series of bivariate correla-

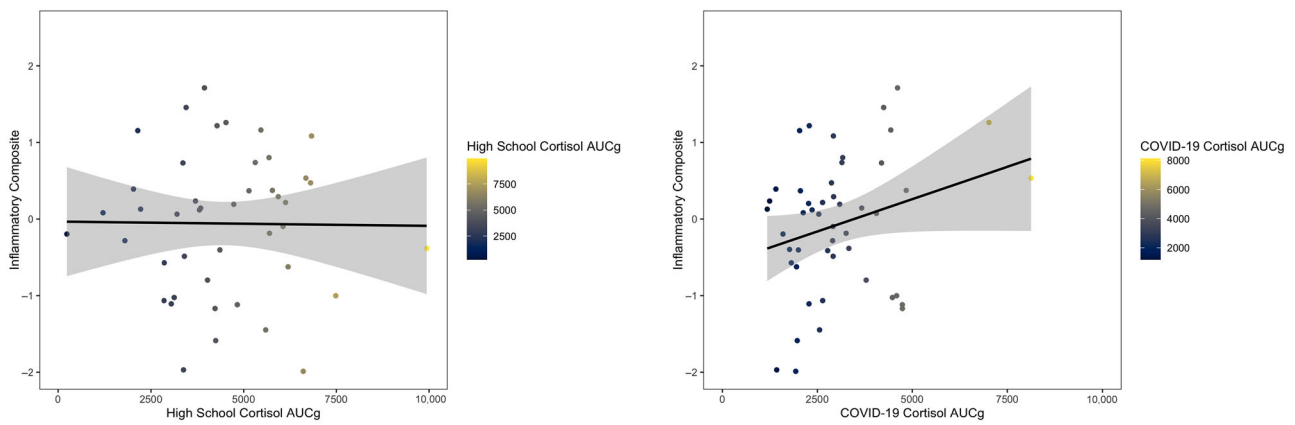
tion analyses examining associations between indices of cortisol and individual inflammatory proteins. Cortisol levels during the COVID-19 pandemic were positively associated with levels of IL-6 ( $r = 0.456$ ,  $p = 0.001$ ), TNF- $\alpha$  ( $r = 0.285$ ,  $p = 0.049$ ), and CRP ( $r = 0.304$ ,  $p = 0.038$ ) but not with levels of IL-1 $\beta$  or IL-8,  $ps \geq 0.151$ . In contrast, cortisol levels during the high school transition were not associated with inflammatory activity,  $ps \geq 0.066$ , and youth's total cortisol risk score across both timepoints was associated only with CRP ( $r = 0.448$ ,  $p = 0.003$ ; all others  $ps \geq 0.150$ ). Associations are illustrated in Figure 2, and a heat map correlation table between all study variables is presented in Figure 3.

## 4 | Discussion

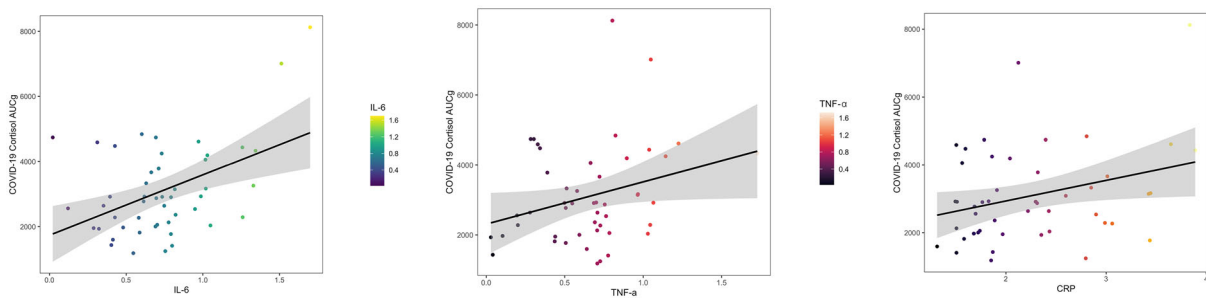
By providing evidence for specificity in associations between the HPA axis and immune system activity, the current work documents a pathway of biological embedding in adolescent youth. Specifically, analyses highlighted that elevated cortisol output across time was associated with higher levels of CRP, while higher cortisol output during the COVID-19 pandemic was associated with overall systemic inflammation, which may be driven by elevations in TNF- $\alpha$ , IL-6, and CRP. By focusing on individual differences in associations between HPA axis activity and inflammatory markers, this analytic approach allows us to assess the impact of meaningful individual differences in HPA axis activity rather than relying on aggregate changes over time.

While our results broadly suggest an association between HPA axis activity and elevated inflammation, it is interesting to consider the specific pattern of findings that highlighted the role of TNF- $\alpha$ , IL-6, and CRP. Our finding of an association between greater longitudinal cortisol output and CRP is consistent with previous work in community samples of adults documenting associations between increased cortisol production and both CRP and IL-6 (Herriot et al. 2017; Knight et al. 2021). We similarly found that higher IL-6 was associated with greater overall cortisol output during the COVID-19 pandemic. This corresponds with work demonstrating that dexamethasone has less of an inhibitory effect on the release of IL-6 among highly exhausted adult men, who also show higher levels of CRP (Wirtz et al. 2003).

It is interesting to consider why CRP and IL-6 appear to be particularly associated with cortisol dysregulation, both in the current study and the broader literature. IL-6, along with other markers, triggers the production of CRP in the liver (Cavailon and Adib-Conquy 2002). In this way, IL-6 and CRP are functionally linked, making a pairing between them biologically credible. Intriguingly, there is also evidence that increased IL-6 and CRP could reflect increased investment in immune readiness, highlighting the importance of considering the role of context in the observed associations (Del Giudice and Gangestad 2018). Inflammatory data were collected at the height of the first wave of the COVID-19 pandemic, a time of increased concern around illness and physical well-being given the uncertainty surrounding the virus. Considering these findings through an evolutionary lens, it is possible that this pattern of biological functioning—in which excessive cortisol over time is associated with increased inflammatory activity—could confer a survival advantage in contexts where threats such as conflict and predation exist (Miller et al. 2009). Indeed, this pattern of HPA axis responsivity supports



**FIGURE 1** | Association between cortisol AUCg and inflammatory composite.



**FIGURE 2** | Associations between COVID-19 cortisol AUCg and inflammatory markers.

the rapid mobilization of energy (via elevated HPA axis activity) as well as the ability to mount a powerful immune response to potential injuries, infections, and, in the case of the pandemic, illnesses. However, in the absence of real threats to personal safety and well-being, long-term biological modulations resulting in dysregulated cortisol and elevated inflammatory activity increase susceptibility to both physical and mental illness across the lifespan. This is particularly true in the context of adolescence, a developmental window for biological recalibration when an individual is particularly vulnerable to the biological embedding of stress (Choudhury et al. 2017).

Our findings also suggest that the relationship between cortisol and inflammation may not reflect a stable, trait-like pattern of glucocorticoid resistance but instead one that fluctuates over time, potentially in response to dynamic biological and/or contextual factors. The fact that cortisol output was not associated with indices of inflammation during the high school pandemic but was associated with multiple individual markers during the COVID-19 pandemic raises the possibility that glucocorticoid resistance may emerge under specific conditions, rather than being permanently embedded in an individual's physiology. Future work should investigate the temporal dynamics of glucocorticoid resistance, identifying factors that modulate its emergence and persistence, to clarify whether it represents a stable biological signature or a context-dependent process.

Given the powerful role of context, it is also interesting to consider why we observed a nonsignificant increase in cortisol from waking to 30-min post-waking during the COVID-19 pandemic. First, the pandemic was experienced by many as a chronic

stressor, which has the potential to result in a blunted CAR (Chida and Steptoe 2009); this may have contributed to a lower response to awakening in the context of the COVID-19 pandemic. Further, changes in sleep patterns may also have played a role in attenuating the cortisol response to awakening. The pandemic led to well-documented altered sleep-wake schedules (e.g., later bedtimes, irregular sleep), which have been shown to result in a blunting of the cortisol awakening response (Backhaus et al. 2004).

Results therefore have implications for health across the lifespan given that elevated levels of the inflammatory markers that emerged as important in addition to systemic inflammation in the current study (i.e., TNF- $\alpha$ , IL-6, and CRP) are linked by both epidemiological and meta-analytic evidence with mortality outcomes related to multiple disease states (Schnabel et al. 2013). Intriguingly, there is also meta-analytic evidence demonstrating that TNF- $\alpha$ , IL-6, and CRP (the proteins identified in the present study as being associated with elevated cortisol output during the pandemic) are the three inflammatory markers most consistently associated with childhood adversity (Baumeister et al. 2016). For some individuals, the pandemic was experienced as a stressful event that was unpredictable, extreme, prolonged, and involved an unknown danger, consistent with recent conceptualizations of adversity and trauma (Griffin 2020).

These findings should be interpreted in the context of several limitations. First, we did not assess inflammatory markers during the high school transition, as inflammatory proteins were only assessed in the context of the COVID-19 pandemic. Given evidence for synergistic associations between the HPA axis and

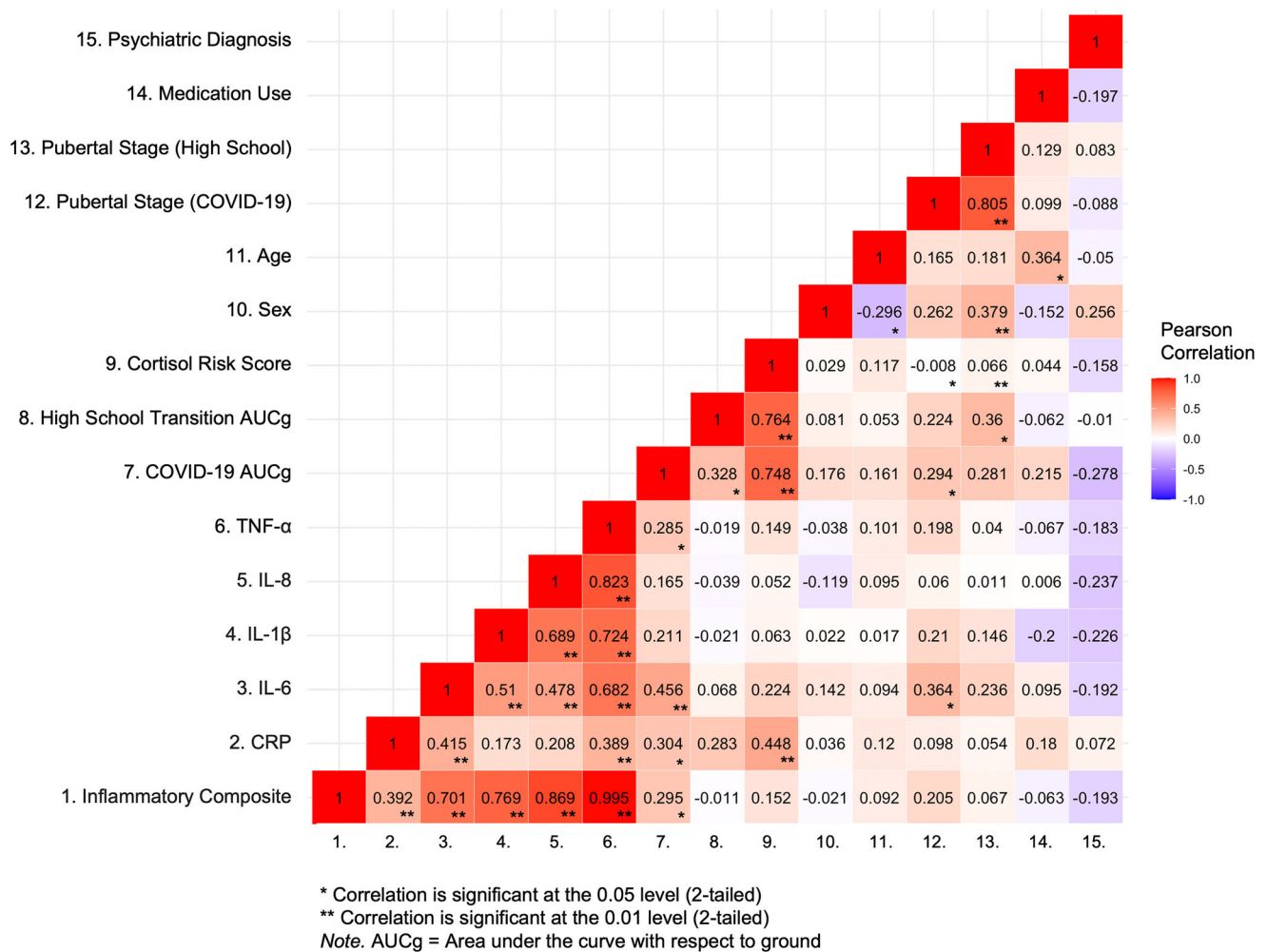


FIGURE 3 | Heat map with zero-order correlations between study variables.

immune system functioning, it will be important for future work to consider longitudinal bidirectional associations of cortisol and inflammatory proteins (Webster et al. 2002). Next, most youth in the present sample identified as White, and many participant households exceeded the median Canadian household income. Therefore, replication of this study in more generalizable samples with greater racial and socioeconomic diversity is important. Further, although our sample size exceeded the recommended sample based on a power analysis, larger samples offer multiple advantages, including more closely replicating the demographics of the population. A larger sample size would also enhance the precision and reliability of distinguishing among varying magnitudes of associations between HPA axis indices and specific inflammatory markers. We also did not assess self-reported stress or distress on the cortisol sampling days during both timepoints, which prevents us from directly examining individual variability in perceived stress during the measurement period. Future research should consider incorporating subjective stress ratings to develop a more comprehensive understanding of the observed associations between cortisol output and inflammatory markers. Further, we did not assess sleep during the high school transition, though we did assess the impact of sleep duration on cortisol levels during the COVID-19 pandemic, which revealed an insignificant association,  $r = -0.227$ ,  $p = 0.117$ . However,

given that sleep and HPA functioning have the potential to be related, this is a limitation of the present work. Finally, this study examined associations between a dynamic measure of cortisol production and tonic measures of systemic inflammation. It will be interesting for future work to further extend the field by examining associations between dynamic diurnal-based measures of both cortisol and inflammation.

## 5 | Conclusions

In summary, we found evidence for specificity in the associations between longitudinal cortisol output and inflammatory markers in a sample of adolescent youth. By elucidating these nuanced associations, results highlight HPA axis dysregulation as a possible pathway by which life stress alters immune system functioning in youth during times of challenge. By documenting associations in which a typically immunosuppressive hormone was associated with elevated levels of inflammatory markers in youth, these findings contribute to the literature on allostatic load and the glucocorticoid-resistance model in youth. In addition, results highlight the importance of taking a multi-biomarker approach to modeling biological system functioning. If replicated, findings could have implications for the development of interven-

tions aimed at restoring the typical anti-inflammatory properties of cortisol, which could in turn have lifelong implications for health and well-being.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Endnotes

<sup>1</sup>The empirically derived factor structure was also compared to a tau-equivalent factor structure, with all inflammatory proteins loading equally onto a single dimension. AIC and BIC statistics both supported the improved fit of the empirically derived factor structure (AIC = 155.34, BIC = 184.02) over the tau-equivalent model (AIC = 218.77, BIC = 237.89), further supported by a significant chi-square difference test,  $\chi^2$  difference (5) = 50.09,  $p < 0.001$ .

<sup>2</sup>Following the calculation of AUCg, one participant was identified as being an outlier during the high school transition, and one participant was identified as being an outlier during the COVID-19 pandemic. The association between the high school transition AUCg and inflammation was nonsignificant both with and without the inclusion of the high school transition outlier ( $ps > 0.923$ ); similarly, the association between COVID-19 AUCg and the inflammatory composite remained significant after excluding the COVID-19 pandemic outlier,  $\beta = 0.299$ ,  $p = 0.039$ .

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.

**Table S1.** Parallel Analysis Results **Table S2.** Protein Loadings **Table S3.** Results of Regression Models **Figure S1.** Parallel Analysis Scree Plots