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Low-Grade Inflammation and Ambulatory Cortisol in Adolescents: Interaction between Interviewer-rated versus Self-rated Acute Stress and Chronic Stress

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Abstract

Objective—To determine whether the association between self-rated or interviewer-rated recent acute stress exposures and low-grade inflammation and daily cortisol production in adolescents is moderated by chronic stress ratings.

Methods—Acute and chronic stress exposures were assessed in 261 adolescents aged 13–16 using a semi-structured life stress interview. The negative impact of acute stressors was independently rated by both adolescents (self-rated) and interviewers (interviewer-rated). Markers of inflammation (IL-6, IL-1ra, CRP) were measured from peripheral blood samples obtained via antecubital venipuncture. Participants collected 4 saliva samples at home on each of six consecutive days for the analysis of diurnal salivary cortisol profiles.

Results—There were no main effects of acute stressors (self- and interviewer-rated) and chronic family or peer stress on adolescent inflammation markers and cortisol ($p > .10$). However, the interaction between interviewer-rated acute stress and chronic family stress was significantly associated with adolescent inflammation markers (IL-6, IL-1ra). Specifically, as chronic family stress increased, the association between acute stressor impact (interviewer-rated) and inflammation markers became more positive (IL-6 ($B = .054$, $SE = .023$, $p = .022$); IL-1ra ($B = .030$, $SE = .014$, $p = .034$)). Interactions between self-rated acute stress and chronic family stress were not associated with any biological measures ($p > .10$). Interactions between acute stressor impact (both self- and interviewer-rated) and chronic peer stress were also not significantly associated with any biological measures ($p > .05$).

Conclusions—Among adolescents, interviewer-based ratings of acute stressor impact may allow for better prediction of health-relevant inflammation markers than adolescents' own ratings.

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Keywords

assessment of stress; inflammation; cortisol; adolescents

INTRODUCTION

Psychological stress has been associated with physiological outcomes relevant to health, such as inflammation markers (Interleukin-6, Interleukin-1 β , C-reactive protein; IL-6, IL-1 β , CRP) and markers reflecting hypothalamic-pituitary-adrenal (HPA) axis activity (e.g., cortisol) (1-6). Acute laboratory-based stressors, i.e. stressors that typically last anywhere from minutes to hours, with a clear onset and offset, such as public speaking and arithmetic tasks, influence HPA axis reactivity and inflammation responses in adults and adolescents (7-9). However, findings linking exposure to acute stressors in naturalistic (rather than laboratory) settings with basal levels of inflammation markers are more mixed, raising a question of how best to assess naturalistic acute stressors in children and adolescents.

Acute naturalistically occurring stressors refer to life events such as school examinations or a short-lived disagreement with a friend. Evidence suggests that the impact of acute stressors is partially contingent on one's levels of chronic stress, i.e. stressful circumstances that continue for a prolonged period of time, often months to years, with no clear ending in sight (e.g., conflictual family relationships). The reserve capacity model (10) suggests that exposure to chronic stress can lead to the depletion of an individual's psychosocial resources, while simultaneously limiting opportunities for the development of resource reserves. In other words, the negative impact of acute stressors may be more marked in the presence of chronic stress.

This is supported by studies investigating interaction effects of acute and chronic stress on health outcomes among children and adolescents. Among youth with asthma, only the combined presence of acute and chronic stress has been found to be detrimental with respect to asthma-related symptoms and inflammation outcomes (11, 12). Other studies focusing on children and adolescents also found no main effects of acute stressors on glucocorticoid receptor mRNA (13) and on incidence of respiratory illness (14) but found interaction effects of acute and chronic stress, such that the physiological consequences of acute stress exposure were moderated by simultaneous exposure to chronic stress. Miller and Chen (13) reported changes in gene expression only in the presence of acute *and* chronic stress; Boyce et al. (14) found that children exposed to more acute stressful life events in the presence of greater chronic stress experienced higher illness rates, whereas acute stressors had seemingly protective effects among children simultaneously exposed to lower levels of chronic stress. Finally, the interaction between acute and chronic stress has also been linked to increased cortisol production and decreased expression of glucocorticoid receptor mRNA among healthy, female adolescents (15). Taken together, these studies suggest that high levels of chronic stress may 'prime' the immune system to respond more strongly to acute stressors.

One question that arises in this literature, however, is how best to assess the impact of an acute event, particularly among children and adolescents. Several assessment tools for life stress are available (16-18). These tools rely either on participant reports of the impact of a

stressful acute event on their lives or on interviewers to provide more standardized ratings of how an acute event has impacted a participant. Perceptions of stress appraisal are inherently subjective, and several studies link perceived stress to inflammation and HPA axis activity in adolescents (6, 19-21) as well as to decreased adolescent well-being in the form of greater emotional distress (22) and lower antibody titers in response to vaccination (23). These patterns suggest that using participants' ratings of life event impact will have predictive utility for health-related outcomes.

However, other research suggests that stressor ratings made by interviewers may be more accurate, as individuals' current psychological states can influence how they report and rate events (24, 25). Participants' histories may also lead to different types of reporting. People who have experienced many negative life events in the past may have developed a different tolerance to events compared to individuals who only rarely experience stressors and hence have differential reporting of event impact. Ratings made by interviewers may also be better able to take into account factors such as the 'normativeness' of an event (e.g., the transition to high school may be reported by an adolescent as stressful, but is also very normative), the long-term consequences, and the contextual factors, i.e. the background factors that can exacerbate or mitigate the impact of an event (26).

Very few studies have compared the effect of self-report vs. interviewer impact ratings of stressful events on outcomes, and almost all focus on adults and psychiatric outcomes. Wagner (25) compared the number of events that children and their parents reported on life event checklists to interviewer-rated events. Although they found the number of events reported by participants and interviewers to be highly correlated, they also found that anxious parents (but not anxious children) tended to report greater event severity. Furthermore, a review by Gorman (27) summarized 12 studies (all in adults) and found overall agreement between respondent-based and investigator-based stress ratings highly varied. A report by McQuaid et al. (28) focused on adults with recurrent major depression further underscores differences between self and interviewer ratings. Among individuals receiving treatment for recurrent major depression, interviewer ratings of life stress significantly predicted future treatment outcome whereas self-report ratings of life stress either did not predict at all or predicted in the opposite direction.

Adolescence is a period marked by the interactive development of neurological, cognitive, and behavioral processes, suggesting that adolescence may be a sensitive period during which exposure to stressful life events may have a particularly large impact and long-lasting implications for future health (29-31). Hence, studying differences between self- and interviewer-rated stress impact among adolescents may be particularly important, as adolescents differ from both children and adults in their processing of the emotional value of stimuli (32); experience increased negative and decreased positive affect (33, 34); and provide reports of their behaviors and psychological problems that are frequently discrepant from reports of other informants, e.g. parents, teachers, and researchers (35). Consequently, adolescent self-reports of event impacts may be divergent from interviewer ratings, raising the question of whether adolescents' perceptions of stressor impact or interviewer ratings of the same more strongly predict adolescent physiological outcomes.

Of the few studies that have set out to make comparisons across informants, none, to our knowledge, have investigated differential effects of self- and interviewer-rated stress on physiological outcomes in adolescents. This study focuses on chronic and acute stress interactions studied in previous research and their relationship to inflammation markers and neuroendocrine activity. Markers of low-grade inflammation (CRP, IL-6, and IL-1ra) have been linked to depression and the early stages of atherosclerosis and diabetes among young adults and adolescents (36, 37). In addition, HPA axis functioning (diurnal, ambulatory salivary cortisol) and disrupted diurnal cortisol patterns have been linked to outcomes including depression, obesity and cancer among adolescents and adults (38-42). As mentioned above, adolescence represents an important developmental period and acute and chronic stress exposures, e.g. on inflammation and HPA axis markers, during this time likely have important implications for individuals' later life psychological as well as physical well-being (43-45). The primary goal of the study was to determine whether self- or interviewer-rated acute stress impact, in combination with chronic stress ratings, is more predictive of chronic, low-grade inflammation and daily cortisol production. We focused on assessing these associations among adolescents because research suggests that discrepancies between self- and interviewer-rated stress impact ratings may be especially large in this age group; because these discrepancies are particularly understudied among adolescents; and because it is important to understand psychosocial influences on physiological outcomes early on as they may represent important contributors to longer-term health. Given that the existing literature reviewed above provides arguments to support both the notion that the interaction between chronic stress and adolescents' self-rated acute stress impact is more strongly associated with adolescent low-grade inflammation and diurnal cortisol than the interaction between chronic stress and interviewer-rated acute stress impact and vice versa, we set out to perform these analyses in an exploratory fashion without a specific directional hypothesis.

Methods

Participants

Participants were 261 adolescents between the ages of 13-16 (14.53 ± 1.07 ; 46.7% male) who were recruited from the larger Vancouver, BC area through advertisements in local media between January 2010 and March 2012. All participants were healthy and fluent in English. Participants with chronic illnesses were not eligible for participation. Interested participants were screened over the telephone and eligible adolescents were scheduled for a late afternoon (after school) visit to the laboratory. In case of acute illness, participants were rescheduled for 4 weeks after the end of symptoms. Participants came from a range of ethnic and socioeconomic backgrounds; see Table 1.

Procedure

Adolescents were briefed about study procedures and provided written assent (parents provided written consent). Next, they were paired up with a trained research assistant who measured their weight and height, conducted the Life Stress Interview and asked questions about demographic information. Participants underwent a peripheral blood draw through antecubital venipuncture, performed by a trained phlebotomist, and were instructed to collect saliva samples at home over six days. Participants were reimbursed for their time and

effort. This study was approved by the Research Ethics Board of the University of British Columbia.

Measures

Life stress interview—We used the University of California Los Angeles Life Stress Interview, Adolescent Version (46), to assess chronic and acute life stress. As part of this semi-structured interview, trained research assistants asked participants about chronic stressors in four domains, including family, peers, school, and home life (which focused on structural aspects of family life, such as finances, parents' work, etc.) over the previous six months. The present study focuses on interactions of acute stressors with chronic stress in the family and peer domains, consistent with previous studies which find that stress in these domains is particularly potent among youth. For example, family chronic stress has been shown to be more strongly related to physiological outcomes than stress in other domains (13, 47) (48). Relationships with peers are also known to be an important aspect of adolescents' lives (49). The chronic family stress rating reflects relational aspects of family life, including levels of closeness, trust, and conflict in the family. The chronic peer stress rating reflects the quality of relationships with peers, closeness to and trust in friends, conflict with friends, and overall social connectedness. Both domains of chronic stress were rated by the interviewer on a 1-5 scale, 1 representing low (e.g., exceptional quality of relationship with all family members) and 5 representing high (e.g., poor quality relationship with family, pervasive problems across family members) levels of chronic stress over the past 6 months. The validity and reliability of this interview have been previously shown (50-52). Our research team has been conducting this interview for the past 8 years, with inter-rater reliabilities ranging from .88-.94 across subscales.

In addition to chronic stress, adolescents were asked to report any acute stressful events they had experienced over the previous 6 months, for example having a close friend move away. Participants were asked to rate the negative impact that each reported acute event had on them at the time it occurred (ranging from 1 = no negative impact to 5 = severe negative impact), providing a self-report rating of event impact. Separately, another impact rating was made by a team of interviewers. Each interviewer presented details of acute events to the team (without mention of the participant's rating), and the team discussed and came to consensus about the negative impact of the event on the same 1-5 scale. These interviewer ratings took into account the context in which events occurred. For example, if a participant failed a class at school, their likelihood of being able to repeat and pass the class, and implications for their social life and future school/career plans were taken into account. Because participants could have experienced more than one event during the interview period, we selected the most severe event for each participant for the analyses below (highest rated event by the interview team). This follows the approach of earlier studies that have focused on severe events (11, 53) and avoids possible problems with participants' differential reporting of minor, less severe events, which could bias sum or average scores derived from all reported events (for example, some participants may be more likely to report many minor events, thereby lowering their sum or average scores, when compared to other participants who may be experiencing the same types of minor events but who focus

on reporting primarily larger events). Participants who reported no acute events over the past 6 months received scores of '0' for both acute stress impact ratings.

Inflammation markers—Participants' peripheral blood was drawn into serum separator tubes (Becton-Dickinson, Franklin Lakes, NJ) and three measures of low-grade inflammation, CRP, IL-6, and IL-1ra, were assessed. Between 60-120 minutes after the blood draw, SST tubes were spun for 10 minutes at 1200 rpm and blood serum was aliquoted and stored at -30°C until further analysis (within 12 months of sample collection). Serum IL-6 was measured using a high-sensitivity ELISA kit (R&D Systems, Minneapolis, MN; intra-assay CV $< 10\%$; detection threshold = .04 pg/mL). CRP assays were conducted using a high-sensitivity, chemiluminescent technique (inter-assay CVs = 2.2%; detection threshold = .20 mg/L). IL-1ra was measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN; intra-assay CV $< 10\%$; detection threshold = 18.3 pg/mL).

Cortisol—Adolescents were instructed to collect saliva samples at home for 6 days following their lab visit. Specifically, they were asked to collect 4 samples on each day, 1, 4, 9, and 11 hours following wake-up, allowing us to capture the diurnal variation in cortisol output (54). Participants were instructed to not eat, drink, or brush their teeth 15 minutes prior to sample collection. Samples were collected by participants placing a sterile cotton dental roll (Salivette, Sarstedt Corp.; Nümbrecht, Germany) in their mouth for 60 seconds. Cotton rolls were then placed in a plastic tube, refrigerated, and at the end of the 6 days, participants returned samples to the lab in a pre-paid envelope. Returned saliva samples were spun at 750g for 5 minutes and saliva samples stored in deep-well plates at -30°C until shipment (on dry ice) for analysis to the laboratory of Drs. Jutta Wolf and Nicolas Rohleder at Brandeis University. Salivary free cortisol concentrations were measured using commercial chemiluminescence immunoassays (CLIA; IBL-International, Toronto, Canada). Intra- and inter-assay CVs were $< 10\%$. Cortisol data were unavailable for 17 adolescents who did not return usable samples. These adolescents did not differ from participants who returned usable samples with respect to age, BMI, chronic and acute stress ratings, ethnicity, and family income (p s $> .10$) but were more likely to be female ($\chi^2(1) = 6.184, p = .013$). On average, adolescents completed $5.47 (\pm 1.03)$ out of the 6 days. To monitor compliance participants were asked to time-stamp salivette labels at the time of collection using a provided stamper (DYMO Datemark) whose time-date function was password protected and could not be changed by participants. Compliance with our schedule was very good; based on stamped times adolescents collected their samples $1.14 (\pm .81)$, $4.38 (\pm 1.28)$, $9.24 (\pm 1.21)$ and $11.50 (\pm 1.27)$ hours following waking.

Covariates—Participants reported their age, sex, and ethnicity. Body mass index (BMI) was computed as kg/m^2 based on height and weight measured at the lab without shoes and outerwear. Total gross family income over the past 12 months was reported by participants' parents using a 9-point scale ranging from 1 (less than \$5,000) to 9 (\$200,000 or more).

Analyses

Levels of inflammation markers and cortisol were not distributed normally and log-transformed to reduce skewness. Two indices of HPA axis activity were computed. First,

total daily cortisol output was computed as the area under the curve (AUC) using the trapezoidal rule (55). For each day and each participant, a line depicting cortisol values at each of the collection times was plotted and the AUC computed as the sum of the three trapezoids below that line. AUCs of all available days were averaged for each participant to provide a more robust estimate of typical daily cortisol output. Higher numbers indicate greater daily total cortisol output. Second, for an index of diurnal cortisol variation, cortisol values were averaged across all available days to increase stability and the slope of the regression line (cortisol values/corresponding time since wake-up) computed. Steeper slopes suggest more rapidly declining cortisol over the course of the day, flatter slopes a slower decline. Cortisol slope represents a commonly used indicator of diurnal cortisol variation which has previously been shown to be influenced by psychosocial stress (56-58).

All analyses adjusted for participant age, sex, ethnicity, and family income. In addition, analyses examining inflammation markers also included BMI as a covariate and analyses examining cortisol levels also included number of completed days of saliva samples. Finally, analyses with cortisol AUC also included adolescents' average self-reported time of waking. Although age was controlled for in all analyses, the main analyses were rerun with puberty added as an additional covariate to see whether this changed the pattern of our findings. First, multiple linear regression analyses were performed to assess the independent main effects of acute stressors (self- and interviewer-rated) and chronic family and peer stress. Second, hierarchical multiple regression analyses were used to investigate the two-way interaction effects of acute stressors (self- or interviewer-rated) and chronic family stress as well as the two-way interaction effects of acute stressors (self- or interviewer-rated) and chronic peers stress on adolescent inflammation markers and cortisol profiles. Covariates and predictor variables were centered at zero, and interaction terms computed by multiplication of centered scores, as recommended by Aiken and West (59). Acute (either self- or interviewer-rated) and chronic stress were entered as main effects in the first step, and the acute x chronic stress interaction term in the second step. Significant interactions were subsequently probed for regions of significance, as recommended by Preacher et al. (60). This technique involves the computation of values of the moderator variable (here, chronic stress) at which the simple slope of the predictor (acute stress) is significantly associated with the outcome (inflammation). Consequently, the resulting upper and lower bounds indicate the values of chronic stress beyond which (that is, above and below which) the effect of acute stress on inflammation outcomes is significant. All analyses were performed using IBM SPSS Statistics version 20.0 (IBM, New York, NY).

Results

Acute (Self- or Interviewer-rated) and chronic stress ratings

One hundred and fifty-three (58.6%) adolescents reported at least one acute stressful event during the past 6 months. Among these adolescents, the correlation between their own acute stressor impact ratings and interviewer ratings of the same events was moderate, $r = .413$. The severity of acute stressful live events was also moderately correlated with chronic family stress, such that adolescents who experienced more chronic family stress were also more likely to experience a more severe acute stressor ($r = .318$ for self-rated acute stress; r

= .327 for interviewer-rated acute stress). See Table S1, Supplemental Digital Content 1, for unadjusted correlations between main study variables.

Main Effects of Acute and Chronic Stress

We assessed the independent main effects of acute stressor impact (self- and interviewer-rated) and chronic (family and peer) stress on adolescent inflammation markers and cortisol.

Acute stress—There were no main effects of adolescents' self-ratings of acute stressor impact (all p s > .30) or interviewer-rated acute stressor impact (p s > .20) on any biological measures.

Chronic family stress—There were no main effects of chronic family stress (p s > .20), on any biological measures.

Chronic peer stress—There were no main effects of chronic peer stress (p s > .10) on any biological measures.

Interaction Effects of Acute Stress (Self- or Interviewer-rated) and Chronic Stress

Next, we assessed whether simultaneous exposure to acute and chronic stress (in the family or peer domain) was associated with adolescent inflammation markers and cortisol, and whether these biological measures were differentially predicted by self- or interviewer-rated acute stressor impact¹.

Interviewer-rated acute stress x chronic family stress—When considering the interaction between interviewer-rated acute stressor impact and chronic family stress, there was a significant effect of the acute x chronic stress interaction on IL-6 ($B = .054$, $SE = .023$, $p = .022$) and IL-1ra ($B = .030$, $SE = .014$, $p = .034$). See Figure 1. Specifically, for the analysis predicting IL-6, the region of significance on chronic stress ranged from -2.88 to 0.45 , suggesting that simple slopes outside this range were significant. As our centered chronic stress variable ranged from -1.10 to 1.90 , this suggests that greater levels of acute stress only resulted in significantly greater IL-6 production in conjunction with exposure to higher levels of chronic background stress. When probing our significant interaction effect on IL-1ra, we found that, notably, the moderating effects of chronic family stress were in opposite directions at low and high levels of chronic stress. At low levels of chronic stress, greater acute stress exposure was associated with marginally lower levels of IL-1ra, whereas at high levels of chronic stress, greater acute stress was associated with marginally greater levels of IL-1ra (region of significance: -0.66 to 1.20). This may be indicative of a stress inoculation effect, suggesting that low levels of stress (such as exposure to an acute stressor in the absence of chronic background stress) may be beneficial with respect to inflammation outcomes. There was no significant effect of the acute x chronic family stress interaction on adolescents' levels of CRP, cortisol slope, or cortisol AUC (p s > .05). See Table 2.

¹Due to the moderate correlations between acute and chronic stressor ratings, multicollinearity diagnostics were run for all models. Review of tolerance and variance inflation factor statistics and variance proportions revealed no evidence of multicollinearity.

Self-rated acute stress x chronic family stress—There was no significant effect of the acute (self-rated) x chronic stress interaction on adolescent CRP, IL-6, IL-1ra, cortisol slope, or cortisol AUC ($ps > .05$). See Table 2.

Interviewer-rated acute stress x chronic peer stress—There was no significant effect of the acute x chronic peer stress interaction on adolescents' levels of CRP, IL-6, IL-1ra, cortisol slope, or cortisol AUC ($ps > .05$).

Self-rated acute stress x chronic peer stress—There were no significant interactions between self-rated acute stressor impact and chronic peer stress on any biological measure ($ps > .10$).

Effect of Puberty

When additionally controlling for puberty, main effects of acute (self- and interviewer-rated) and chronic (family and peer) stress remained nonsignificant (all $ps > .20$). Interactions between interviewer-rated acute stressor impact and chronic family stress continued to significantly predict adolescent IL-6 ($B = .056$, $SE = .024$, $p = .021$) and IL-1ra ($B = .030$, $SE = .014$, $p = .041$). The interaction effect between self-rated acute stressor impact and chronic family stress significantly predicted ($B = -.003$, $SE = .001$, $p = .046$) cortisol slope. All other interactions remained non-significant ($ps > .05$).

Discussion

To our knowledge, this is the first study to compare how self- and interviewer-rated acute stress impact ratings predict inflammation markers and cortisol levels among adolescents. Consistent with previous literature, we found that the interaction between acute and chronic stress predicted inflammation markers (12), even in the absence of main effects of chronic and acute stress, highlighting the need to consider synergistic effects of acute and chronic stress exposures. Other studies have previously reported interaction effects of acute and chronic stressors on physiological health outcomes in the absence of main effects (12, 15) or markedly weaker main effects (13, 14), perhaps because the effect of acute stressors depends in large parts on simultaneously existing levels of chronic stress. This has direct relevance, for example, to allostatic load theory which suggests that repeated and/or ongoing exposure to psychological stressors results in the overactivation or dysregulation of important physiological systems, including inflammation processes, thereby raising the risk of future ill health (61). When considering self- vs. interviewer-ratings of acute stressor impact, we found that the interaction between interviewer-rated acute stress and chronic family stress was a more robust predictor of markers of low-grade inflammation. The interviewer-rated acute stress x chronic family stress interaction significantly predicted adolescent levels of IL-6 and IL-1ra. Specifically, we found that as chronic family stress increased, the association between acute stressor impact (interviewer-rated) and inflammation markers became more positive. Conversely, the self-rated acute stress x chronic family stress interaction and interactions between either acute stress rating and chronic peer stress did not significantly predict any biological measures.

These results suggest that interviewer assessments of the impact of acute stressful life events are stronger predictors of adolescent low-grade inflammation (in combination with chronic family stress ratings) than adolescents' own evaluation of the impact of the very same stressful life events, paralleling findings from similar studies that have focused on psychological outcomes in adults (27). Several reasons may explain these findings. First, adolescence has been associated with an increased experience of both negative and positive affect (33, 34) as well as overall heightened emotional reactivity (62, 63). In other words, adolescents experience stronger fluctuations in mood on a moment to moment basis. As self-ratings of stressful life events have previously been shown to be influenced by current mood states (24, 25), it is possible that interviewer ratings are more predictive of adolescent low-grade inflammation because they use a standardized approach to quantifying impact across all participants, whereas adolescents' own ratings may be more reflective of their current mood state at the time of the interview (and hence somewhat less reliable and stable indicators of the event itself). Second, and relatedly, adolescents may give disproportional weight to particular, e.g. social, aspects of past events. Interviewer-based ratings, in contrast, are presumably able to more objectively judge impacts across a variety of domain (e.g., social, financial, academic). Third, interviewers make ratings that are standardized across participants (i.e., events must have certain objective qualities to be rated above a certain impact level), and these more standardized impact ratings may be more predictive of low-grade inflammation than individuals' own perceptions of impact.

Our results further emphasize the importance of ongoing family stress in the lives of adolescents. In this study, interactions between interviewer-rated acute stress impact and chronic family stress, but not chronic peer stress, were associated with adolescent low-grade inflammation. This is in line with previous research emphasizing the importance of chronic family stress with respect to adolescent inflammation markers (12, 48). Although the adolescent years are marked by an increasing importance of and focus on peer relationships in addition to family relationships (49), adolescents continue to rely on relationships with family members and are negatively impacted by family stressors, even as they begin to build a more complex social network outside of their family home. It is possible, however, that chronic peer stress influences other relevant outcomes, such as adolescent psychological well-being.

Our results depict a crossover interaction, especially for IL-1ra, such that, at lower levels of chronic background stress, the association between acute event impact and low-grade inflammation becomes more negative, and, at higher levels of chronic background stress, becomes more positive. Although this may seem counterintuitive, some research suggests that the experience of acute stressors may be more challenging to those who typically are not used to dealing with stress in their lives (64, 65). In monkeys, stress inoculation research demonstrates that exposure to brief stressors is beneficial to later arousal regulation and resilience (66, 67). Moderate amounts of adversity may aid in the development of resilience because exposure to some adversity followed by a period of recovery may provide individuals with the opportunity to learn to better manage adverse situations and in the process enhance their ability to deal with future stressors. Conversely, individuals experiencing very low levels of stress may find themselves ill-equipped to deal with acutely stressful situations when they do arise, as they have not had the same opportunities to

develop appropriate responses to such situations. This is supported by studies reporting opposite effects of acute stress exposure in the context of low compared to high chronic stress among children and adolescents (14, 15).

The present study has important strengths. First, we were able to collect participant- and interviewer-based stress impact ratings in reference to the same stressful life events, allowing for a valid comparison of such ratings. Many studies comparing participant-based and interviewer-based stress impact ratings to date suffer from the use of multiple instruments, making comparisons of the self- and interviewer-ratings difficult as they may in fact also reflect different events (28). Second, we were able to extend the previous literature in this area on psychological outcomes to physiological measures obtained via blood and saliva. Third, we were able to do so among a sample of adolescents, an age group that is less commonly the focus of psychoneuroimmunology research.

Nonetheless, some limitations of our study include the lack of participant-derived ratings of chronic stress (these were not collected because the original interview was designed to collect participant ratings of only acute events). Similarly, it would be of interest to conduct analyses differentiating between interpersonal and noninterpersonal stressors or to investigate whether discrepancies between self- and interviewer-based ratings predict adolescent low-grade inflammation. Future research should also investigate the role of adolescents' mental health and the possibility that adolescents' own impact ratings of acute stress are less predictive (together with chronic stress) of low-grade inflammation because adolescents more heavily emphasize certain aspects of events, e.g. their social components, as previous research has shown that adolescent emotion regulation is particularly challenged by negative social, rather than non-social, stimuli (68). The current study also does not compare the effects of self- and interviewer-based acute stress impact ratings on longer-term trajectories of low-grade inflammation or on clinical health outcomes and clinically used cut-off points (e.g. for CRP), and the cross-sectional nature of this study precludes inferences about causality. Because our study focused on 13-16 year olds, these associations should also be further studied among youth of different ages. It is currently unknown how these adolescent patterns would compare to those of younger children or of adults. Finally, we note that the reliability of these findings needs to be established through future replication. Given the exploratory nature of our analyses and the number of effects examined, our results should be interpreted with caution until examined further.

Conclusions

The present results suggest that interviewer-based impact ratings of acute stressful life events to which participants are exposed may be more predictive of markers of low-grade inflammation in adolescents than adolescents' own impact ratings. This may be particularly true for studies involving adolescents because their emotion regulation abilities are still developing and adolescents' own impact ratings of acute stressors consequently may be especially discrepant from interviewer-based ratings. Our study also draws further attention to the importance of considering acute stress in the context of ongoing chronic stress, particularly chronic family stress. In contrast, interactions with chronic peer stress were not associated with adolescent low-grade inflammation, highlighting the continued importance

of family relationships during the adolescent period for biological markers relevant to health. Researchers interested in assessing the influence of stress exposures on markers of low-grade inflammation in youth should consider obtaining interviewer-rated assessments of the impact of stressful life events that their participants experience.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Acronyms used in text

AUC	area under the curve
BMI	body mass index
CRP	C-reactive protein
HPA	hypothalamic-pituitary-adrenal
IL	Interleukin

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Panel a)

Panel b)

Figure 1. Interactions between Interviewer-based Ratings of Acute Stress and Chronic Stress Predicting Levels of IL-6 and IL-1ra among Adolescents

Interactions between interviewer-rated acute stress ratings and chronic family stress significantly predict adolescent levels of IL-6 [$B = .054$, $SE = .023$, $p = .022$; Panel a)] and levels of IL-1ra [$B = .030$, $SE = .014$, $p = .034$; Panel b)]. Chronic and acute stress are depicted at $\pm 1SD$.

Table 1**Participant Characteristics**

N = 261

	M ($\pm$ SD)	n (%)
Male		122 (46.7)
Female		139 (53.3)
Ethnicity		
European		129 (49.4)
Asian		94 (36.0)
Other		38 (14.6)
BMI	21.37 $\pm$ 3.70	
Age (years)	14.53 $\pm$ 1.07	
Total family income		
< \$5,000		4 (1.5)
\$5,000 - \$19,999		12 (4.6)
\$20,000 - \$34,999		21 (8.0)
\$35,000 - \$49,999		34 (13.0)
\$50,000 - \$74,999		59 (22.6)
\$75,000 - \$99,999		36 (13.8)
\$100,000 - \$149,999		52 (19.9)
\$150,000 - \$199,999		28 (10.7)
> \$200,000		13 (5.0)
Chronic family stress	2.10 $\pm$ 0.70	
Highest acute event rating (self)	3.07 $\pm$ 1.09	
Highest acute event rating (interviewer)	2.16 $\pm$ 0.73	
Average wake-up time (hh:mm)	08:30 $\pm$ 01:13	
Cortisol AUC (nmol/L; log)	8.68 $\pm$ 1.73	
(log)Cortisol slope	-0.04 $\pm$ 0.02	
CRP (mg/L)	1.01 $\pm$ 3.44	
IL-6 (pg/ml)	1.01 $\pm$ 1.41	
IL-1ra (pg/ml)	329.25 $\pm$ 197.96	

BMI = body mass index; AUC = area under the curve; CRP = C-reactive protein; IL-6 = Interleukin-6; IL-1ra = Interleukin-1 receptor antagonist

Note: Means and standard deviations for CRP, IL-6, and IL-1ra are based on untransformed values. Information provided about highest acute and self-rated event ratings reflects ratings regarding only participants who reported any acute events ($n = 153$). Possible range of values for both chronic and acute stress is 1-5.

Ratings of chronic family stress ranged from 1-4; highest self-rated acute event ratings ranged from 0 (no event) to 5 and highest interviewer-rated acute event ratings ranged from 0 (no event) to 4.5

Table 2

Hierarchical Multiple Regression Analyses of Chronic Family and Acute Stress Predicting Adolescent Inflammatory Biomarkers

	Self-rated Acute Stress			Interviewer-rated Acute Stress		
	B	SE	p	B	SE	p
IL-6						
Intercept	−.163	.020	<.001	−.168	.020	<.001
Chronic Family Stress	−.023	.030	.45	−.025	.030	.407
Acute Stress	.010	.012	.43	.014	.018	.42
Chronic × Acute Stress	.025	.016	.120	.054	.023	.022
Overall Model	R ² = .18; F(8,246) = 6.56, p = <.001			R ² = .19; F(8,246) = 7.01, p = <.001		
IL-1ra						
Intercept	2.47	.012	<.001	2.46	.012	<.001
Chronic Family Stress	.010	.018	.60	.009	.019	.61
Acute Stress	−.004	.007	.61	−.005	.011	.63
Chronic × Acute Stress	.016	.010	.11	.030	.014	.034
Overall Model	R ² = .15; F(8,247) = 5.45, p = <.001			R ² = .16; F(8,247) = 5.71, p = <.001		
CRP						
Intercept	−.407	.027	<.001	−.409	.027	<.001
Chronic Stress	−.041	.039	.30	−.037	.040	.35
Acute Stress	.020	.016	.21	−.15	.024	.52
Chronic × Acute Stress	−.011	.021	.61	−.007	.031	.82
Overall Model	R ² = .17; F(8,247) = 6.16, p = <.001			R ² = .16; F(8,247) = 5.97, p = <.001		

Note: Significant ($p < .05$) associations in bold; all analyses controlled for age, sex, ethnicity, body mass index, and income (not included in table).