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Title: Adolescent Temperament Dimensions as Stable Prospective Risk and Protective Factors for Salivary C-Reactive Protein

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Title: Adolescent Temperament Dimensions as Stable Prospective Risk and Protective Factors for Salivary C-Reactive Protein

Abstract

Objective: Temperament has associations with later physical health outcomes, yet there is a dearth of research exploring the connection between temperament and mechanisms that have known associations with these health outcomes. Recent research has delineated a connection between personality and inflammation during adulthood, but this association has not yet been studied in adolescent samples. **Design:** We investigated whether stable adolescent temperament

(averaged over two years), specifically effortful control and negative emotionality, provided a more robust prediction of inflammation as measured by salivary C-reactive protein (sCRP), than depressive symptoms. **Methods:** Temperament and depressive symptoms were measured in a sample of sixty-three adolescents (37 males) when they were approximately 12 years old (mean age = 12.30, SD = .69) and again when they were approximately 14 years old (mean age = 14.84, SD = .49). Levels of sCRP were determined approximately 7 months later (mean = 6.77, SD = 2.99) when participants were approximately 15 years old (mean age = 15.49, SD = .49). **Results:** Regression analyses revealed that effortful control (EC) was significantly associated with lower sCRP levels, while higher negative emotionality (NE) was significantly associated with higher sCRP levels. Furthermore, these associations were larger than those for depressive symptoms and were differentially impacted by the addition of covariates. Implications for the role of stable risk and protective factors in inflammatory processes are discussed. **Conclusions:** These findings are the first to show associations between adolescent temperament and inflammation. Furthermore, these findings extend previous personality research to temperamental research in a younger sample of adolescents.

Keywords: adolescence; depressive symptoms; effortful control; inflammation; negative emotionality; salivary C-reactive protein; temperament

Introduction

Factors associated with health and disease across the lifespan are partly laid down during the critical developmental period of adolescence (Lupien, McEwen, Gunnar, & Heim, 2009). Therefore, early adolescence is an important period for studying factors that are associated with adolescent health in order to better understand protective and risk processes. While there is a well-established literature on the association between mental health status, such as depression, and inflammation (e.g., Slavich & Irwin, 2014), there is a paucity of research examining the relationship between more stable psychological traits, such as temperament, and inflammation.

Temperament Dimensions as Stable Protective and Risk Factors for Inflammation?

Temperament refers to individual differences in emotional, biological, and behavioral reactivity that emerge early in life and are relatively stable across environmental contexts (Rothbart, 2007). Starting as far back as the 1970's research has elucidated the association between temperament and health outcomes, such as premature disease (Betz & Thomas, 1979). Since that time a number of studies have found that temperament predicts both heart disease

(Chida & Steptoe, 2009; Williams, Nieto, Sanford, & Tyroler, 2001) and mortality (Graves, Mead, Wang, Liang, & Klag, 1994; Mccarron, Gunnell, Harrison, Okasha, & Smith, 2003). For example, Mccarron et al. (2003) conducted a large prospective observational study in which they examined over 9,000 young adult males (aged 16-30) to see whether temperament predicted later mortality. After controlling for potential confound variables, findings indicated that when participants with "stable" temperament or those described by words such as, "cheerful" or "pleasant", were compared to those who were classified to have an "atypical temperament" (e.g., anxious, depressed), those with atypical temperament were at increased risk of all cause mortality and had double the risk of dying from a stroke.

While there has been an abundance of research delineating the associations between temperament and depression (Yap et al., 2011) as well as depressive symptoms and inflammation (Stewart, Rand, Muldoon, & Kamarck, 2009), research has not yet examined the potential links between adolescent temperament and biological mechanisms that are associated with later physical health problems, such as inflammation. This may be a particularly important question for two reasons. First, temperamental dimensions may act as common underlying psychological factors that may predispose or protect against both depressive symptomatology and inflammation. Second, c-reactive protein, a measure of inflammation, has been shown to display stability for up to 5 years ($r = .66$, $p < .001$; see Deverts et al. 2010), which is similar to temperament's stable trait-like characteristics. In contrast, depressive symptoms are more variable over time (i.e., state-like). For these two reasons temperament may be tapping into a more common underlying relationship to CRP than more state-like depressive symptoms.

Temperament has associations with both the autonomic nervous system (ANS; Spangler & Friedman, 2015) and the hypothalamic-pituitary-adrenal (HPA) axis (Mayer, Abelson, & Lopez-Duran, 2014) - two fundamental stress response systems that, when dysregulated, may predispose individuals to increased risk of inflammation (Slavich & Irwin, 2014) and poor general health outcomes (McEwen, 2012). Two important dimensions of temperament that may influence risk for both depression (Hankin, 2015; Yap et al., 2011) and physiological processes related to disease (McEwen, 2012), are effortful control (EC) and negative emotionality (NE). EC, often alternatively referred to as self-regulation (Eisenberg, Smith, Sadovsky, & Spinrad, 2004), has been defined as "the efficiency of executive attention—including the ability to inhibit a dominant response and/or to activate a subdominant response, to plan, and to detect errors"

(Rothbart & Bates, 2006, p. 129). Self-regulatory failure has recently been proposed to be associated with inflammation, as inflammatory processes can impact cognitive, neural, and motivational processes (Shields et al., 2017). EC allows for the regulation and modulation of both affective reactivity (Snyder et al., 2015) and physiological reactivity to stress (Oldehinkel, Hartman, Nederhof, Riese, & Ormel, 2011). Greater EC is related to lower levels of psychopathology and greater emotion regulation ability and resilience (Eisenberg, Smith, Sadovsky, & Spinrad, 2004; Snyder et al., 2015; Vijayakumar et al., 2014). Furthermore, temperamental EC is strongly associated with the personality dimension of conscientiousness (Jensen-Campbell et al., 2002), which is a dimension of personality that has been shown to be negatively correlated with inflammation across the adult lifespan (Luchetti, Barkley, Stephan, Terracciano, & Sutin, 2014). In contrast, NE may be understood to be a dysregulated form of emotionality (Snyder et al., 2015; Yap et al., 2011), in which individuals exhibit greater vigilance, inflexible engagement with the environment (Snyder et al., 2015), and greater negative emotions and emotional distress (Yap et al., 2011). Furthermore, research has indicated that psychological distress is associated with inflammation (Maes et al., 1998), while trait negative affect may be a common risk factor for depression and impaired cardiac functioning (Bleil, Gianaros, Jennings, Flory, & Manuck, 2008). Indeed, individuals high in NE and low in EC have higher levels of depressive symptoms (Yap et al., 2011) and risk for depressive disorders (Snyder et al., 2015; Vijayakumar et al., 2014).

Context appropriate regulatory responses, potentially due to high EC, may allow individuals to have dynamic physiological responses that meet environmental demands, while also allowing the individual to return to homeostasis after a regulatory goal is met, as a process of allostasis (McEwen, 2012). In contrast, poor regulation, a characteristic of high NE, may lead to a greater duration, frequency, and intensity of physiological arousal, which may contribute to an inflammatory profile that puts an individual at risk for the early onset of disease through increased allostatic load (McEwen, 2012). Therefore, temperament EC and NE may be distal protective and risk factors, respectively, for inflammation.

One measure of the immune system that has received much attention for its role in physical and mental health processes is c-reactive protein (CRP; Ridker, Stampher, & Rifai, 2001; Valkanova, Ebmeier, & Allan, 2013). This acute phase protein produced by the liver (Hurlimann, Thorbecke, & Hochwald, 1966) has been widely used as an index of systemic

inflammation (Karadag, Kirdar, Karul, & Ceylan, 2008) and with adolescent populations (Byrne et al., 2013). While the immune system can be activated in response to viral infection and physical threat, recent research has demonstrated that the immune system can also respond to psychological factors such as perceived, imagined, or real conditions that involve social threat, conflict, isolation, rejection, and exclusion (Slavich & Irwin, 2014). CRP levels are associated not only with mental health outcomes (e.g., depression, distress, anxiety; Slavich & Irwin, 2014; Wium-Andersen et al., 2013), but also physical disease (e.g., cardiovascular disease, diabetes, and cancer; Emerging Risk Factors Collaboration, 2012; Pradhan, Manson, Rifai, Buring, & Ridker, 2001; Ridker, Stampher, & Rifai, 2001) and mortality (Emerging & Factors, 2010), which makes it a prime candidate for studying how psychological factors influence mechanisms associated with disease. CRP can be detected in saliva (sCRP) and research has shown that CRP can pass from blood to saliva through gingival crevicular fluid, indicating that sCRP can be indicative of both local and systemic inflammation (Megson, Fitzsimmons, Dharmapatni, & Mark Bartold, 2010). Furthermore, several studies have found that sCRP is associated with measures of both psychological and physical health in children and adults (Cicchetti, Handley, & Rogosch, 2015; Goodson et al., 2014; Laurent, Lucas, Pierce, Goetz, & Granger, 2016; Lucas et al., 2016; Naidoo, Konkol, Biccard, Dudose, & McKune, 2012) as well as neural responses to emotional processing of stress (O'Connor, Irwin, & Wellisch, 2009) suggesting that it is also a useful measure of not only oral inflammation, but also more systemic health status.

There are few studies that have examined the direct effect of more stable psychological characteristics and inflammation in humans. One recent meta-analysis showed that the personality dimension of conscientiousness, which is highly correlated with temperamental EC (Jensen-Campbell et al., 2002), has a protective effect against inflammation across adulthood (Luchetti et al., 2014) suggesting that the temperamental features during adolescence may be related to immune system functioning and serve as stable protective and risk factors for inflammation.

Current Study

Adolescence is a critical stage of life for the emergence of both valid and stable self-reported temperament (Capaldi & Rothbart, 1992) and lifelong patterns of mental and physical health related behaviors (Lewinsohn, Rohde, & Brown, 1999; Paavola, Vartiainen, & Haukkala, 2004). As such, the current study investigated the association between stable temperamental EC

and NE, averaged over two years, as compared to depressive symptoms in predicting subsequent sCRP. First, we hypothesized that EC and NE would be negatively associated with one another at both Time 1 (T1) and Time 2 (T2) and that they would stay significantly correlated with themselves over that time period, indicating temperamental stability. Second, we hypothesized that average EC and NE over T1 and T2 would be negatively and positively associated with depressive symptoms at T2, respectively. Finally, we hypothesized that greater average EC would be related to lower sCRP, while greater average NE would be related to higher sCRP. Furthermore, we hypothesized that these stable measures of temperament would be more robust predictors of sCRP, than depressive symptoms. We did not have hypotheses for the temperamental factors of affiliation and surgency, and therefore they were not included in analyses.

Methods

Participants and Recruitment

The sample described in the current study was derived from the XXX [study name removed for blind submission], a large longitudinal study of risk and resilience for the development of psychopathology, conducted in Melbourne, Australia.

The XXX [study name removed for blind submission] recruited 2,453 students in their final year of primary school (i.e., elementary school) across metropolitan Melbourne to participate in an initial school-screening phase. Through this process, 2,280 students completed the Early Adolescent Temperament Questionnaire–Revised (see below EATQ–R; Capaldi & Rothbart, 1992; Ellis & Rothbart, 2001) in the classroom. Selection into the OADS was based on scores on two temperamental dimensions, NE and EC, based on their hypothesized role as risk factors for emotional and behavioral disorders. Accordingly, equal numbers of male and female students were selected from high, medium, and low risk ranges of scores (i.e. “bins”) on each dimension: 0–1, 1–2, 2–2.5, and greater than 2.5 SD above and below the mean. This produced a smaller risk-enriched sample of 414 (16%) that showed a relatively even distribution across EC and NE, while maintaining the range of temperament scores evident in the initial school screening sample (Yap, Allen, & Ladouceur, 2008).

Of the selected adolescents, 245 (58%) agreed to participate in further research. These participants were screened for DSM-IV Axis-I disorders using the Kiddie Schedule for Affective Disorder and Schizophrenia for School-Aged Children: Present and Lifetime Version (K-SADS-

PL; Kaufman et al., 1997) and those who met the criteria for current or past major depressive disorder (N = 2) were excluded due to the broader aims of the study. Of those that agreed to participate 245 filled out the Center for Epidemiological Studies Depression Scale (CES-D) and 228 filled out EATQ-R questionnaires. A subset of 82 participants were selected, based on the order of their participation in the T2 assessment, to participate in the immune analyses using saliva samples for sCRP. Participants that had reported taking medication (N=14) in the 24 hours prior to saliva collection were excluded from analyses. These medications included antihistamines, ibuprofen, and cold and flu tablets, which can affect inflammatory processes (Assanasen & Naclerio, 2002; El-Sharrawy, El-Hakim, & Sameeh, 2006; Mainous III, & Pearson, 2003; Nettis, Colanardi, Ferrannini, & A.Tursi, 2005; Vena, Cassano, Buquicchio, & Ventura, 2008). Furthermore, one additional participant's sCRP value was considered to be an outlier as it was > 5 standard deviations (8.08 SD) above the mean so this participant was excluded from the analyses, leaving 67 usable saliva samples. Lastly, time of day of saliva collection was missing for four subjects. This resulted in a final sample size of 63 participants (37 males). All sCRP levels were detectable. One-way ANOVAs showed that the subgroup of 63 adolescents did not differ from the risk-enriched sample of 415 on temperamental dimensions of EC ($F(1, 413) = .777$, $p = .379$) or NE ($F(1, 407) = .234$, $p = .628$). In addition, one-way ANOVAs showed that the subgroup of 63 adolescents did not differ significantly from the overall sample of 245 adolescents on temperamental dimensions of EC ($F(1, 226) = .000$, $p = .983$) or NE ($F(1, 225) = .027$, $p = .869$) and depressive symptoms ($F(1, 225) = .260$, $p = .611$). Table 1 lists the percentages of ethnicity (identified by the adolescent) and household composition (identified by the parent).

This project was approved by the Human Research Ethics Committee at the XXX [university removed for blind submission], Australia, and all procedures were consistent with National Health and Medical Research Council ethical guidelines. Consent to participate in the study was obtained from both the child and at least one parent at all time points, and reimbursements were provided for child and parent participation.

Procedure

At T1 adolescents (mean age = 12.30, SD = .69) filled out the Early Adolescent Temperament Questionnaire Revised (EATQ-R) and the Center for Epidemiological Studies Depression Scale (CES-D). At T2, approximately 2.5 years later (mean time = 2.49, SD = .25),

adolescents (mean age = 14.84, SD = .49) again filled out the EATQ-R and CES-D and we collected measures on body mass index, pubertal development, and socioeconomic status. Finally, at Time 3 (T3), approximately 7 months later (mean time = 6.77, SD = 2.99) or 3.19 years after T1, adolescents (mean age = 15.49, SD = .49) provided a saliva sample that was assayed for sCRP and we collected measures on smoking status and family history of cardiovascular disease.

Measures

Adolescent Temperament

The EATQ-R consists of 65 items used to derive ten subscales that load onto four factors (EC, NE, surgency, and affiliation, see Ellis & Rothbart, 2001). Of interest to the current study are the EC and NE factors. In the current sample, activity control, inhibitory control, and attentional control subscales loaded onto the EC factor. Attentional control is the capacity to focus and shift attention as required, inhibitory control refers to the capacity to plan and suppress inappropriate behavior, and activity control is the ability to perform an action when there is a strong desire/tendency to avoid it. Higher scores reflect higher EC. In the current sample, only the frustration subscale loaded onto NE. Frustration consists of negative affect related to interruption of ongoing tasks or goal blocking. In order to obtain a more accurate measure of the aspects of self-reported temperament that are stable across time, scores were averaged over the T1 and T2 assessments (i.e., across a 2.5 year period). Reliability for EC at T1 was α = .815 and at T2 was α = .832. Reliability for NE at T1 was α = .950 and at T2 was α = .956 (see supplementary material for scoring syntax).

Depressive Symptoms

The CES-D consists of 20 items used to measure depressive symptoms (Radloff, 1977) and has been found to be a valid and reliable measure for use in adolescent populations (Garrison, Addy, Jackson, McKeown, & Waller, 1991). Cronbach's alphas for T1 was α = .855 and for T2 was α = .876 (see supplementary material for scoring syntax).

Inflammation

Two mL of whole, unstimulated saliva was collected from 82 participants at T3 using the passive drool method to analyze peripheral concentrations of sCRP. Saliva is easier and safer to collect as compared to blood in research studies (Granger et al., 2007). In particular, some studies show that sCRP may be correlated with systemic or major sources of the general

inflammatory marker, CRP (Byrne et al., 2013; Out, Hall, Granger, Page, & Woods, 2012). These studies have demonstrated that CRP can be detected in saliva as well as in blood, and the measures in these two tissues correlate with medium to large effect sizes. One study found that many inflammatory markers had higher detection rates in saliva when compared to blood in an adolescent cohort (Byrne et al., 2013). However, some studies show no significant correlation between these measures (e.g., Kopanczyk et al., 2010).

Collection time varied by participant, however, research is mixed as to whether CRP has a diurnal variation with some studies showing no diurnal variation (Miles et al., 2008) as was found in our sample between morning and afternoon samples ($F(1,8) = .627$, $p = .451$), morning and evening samples ($F(1,8) = .301$, $p = .598$), and afternoon and evening ($F(1,8) = .983$, $p = .351$), although some research has shown diurnal variability in healthy populations (Rudnicka, Rumley, Lowe, & Strachan, 2007), those with coronary artery disease (Koc, Karaarslan, Abali, & Batur, 2010), and those with obstructive sleep apnea (Mills, Natarajan, von Känel, Ancoli-Israel, & Dimsdale, 2009). sCRP analysis was performed according to manufacturer's instructions by the Bio-Plex multiplex bead array immunoassay system of human cytokine panel and plates read on a Bio-Plex Array Reader (Bio-Plex 200 System and Bio-Plex Manager Version 4.0, Bio-Rad Laboratories, Inc., New South Wales, Australia). Saliva samples were frozen immediately at -20°C after collection and stored for 24-36 months prior to analysis. After thawing to room temperature (24°C), samples were first vortexed with a protease inhibitor cocktail (PIC), "Complete, Mini" (Roche, Castle Hill; NSW, Australia) in order to protect the integrity of the acute-phase proteins. Samples were then centrifuged at 10,000 g for 10 minutes, to isolate the precipitate and debris from the supernatant. The supernatant was extracted and divided into 3 test tubes before being snap-frozen in liquid nitrogen and stored at -80°C overnight. Samples were again thawed to room temperature the following day and centrifuged once more at 10,000 g for 10 minutes and supernatants analyzed for sCRP by Bio-Plex assay, described elsewhere (Byrne et al., 2013). Pilot testing showed that a second centrifugation resulted in much lower viscosity, with less likelihood of clogging the Bio-Plex suspension array system. Furthermore, the lower viscosity enabled us to analyze the samples without further dilution with the Bio-Plex immunoassays.

Saliva sample supernatant was assayed in duplicate, undiluted, and analyzed by the flow-based Bio-Plex suspension array system. Intra-assay CV was <20%, consistent with other studies

of sCRP (Byrne et al., 2013). For the assays, the test volume was 50 μL, with a range of standards from 10 – 79560 pg/mL. The mean of recovery percentages ($\frac{Observed\ Concentration}{Expected\ Concentration}$) from standards was 99.42%, S.D. = 11.31%, range: 75% - 116%. On the day of saliva collection, time of saliva collection was recorded and participants were asked to complete a "diary", which included questions about any medication or substance use in the past 24 hours prior to collection, and the type and dose. As mentioned previously, anyone who took medications were excluded from analyses.

Covariates

Gender. Adolescent gender was collected as there are known gender differences in inflammatory reactivity in response to stress (Bouman, Jan Heineman, & Faas, 2005).

Puberty. The Pubertal Development Scale (PDS) was used to assess pubertal development using the self-reported PDS (Petersen, Crockett, Richards, & Boxer, 1988). For females, this measure includes 8 items assessing the stage of breast development, hair growth, acne presence, and hip width, and we added an additional question on menarcheal status. For males, this measure includes 11 items assessing genitalia development, hair growth, acne presence and voice change. Reliability and validity of the PDS has been well established (Petersen et al., 1988). For descriptive purposes, the PDS data was coded into a 5-point scale in accordance with the Tanner stages based on prior work (Shirtcliff, Dahl, & Pollak, 2009).

Socioeconomic Status. A robust measure of socio-economic status (SES) was calculated for participants by using the Australian National University-4 (ANU4) scale for occupations (Jones & McMillan, 2001), which provides a score between 0 and 100. Parents were asked about their occupation and education. For parents that had missing data or reported an occupational status that could not be coded according to ANU4 (e.g., unemployed or small business owner), data on education was used as a substitute, in number of years of education, scaled to reflect ANU4 codes. This method of measuring socio-economic status has been recommended in Australia by the National Education Performance Monitoring Taskforce.

Family History of Cardiovascular Disease. A family history of cardiovascular disease (CVD) is an independent risk factor for developing cardiac disease (Williams et al., 2001); therefore, it was collected as a covariate to account for shared heritable contribution to inflammatory diseases. Information about incidence of CVD in the participant's first- and second-degree relatives was obtained from parents in an interview. A family history proportion

score was calculated for each person to assess familial risk of developing CVD (Murad, Kalter-Leibovici, Chetrit, & Freedman, 2007).

Body Mass Index. Body-mass index (BMI) was measured by researchers by weighing the participant on a scale and measuring height, and calculating BMI equal to the weight (kg) divided by the height (m) squared. BMI has been shown to be associated with levels of CRP in children (Gillum, 2003).

Smoking Status. On the day of saliva collection, participants were asked to complete a "diary", which included questions about any medication or substance use in the past 24 hours prior to collection, and the type and dose. We also collected smoking status as this has been shown to influence sCRP (Azar & Richard, 2011).

Time of Day of Saliva Collection. Research is mixed on whether CRP has diurnal variation with some studies showing no variation in healthy adults (Miles et al., 2008), while others studies show the contrary (Koc, Karaarslan, Abali, & Batur, 2010; Mills et al., 2009; Rudnicka, Rumley, Lowe, & Strachan, 2007). Therefore, time of day was collected using morning (< 12:00 pm), afternoon (12:00 to 5:00 pm), and evening (> 5:00 pm).

Statistical Analysis

All statistical transformations and analyses were conducted with IBM SPSS Statistics for Mac, version 23 (SPSS Inc., Chicago, IL, USA). See Table 2 for descriptive statistics on study variables. All reported p-values are exact two-sided significance levels. Statistical significance was defined as $p < 0.05$.

Exploratory statistics of histograms as well as skew and kurtosis statistics were run for each variable to check for normality. These analyses revealed no significant deviations from normality. However, sCRP was positively skewed, so these values were log transformed.

Data was missing for pubertal stage from three participants (4.5%), CES-D from three participants at T1 (4.5%), CES-D from two participants at T2 (3%), and EC and NE from three participants at T1 (4.5%) and EC and NE from one participant at T2 (1.5%). To preserve statistical power lost through deletion methods, single imputation with the EM algorithm was used to estimate missing data (Little & Rubin, 1987). Little's MCAR test indicated that the null hypothesis that the data was missing in a random fashion could not be rejected, $\chi^2 = 98.43$ (df = 92; $p = 0.30$).

Covariates. Correlations were run between sCRP and covariates (see Table 3). In addition, we looked at correlations between covariates to assess multicollinearity. Correlations were also run between EC and NE at both T1 and T2 to check for temperamental stability.

Regression. For our main analyses, we ran three separate, increasingly stringent, statistical tests of the of the association between temperament scores (i.e., averaged across T1 and T2) and depressive symptoms at T2 and sCRP. For detailed tables describing full model statistics for each block and change statistics see Supplementary Tables S1-S8. In the first set of models, we ran three different regression models between each of the predictors (i.e., EC, NE, depressive symptoms) and sCRP. In the second set of models, we ran these same regressions, but in block one of each regression we added covariates (BMI, SES, age, gender, family history of cardiovascular disease, pubertal development, smoking status, and time of day of saliva collection) and in block two we separately entered each of the three predictors. Finally, in the third set of models we tested whether temperament variables would predict variance in sCRP over and above that predicted by depressive symptoms (i.e., the most stringent test of the independent contribution of temperament), so we entered covariates into block one, depressive symptoms into block two, and in two separate models we entered EC and NE into block three respectively.

Results

Preliminary Analyses. EC at T1 was significantly associated with EC at T2 ($r = .618$, $p < .001$) and NE at T1 was significantly associated with NE at T2 ($r = .454$, $p < .001$). EC and NE were significantly inversely related at both T1 ($r = -.409$, $p = .001$), and T2 ($r = -.520$, $p < .001$). Furthermore, higher average EC was significantly associated with lower depressive symptoms at T2 ($r = -.435$, $p < .001$), while higher average NE was significantly associated with higher depressive symptoms at T2 ($r = .398$, $p = .001$).

Regressions. In our first set of models, average EC and NE as well as depressive symptoms at T2 were entered as predictors, in three separate models, of sCRP. As shown in Table 4a, higher average EC was significantly associated with lower sCRP levels ($b = -.042$, $SE = .016$, 95% CI [-.074, -.011], $p = .010$; overall model $R^2 = .104$, $F(1, 61) = 7.118$, $p = .010$)). As shown in Table 4b, the association between average NE and sCRP showed a non-significant trend ($b = .053$, $SE = .028$, 95% CI [-.004, .110], $p = .068$; overall model $R^2 = .053$, $F(1, 61) = 3.447$, $p = .068$). As shown in Table 4c, higher depressive symptoms at T2 were significantly

associated with higher sCRP (b = .041, SE = .018, 95% CI [.006, .077], p = .024; overall model R^2 = .081, F(1, 61) = 5.369, p = .024). These findings indicate that prior to the inclusion of covariates, greater EC is associated with lower sCRP, greater depressive symptoms are associated with higher sCRP levels, whereas higher NE shows a non-significant trend toward an association with sCRP.

In our second set of models, after controlling for covariates in step one, either average EC, NE, or depressive symptoms at T2 were entered into step two. As shown in Table 5a, after controlling for covariates, higher average EC was significantly related to lower sCRP (b = -.044, SE = .017, 95% CI [-.078, -.010], p = .012; overall model R^2 = .258, F(9, 53) = 2.043, p = .052), but the overall model had a non-significant trend. In addition, as shown in Table 5b, after controlling for covariates, higher average NE was significantly associated with higher sCRP (b = .064, SE = .029, 95% CI [.007, .121], p = .029; overall model R^2 = .236, F(9, 53) = 1.815, p = .087), although the overall model had a non-significant trend. In contrast and as shown in Table 5c, higher depressive symptoms at T2 showed a non-significant tend towards being associated with higher sCRP (b = .035, SE = .019, 95% CI [-.004, .074], p = .078; overall model R^2 = .212, F(9, 53) = 1.574, p = .145), with a non-significant overall model. It is important to note that while the EC and NE predictor variables were significant, the overall effect was trending, but not significant. Multicollinearity is one potential explanation for this pattern of findings, but is unlikely to be the cause in this context as all model VIFs were below 2 (the cutoff for multicollinearity usually ranges from 5-10; see Hair, Anderson, Tatham, & Black, 1995). Lack of power is more likely to be the issue. When analyses were rerun with only significant covariates (BMI and PDS) both EC (b = -.038, SE = .015, 95% CI [-.068, -.008], p = .015; overall model R^2 = .182, F(3, 59) = 5.601, p = .002) and NE (b = .058, SE = .027, 95% CI [.005, .112], p = .033; overall model R^2 = .203, F(3, 59) = 5.016, p = .004) were significantly associated with sCRP.

Lastly, our most stringent set of models entered covariates in step one, depressive symptoms at T2 in step two, and average temperament ratings in step three. As shown in Table 6a, higher average EC showed a non-significant trend towards being associated with lower sCRP (b = -.037, SE = .019, 95% CI [-.075, .000], p = .051; overall model R^2 = .268, F(10, 52) = 1.901 p = .066). In addition, as shown in Table 6b, NE was not significantly associated with sCRP (b = .051, SE = .032, 95% CI [-.013, .115], p = .114). These findings indicate that in the most stringent models, which included both covariates and depressive symptoms, EC showed a non-

significant trend level association with sCRP, while the association between NE and sCRP was non-significant. Similar to the previous models, small sample size combined with the inclusion of 9 covariates may have led to an issue of power resulting in a failure to capture a true effect (i.e., type 2 error; Button et al., 2013). In contrast, it is possible that the inclusion of these covariates explained more variance in sCRP leading to the additional variance of temperament being too small to significantly improve the model. As noted above, multicollinearity was not likely to be the cause of this discrepancy.

Discussion

The results of the current study provide partial support for our hypotheses. Consistent with our hypotheses, repeated measures of EC and NE over a two year period were highly correlated, indicating a degree of stability in temperament over early to mid-adolescence. In addition, average EC and NE significantly predicted depressive symptoms at T2, with higher EC and lower NE being associated with lower depressive symptoms.

In our first set of main analyses, which did not control for covariates, higher adolescent EC was significantly associated with lower sCRP levels as was hypothesized. In contrast, higher adolescent NE had a non-significant trend level association with higher sCRP. Finally, as was hypothesized and as has been shown in previous research, higher levels of depressive symptoms were related to higher sCRP.

In our second set of analyses, as we hypothesized, after controlling for all covariates, higher EC was still significantly related to lower sCRP, although the overall model was trending, but not significant, which may have been due to a lack of power when including 8 covariates. When analyses were rerun with only significant covariates (BMI and PDS), then the overall model became significant. In addition, after the inclusion of covariates higher NE was significantly related to higher sCRP. Again, the overall model was trending, but not significant, which may have resulted from lack of power. When the model was rerun with only significant covariates, then the overall models became significant as well. In contrast to hypotheses, depressive symptoms had a trending, but nonsignificant association with higher sCRP, indicating that after the inclusion of covariates, depressive symptoms no longer explained a significant amount of variance in later sCRP.

Lastly, in our most stringent test of the association between temperament and sCRP, which controlled for covariates and depressive symptoms before testing the temperamental

predictors, both higher EC and lower NE had trending, but nonsignificant associations with lower sCRP. These findings, which were in contrast to our hypotheses, indicate that either 1) after including all covariates as well as depressive symptoms, temperamental variables no longer explained sufficient variance in sCRP or 2) issues related to sample size and power after adding 9 additional variables to the model reduced the ability to find a true effect (Button et al., 2013).

The observed relationship between higher average adolescent EC and lower inflammation (indicated by lower sCRP levels), both with and without covariates, as well as the finding of higher average adolescent NE and higher inflammation after the inclusion of covariates are novel findings, especially given the relatively long time period (3.19 years) between the T1 measures and immune assessment. There are a few pathways through which EC and NE may be associated with inflammation. First, research indicates that EC is likely related to better executive functioning (Blair & Razza, 2007), which has neurobiological underpinnings, including prefrontal regions (O'Connor et al., 2009) and peripheral psychophysiology measures of heart rate variability (Singh, Hawkley, McDade, Cacioppo, & Masi, 2009), that have known associations with peripheral immunity. Therefore, EC may alter these biological pathways reducing inflammation. Second, previous literature has shown that the personality dimension of conscientiousness, which is highly correlated with temperamental EC (Jensen-Campbell et al., 2002), has a protective effect against inflammation across adulthood (Luchetti et al., 2014) and these authors proposed that a likely mechanism for this finding may be lower HPA axis activation to stress. It is important to note that while cortisol is initially an anti-inflammatory agent, overtime, chronic exposure of cortisol is associated with increased inflammation (McEwen, 2012). Therefore, lower cortisol secretion overtime may be associated with lower inflammation. Similarly, greater NE is associated with dysregulated emotionality (Snyder et al., 2015; Yap et al., 2011), which may cause chronic HPA axis activation to stress and therefore impaired regulation (Shields et al., 2017), greater allostatic load, and, overtime, inflammation. Third, from a cognitive framework, the ability to modify thoughts, emotions, and behavior by focusing and shifting attention in order to either inhibit a dominant response or activate a subdominant response as is found in those with higher EC (Rothbart & Bates, 2006), may have protective effects on health related outcomes, while the greater vigilance and inflexible engagement with the environment found in those with higher NE (Snyder et al., 2015) may have an adverse effect on health related outcomes. Research supports this potential interpretation as

greater EC has been associated with decreased risk for depression, while the opposite has been found for NE (Yap et al., 2011). Fourth, individuals lower in EC have also been shown to have greater risk-taking behaviors, including illicit drug use (Lafreniere, Menna, & Cramer, 2013), which may increase levels of inflammation. Finally, another potential explanation is that the temperament scales may have tapped into more trait-like aspects of adolescents' functioning that underpins both depressive symptoms and inflammation, therefore rendering it a more robust predictor of longitudinal outcomes than depressive symptoms, which are considered to reflect more state like aspects of functioning that may not have such a strong association with immune functioning later in time (see Stewart et al., 2009). The lack of an association between depressive symptoms and subsequent sCRP after controlling for covariates is in line with previous research showing a null relationship between depressive symptoms and later CRP levels (Stewart et al., 2009). In other words, it is possible that depressive symptoms vacillated during the 7 month gap between the indexing of depressive symptoms and the collection of sCRP, and therefore, did not act as a strong predictor for later inflammation.

Somewhat in contrast to our hypotheses, after controlling for all covariates as well as depressive symptoms EC was trending, but not significantly associated with lower sCRP, while the relationship between NE and sCRP was no longer statistically significant. There are a number of potential reasons for this finding. First, from a statistical standpoint, there may have not been enough power in a sample of 63 participants to be able to robustly predict sCRP with the number of variables we were controlling for once we added depressive symptoms to our covariates. In other words, insufficient power may have reduced the likelihood of finding a true effect. Second, it is possible that after controlling for all covariates and depression, temperamental variables no longer explained a significant amount of variance over that already provided by covariates and depressive symptoms. Third, temperamental factors and depressive symptoms were correlated, so the shared variance between temperament and depressive symptoms was already accounted for in the equation when the temperament variable was entered (i.e., temperament is entered in the third block, whereas depression is entered in the second block). This means that only the unique variance associated with temperament (i.e., that not shared the measures of depression) was being tested. These models therefore may represent a test of a different temperament construct (i.e. that aspect of temperament that is not associated with depressed symptoms) than the previous models, which used measures of NE and EC that include

this variance. However, these models suggest that the unique variance in EC does not predict sCRP, but does come close to significance even in this very stringent test.

Limitations and Future Directions

While this study provided new insights into the psychological factors that may predispose individuals to greater inflammation, it is important to note a number of limitations. First, our study had a sample size of 63 participants, which only allows for initial preliminary evidence for the associations between temperament and inflammation. Small sample size combined with the inclusion of 8 covariates may have led to an issue of power leading to significant predictors, but trending non-significant overall models that may have reduced the likelihood of finding a true effect (Button et al., 2013). Future studies should attempt to replicate this study with a larger sample size in order to draw stronger conclusions. Second, sCRP was only collected once, which did not allow for a proper prospective design. Future studies should provide repeated measures of sCRP in order to draw stronger conclusions on how temperament might be associated with changes in sCRP over time. Third, although research has found strong stability between CRP levels collected 5 years apart ($r = .66$, $p < .001$; see Deverts et al 2010), the collection of sCRP 7 months after the second assessment was not ideal and didn't allow us to assess concurrent inflammation, depressive symptoms, and temperament. As a result, other explanatory variables at the collection of sCRP may have better accounted for sCRP levels than either depressive symptoms or stable temperament. Future studies should make sure to collect all variables of interest concurrently. Fourth, BMI, which is associated with low-grade inflammation and CRP, was only collected at our second time point and not also during sCRP collection, which prevented addressing the possible role of changes in BMI as it might relate to sCRP. Future studies should attempt to collect BMI at the time of sCRP collection. Fifth, although we found an initial relationship between average EC and NE with sCRP, future research should include other biological (e.g., autonomic, endocrine) and behavioral (e.g., risk-taking behavior, drug use, diet, exercise) factors in order to better understand how EC and NE relate to chronic inflammation as these factors may serve as explanatory variables. However, it is important to note that the inclusion of alcohol consumption and smoking in a prospective observational study did not change the association between temperament and later mortality (McCarron et al., 2003). Sixth, the current study was limited in examining sCRP as the sole marker of inflammation. Future studies should examine multiple pro-inflammatory and anti-inflammatory biomarkers in order to

provide a more complete understanding of how EC and NE may "get under the skin" to influence inflammatory processes. Seventh, while our study excluded participants taking medication, we did not measure temperature or assess illness or dental hygiene, which could be relevant for any salivary inflammatory markers, although these phenomena are less frequent in younger samples. Therefore, it is possible that the associations we found between temperament and sCRP are due to temperamental differences in oral hygiene. Physical illness and dental hygiene should be carefully assessed in future studies. Lastly, we did not have the means to address associations of sCRP with later physical health outcomes, which should also be a focus of future studies.

Conclusion

The present study is the first to our knowledge to provide initial data on the relationship between adolescent temperament and inflammation. Overall, our findings suggest that temperamental factors are stable across early adolescence and adolescents with low EC or high NE are at greater risk of depressive symptoms and later salivary inflammation, although this relationship may weaken after accounting for covariates. In addition, these findings extend previous personality research examining the protective effects of conscientiousness, a construct related to temperamental EC, to a younger sample of adolescents. More research is needed to refine our understanding of the biological and behavioral mechanisms that may explain the association between EC and NE with inflammatory processes. This understanding may lead to new prevention and intervention efforts to identify adolescents at risk in order to decrease the incidence of inflammatory-related physical and mental health problems.

References

Assanasen, P., & Naclerio, R. M. (2002). Antiallergic anti-inflammatory effects of H1-antihistamines in humans. Clinical Allergy and Immunology, 17, 101–139.

Azar, R., & Richard, A. (2011). Elevated salivary C-reactive protein levels are associated with active and passive smoking in healthy youth: A pilot study. Journal of Inflammation, 8(1), 37.

Bouman, A., Heineman, M. J., & Faas, M. M. (2005). Sex hormones and the immune response in humans. Human Reproduction Update, 11(4), 411–423.

Byrne, M. L., O'Brien-Simpson, N. M., Reynolds, E. C., Walsh, K. A., Laughton, K., Waloszek,

J. M., Woods, M. J., Trinder, J., & Allen, N. B. (2013). Acute phase protein and cytokine levels in serum and saliva: A comparison of detectable levels and correlations in a depressed and healthy adolescent sample. Brain, Behavior, and Immunity, 34, 164–175.

Capaldi, D. M., & Rothbart, M. K. (1992). Development and Validation of an Early Adolescent Temperament Measure. The Journal of Early Adolescence, 12(2), 153–173.

Cicchetti, D., Handley, E. D., & Rogosch, F. A. (2015). Child maltreatment, inflammation, and internalizing symptoms: Investigating the roles of C-reactive protein, gene variation, and neuroendocrine regulation. Development and Psychopathology, 27(2), 553–566.

Eisenberg, N., Smith, C. L., Sadovsky, A., & Spinrad, T. L. T. (2004). Effortful control: Relations with emotion regulation, adjustment, and socialization in childhood. Handbook of Self-Regulation: Research, Theory and Applications, (November), 259–282.

El-Sharrawy, E. a, El-Hakim, I. E., & Sameeh, E. (2006). Attenuation of C-reactive protein increases after exodontia by tramadol and ibuprofen. Anesthesia Progress, 53(3), 78–82.

Ellis, L. K., & Rothbart, M. K. (2001). Revision of the early adolescent temperament questionnaire. In Poster presented at the 2001 biennial meeting of the society for research in child development, Minneapolis, Minnesota.

The Emerging Risk Factors Collaboration (2010). C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. The Lancet, 375(9709), 132–140.

Garrison, C. Z., Addy, C. L., Jackson, K. L., McKeown, R. E., & Waller, J. L. (1991). The CES-D as a screen for depression and other psychiatric disorders in adolescents. Journal of the American Academy of Child & Adolescent Psychiatry, 30(4), 636–641.

Gillum, R. F. (2003). Association of serum C-reactive protein and indices of body fat distribution and overweight in Mexican American children. Journal of the National Medical Association, 95(7), 545–552.

Goodson, J. M., Kantarci, A., Hartman, M. L., Denis, G. V., Stephens, D., Hasturk, H., ... & Barake, R. (2014). Metabolic disease risk in children by salivary biomarker analysis. PloS one, 9(6), e98799.

Granger, D. A., Kivlighan, K. T., Fortunato, C., Harmon, A. G., Hibel, L. C., Schwartz, E. B., & Whembolua, G. L. (2007). Integration of salivary biomarkers into developmental and behaviorally-oriented research: Problems and solutions for collecting specimens.

Physiology and Behavior, 92(4), 583–590.

Hankin, B. L. (2015). Depression from childhood through adolescence: Risk mechanisms across multiple systems and levels of analysis. Current Opinion in Psychology, 4, 13–20.

Hurlimann, J., Thorbecke, G. J., & Hochwald, G. M. (1966). The liver as the site of C-reactive protein formation. The Journal of experimental medicine, 123(2), 365-378.

Jensen-Campbell, L. A., Rosselli, M., Workman, K. A., Santisi, M., Rios, J. D., & Bojan, D. (2002). Agreeableness, conscientiousness, and effortful control processes. Journal of Research in Personality, 36(5), 476–489.

Jones, F. L., & McMillan, J. (2001). Scoring occupational categories for social research: A review of current practice, with australian examples. Work, Employment & Society, 15(3), 539–563.

Karadag, F., Kirdar, S., Karul, A. B., & Ceylan, E. (2008). The value of C-reactive protein as a marker of systemic inflammation in stable chronic obstructive pulmonary disease. European Journal of Internal Medicine, 19(2), 104–108.

Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., Williamson, D., Ryan, N. (1997). Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime version (K-SADS-PL): Initial reliability and validity data. Journal of the American Academy of Child and Adolescent Psychiatry, 36, 980–988.

Koc, M., Karaarslan, O., Abali, G., & Batur, M. K. (2010). Variation in high-sensitivity C-reactive protein levels over 24 hours in patients with stable coronary artery disease. Texas Heart Institute Journal, 37(1), 42–48.

Kopanczyk, R., Opris, D. C., Lickliter, J., Bridges, E. G., Nazar, A. M., & Bridges, K. G. (2010). C-reactive protein levels in blood and saliva show no correlation in young, healthy adults. FASEB, 24(1), b409–lb409.

Lafreniere, K. D., Menna, R., & Cramer, K. M. (2013). Rebelliousness , Effortful Control , and Risky Behavior: Metamotivational and Temperamental Predictors of Risk-Taking in Older Adolescents. Journal of Motivation, Emotion, and Personality, 1(1), 17–26.

Laurent, H. K., Lucas, T., Pierce, J., Goetz, S., & Granger, D. A. (2016). Coordination of cortisol response to social evaluative threat with autonomic and inflammatory responses is moderated by stress appraisals and affect. Biological Psychology, 118, 17–24.

Lewinsohn, P. M., Rohde, P., & Brown, R. A. (1999). Level of current and past adolescent

cigarette smoking as predictors of future substance use disorders in young adulthood. Addiction, 94(6), 913–921.

Little, RJA and Rublin, D. (1987). Statistical Analysis with Missing Data. Wiley, New York., 381.

Lucas, T., Lumley, M. A., Flack, J. M., Wegner, R., Pierce, J., & Goetz, S. (2016). A preliminary experimental examination of worldview verification, perceived racism, and stress reactivity in African Americans. Health Psychology, 35(4), 366-375.

Luchetti, M., Barkley, J. M., Stephan, Y., Terracciano, A., & Sutin, A. R. (2014). Five-factor model personality traits and inflammatory markers: New data and a meta-analysis. Psychoneuroendocrinology, 50, 181–193.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature Reviews Neuroscience, 10(6), 434–45.

Mainous III, A. G., & Pearson, W. S. (2003). Aspirin and ibuprofen: Potential mediators of the cardiovascular risk due to smoking? Family Medicine, 35(2), 112–118.

Mayer, S. E., Abelson, J. L., & Lopez-Duran, N. L. (2014). Effortful control and context interact in shaping neuroendocrine stress responses during childhood. Hormones and Behavior, 66(2), 457–465.

McCarron, P., Gunnell, D., Harrison, G. L., Okasha, M., & Smith, G. D. (2003). Temperament in young adulthood and later mortality: prospective observational study. Journal of Epidemiology and Community Health, 57(11), 888-892.

McEwen, B. S. (2012). Brain on stress: How the social environment gets under the skin. Proceedings of the National Academy of Sciences, 109(Supplement 2), 17180-17185.

Megson, E., Fitzsimmons, T., Dharmapatni, K., & Mark Bartold, P. (2010). C-reactive protein in gingival crevicular fluid may be indicative of systemic inflammation. Journal of clinical periodontology, 37(9), 797-804.

Miles, M. P., Andring, J. M., Pearson, S. D., Gordon, L. K., Kasper, C., Depner, C. M., & Kidd, J. R. (2008). Diurnal variation, response to eccentric exercise, and association of inflammatory mediators with muscle damage variables. Journal of Applied Physiology, 104(2), 451–458.

Mills, P. J., Natarajan, L., von Känel, R., Ancoli-Israel, S., & Dimsdale, J. E. (2009). Diurnal

variability of C-reactive protein in obstructive sleep apnea. Sleep and Breathing, 13(4), 415–420.

Murad, H., Kalter-Leibovici, O., Chetrit, A., & Freedman, L. S. (2007). A statistical comparison of different family history scores. Statistics in Medicine, 26(14), 2785–2798.

Naidoo, T., Konkol, K., Biccard, B., McKune, A. J., & Dubose, K. (2012). Elevated salivary C-reactive protein predicted by low cardio-respiratory fitness and being overweight in African children: cardiovascular topic. Cardiovascular journal of Africa, 23(9), 501-506.

Nettis, E., Colanardi, M. C., Ferrannini, A., & A.Tursi. (2005). Antihistamines as important tools for regulating inflammation. Current Medicinal Chemistry, 4, 81–89.

O'Connor, M. F., Irwin, M. R., & Wellisch, D. K. (2009). When grief heats up: pro-inflammatory cytokines predict regional brain activation. Neuroimage, 47(3), 891-896.

Oldehinkel, A. J., Hartman, C. A., Nederhof, E., Riese, H., & Ormel, J. (2011). Effortful control as predictor of adolescents' psychological and physiological responses to a social stress test: The Tracking Adolescents' Individual Lives Survey. Development and Psychopathology, 23(2), 679–688.

Out, D., Hall, R. J., Granger, D. A., Page, G. G., & Woods, S. J. (2012). Assessing salivary C-reactive protein: longitudinal associations with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence. Brain, Behavior, and Immunity, 26(4), 543–551.

Paavola, M., Vartiainen, E., & Haukkala, A. (2004). Smoking, alcohol use, and physical activity: a 13-year longitudinal study ranging from adolescence into adulthood. The Journal of Adolescent Health, 35(3), 238–244.

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. Journal of Youth and Adolescence, 17(2), 117–133.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Jama, 286(3), 327-334.

Radloff, L. S. (1977). The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. Applied Psychological Measurement, 1(3), 385–401.

Ridker, P., Stampher, M., & Rifai, N. (2001). Novel risk factors for atherosclerosis. Journal of

American Medical Association, 285(19), 2481–2485.

Rothbart, M. K., & Bates, J. E. (2006). Temperament. In N. Eisenberg & W. Damon (Eds.), Handbook of child psychology: Vol. 3. Social, emotional, and personality development (pp. 99–166). New York: Wiley.

Rothbart, M. K. (2007). Temperament, development, and personality. Current Directions in Psychological Science, 16(4), 207–212.

Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: Correspondence between hormonal and physical development. Child Development, 80(2), 327–337.

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. Psychological Bulletin, 140(3), 774–815.

Snyder, H. R., Gulley, L. D., Bijttebier, P., Leuven, K., Hartman, C. A., Oldehinkel, A. J., Mezulis, A., Young, J. F., & Hankin, B. L. (2015). Adolescent Emotionality and Effortful Control: Core Latent Constructs and Links to Psychopathology and Functioning. Journal of Personality and Social Psychology, 109(6), 1132–1149.

Spangler, D. P., & Friedman, B. H. (2015). Effortful control and resiliency exhibit different patterns of cardiac autonomic control. International Journal of Psychophysiology, 96(2), 95–103.

Stewart, J. C., Rand, K. L., Muldoon, M. F., & Kamarck, T. W. (2009). A prospective evaluation of the directionality of the depression–inflammation relationship. Brain, behavior, and immunity, 23(7), 936-944.

Valkanova, V., Ebmeier, K. P., & Allan, C. L. (2013). CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. Journal of affective disorders, 150(3), 736-744.

Vena, G. A., Cassano, N., Buquicchio, R., & Ventura, M. T. (2008). Antiinflammatory effects of H1-antihistamines: clinical and immunological relevance. Current Pharmaceutical Design, 14(27), 2902–2911.

Vijayakumar, N., Whittle, S., Dennison, M., Yucel, M., Simmons, J., & Allen, N. B. (2014). Development of temperamental effortful control mediates the relationship between maturation of the prefrontal cortex and psychopathology during adolescence: A 4-year longitudinal study. Developmental Cognitive Neuroscience, 9, 30–43.

Williams, R. R., Hunt, S. C., Heiss, G., Province, M. A., Bensen, J. T., Higgins, M., Chamberlain, R.M., Ware, J., Hopkins, P. N. (2001). Usefulness of cardiovascular family history data for population-based preventive medicine and medical research (The Health Family Tree Study and the NHLBI Family Heart Study). American Journal of Cardiology, 87(2), 129–135.

Wium-Andersen, M. K., Ørsted, D. D., Nielsen, S. F., & Nordestgaard, B. G. (2013). Elevated C-reactive protein levels, psychological distress, and depression in 73 131 individuals. JAMA Psychiatry, 70(2), 176-184.

Yap, M. B. H., Allen, N. B., & Ladouceur, C. D. (2008). Maternal socialization of positive affect: The impact of invalidation on adolescent emotion regulation and depressive symptomatology. Child Development, 79(5), 1415–1431.

Yap, M. B. H., Allen, N. B., O'Shea, M., di Parsia, P., Simmons, J. G., & Sheeber, L. (2011). Early adolescents' temperament, emotion regulation during mother-child interactions, and depressive symptomatology. Development and Psychopathology, 23(1), 267–282.

Conflict of Interest

All authors declare no conflicts of interest.

Table 1

Demographics and Household Composition

	Percentage
Ethnicity	
White/Caucasian	77.8%
Asian	4.8%
More than one race	7.9%
Household composition	
Two-parent households with siblings and/or other relatives	73.0%
Two-parent households with no siblings or other relatives	3.2%

Adoptive Parents and siblings	1.6%
Single-parent (mother) households with siblings and/or other relatives	1.6%
Single-parent (father) households with siblings and/or other relatives	1.6%
Single-parent (mother) households with no other siblings or other relatives	6.3%
Single-parent (father) households with no other siblings or other relatives	1.6%
Relatives other than biological parents, stepparents, adoptive parents, or grandparents (e.g., aunts or uncles as parental figures)	1.6%
Missing	9.5%

Table 2. Descriptive Statistics

Variable	Mean	SD
sCRP (log)	-3.693	1.039
EC T1	48.905	9.386
NE T1	21.464	5.203
CES-D T1	32.224	10.104
EC T2	47.338	8.225
NE T2	20.692	5.484
CES-D T2	29.305	7.142
BMI at T2	22.851	4.377
PDS T2	24.510	5.752
ANU4	57.772	21.597
CVD Hx	.108	.104
Age at Biosamples	15.487	.493
Variable	**Frequency (n, percentage)**	
Sex	Male: 37, 58.7%	
	Female: 26, 41.3%	
Regular Smoker	No: 60, 95.2%	
	Yes: 3, 4.8%	
Time of Day of Saliva Collection	Morning: 29, 46.0%	
	Afternoon: 25, 39.7%	

Evening: 9, 14.3%

Note. EC = Effortful Control, NE = Negative Emotionality, sCRP (log) = salivary c-reactive protein log transformed, ANU4 = Australian National University-4 (measure of socioeconomic status), BMI = body mass index, CVD Hx =cardiovascular disease history, PDS = pubertal development scale, T1 = Time 1, T2 = Time 2.

Table 3.

Bivariate Correlations Between sCRP and Covariate

Variable	Sex	Age	BMI T2	Time of Day	CVD Hx	PDS T2	ANU4	Smoking Status
sCRP (log)	0.221	-0.087	.318*	-0.014	-.004	-.249*	0.038	.022

Note. ANU4 = Australian National University-4 (measure of socioeconomic status), BMI = body mass index, CVD Hx =cardiovascular disease history, PDS = pubertal development scale.
* = $p \leq .05$

Table 4a.

Effortful Control Predicting sCRP

Variables	β	SE	p	95% CI (LL, UL)
Constant	-1.653	.775	.037*	-3.202, -.104
Effortful Control	-.042	.016	.010**	-.074, -.011

Table 4b.

Negative Emotionality Predicting sCRP

Variables	β	SE	p	95% CI (LL, UL)
Constant	-4.805	.612	< .000***	-6.030, -3.580
Negative Emotionality	.053	.028	.068†	-.004, .110

Table 4c.

Depressive Symptoms Predicting sCRP				
Variables	β	SE	p	95% CI (LL, UL)
Constant	-4.906	.538	< .000***	-5.983, -3.829
Depressive Symptoms	.041	.018	.024*	.006, .077

Note. *p < .05, **p < .01; *** p < .001; † = p < .09.

Table 5a.

Effortful Control Predicting sCRP Controlling for Covariates

Variable	β	SE	p	95% CI (LL, UL)
Constant	-2.433	4.197	.565	-10.851, 5.985
Gender	.397	.306	.200	-.217, 1.010
Age	-.021	.272	.940	-.566, .525
BMI	.059	.030	.056†	-.002, .119
Time of Day	-.064	.183	.727	-.431, .303
Family Cardiovascular Hx	1.123	1.238	.369	-1.361, 3.607
Pubertal Status	-.019	.026	.464	-.072, .033
ANU4	.001	.006	.889	-.012, .013
Smoking Status	.123	.592	.837	-1.064, 1.310
Effortful Control	-.044	.017	.012**	-.078, -.010

Note. BMI= Body mass index; Hx= history, ANU4 = Australian National University-4 (measure of socioeconomic status); LL = lower limit; UL = upper limit.

** = p < .01; † = p < .09.

Table 5b.

Negative Emotionality Predicting sCRP Controlling for Covariates

Variable	β	SE	p	95% CI (LL, UL)
Constant	-4.870	4.395	.273	-13.685, 3.946
Gender	.380	.310	.226	-.242, 1.002

Age	-.121	.271	.658	-.664, .423
BMI	.077	.030	.014**	.016, .138
Time of Day	.040	.180	.825	-.320, .400
Family Cardiovascular Hx	.905	1.256	.474	-1.615, 3.425
Pubertal Status	-.015	.027	.577	-.069, .039
ANU4	.000	.006	.980	-.013, .012
Smoking Status	.494	.608	.420	-.725, 1.714
Negative Emotionality	.064	.029	.029*	.007, .121

Note. BMI= Body mass index; Hx= history, ANU4 = Australian National University-4 (measure of socioeconomic status); LL = lower limit; UL = upper limit.
* = p < .05; ** = p < .01.

Table 5c.

Depressive Symptoms Predicting sCRP Controlling for Covariates

Variable	β	SE	p	95% CI (LL, UL)
Constant	-2.734	4.328	.530	-11.416, 5.947
Gender	.332	.313	.308	-.306, .950
Age	-.201	.276	.469	-.754, .352
BMI	.056	.032	.086†	-.008, .119
Time of Day	-.008	.186	.967	-.381, .365
Family Cardiovascular Hx	1.099	1.276	.393	-1.461, 3.659
Pubertal Status	-.018	.027	.510	-.072, .036
ANU4	.001	.006	.919	-.012, .014
Smoking Status	.369	.611	.548	-.856, 1.595
Depressive Symptoms	.035	.019	.078†	-.004, .074

Note. BMI= Body mass index; Hx= history, ANU4 = Australian National University-4 (measure of socioeconomic status); LL = lower limit; UL = upper limit.
* = p < .05; ** = p < .01; † = p < .09.

Table 6a.

Effortful Control Predicting sCRP Controlling for Covariates and Depressive Symptoms

Variable	β	SE	p	95% CI (LL, UL)
Constant	-2.584	4.212	.542	-11.035, 5.868
Gender	.393	.307	.206	-.222, 1.009
Age	-.063	.277	.821	-.619, .493
BMI	.053	.031	.092	-.009, .115
Time of Day	-.079	.184	.670	-.449, .291
Family Cardiovascular Hx	1.152	1.242	.358	-1.341, 3.644
Pubertal Status	-.017	.026	.519	-.070, .036
ANU4	.002	.006	.778	-.011, .014
Smoking Status	.201	.601	.739	-1.004, 1.406
Depressive Symptoms	.018	.021	.401	-.024, .059
Effortful Control	-.037	.019	.051†	-.075, .000

Note. BMI= Body mass index; Hx= history, ANU4 = Australian National University-4 (measure of socioeconomic status); LL = lower limit; UL = upper limit.

† = $p < .09$.

Table 6b.

Negative Emotionality Predicting sCRP Controlling for Covariates and Depressive Symptoms

Variable	β	SE	p	95% CI (LL, UL)
Constant	-4.547	4.411	.307	-13.399, 4.305
Gender	.377	.310	.231	-.246, .999
Age	-.153	.273	.578	-.702, .396
BMI	.067	.032	.041*	.003, .132
Time of Day	.006	.183	.976	-.362, .373
Family Cardiovascular Hx	.980	1.260	.440	-1.548, 3.508
Pubertal Status	-.014	.027	.617	-.067, .040
ANU4	.001	.006	.872	-.012, .014
Smoking Status	.513	.609	.403	-.709, 1.734

Depressive Symptoms	.020	.021	.341	-.022, .063
Negative Emotionality	.051	.032	.114	-.013, .115

Note. BMI= Body mass index; Hx= history, ANU4 = Australian National University-4 (measure of socioeconomic status); LL = lower limit; UL = upper limit.

* = $p < .05$.