Title: Parenting and adolescent health: Observed parenting behavior is associated with inflammation two and half years later in a community sample

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Abstract:

Objective: Family environments have an effect on physical health during adolescence, and one possible underlying mechanism is inflammation. However, little is known about the association between observed parenting behaviors and immune system functioning. The current study examined whether positive and negative emotional parental behaviors observed during family interactions were associated with inflammation in adolescents. Methods: Sixty-one parent-adolescent dyads (37 male adolescents, 60.6%; 15 male parents, 24.6%) were observed during two laboratory-based interaction tasks designed to elicit positive and conflictual emotional behaviors, respectively. Frequency of aggressive and positive parental behavior was coded. Adolescents were followed up approximately 2.5 years later and salivary concentrations of the inflammatory biomarker C-reactive protein (sCRP) were measured. Results: Controlling for BMI and depressive symptoms, lower sCRP was associated both with greater frequency of positive parental behaviors ($t = -3.087, p = 0.003$) and less frequency of aggressive parental behavior ($t = 2.087, p = 0.041$) in the conflictual task. Trend associations between positive behavior during the positive task and lower sCRP were also found. Conclusions: This is the first study to show that observed positive parenting is associated with lower levels of inflammation in adolescents.

Keywords: Adolescence; parenting emotional behavior; positive behavior; inflammation; salivary C-reactive protein; saliva; family environments

Abbreviations: ADS = Adolescent Development Study; ANU4 = Australian National University-4 scale; BMI = Body-mass index; CES-D = Center for Epidemiological Studies Depression scale; EC = effortful control; EPI = Event-Planning Interaction; NE = negative emotionality; PSI = Problem-Solving Interaction; RPM = Rate per minute; sCRP = salivary C-reactive protein; SLE = stressful life events
There is compelling evidence that the social environment influences physical health (Cohen, 2004), predicting morbidity and mortality to a similar extent to known risk factors, such as cigarette smoking and obesity (Holt-Lunstad, Smith, & Layton, 2010). The inflammatory response may underpin links between early social environments and disease (Fagundes, Bennett, Derry, & Kiecolt-Glaser, 2011), as chronic inflammation is a factor leading to medical illness (Out, Hall, Granger, Page, & Woods, 2012). Inflammation, which is a process by which the body attempts to remove pathogens and heal tissue, is known to respond to stress regardless of whether the stressor is physical or psychosocial (Hennessy et al., 2004; Selye, 1956). When this process is persistent and does not resolve, it becomes chronic inflammation, which is associated with a range of adverse medical outcomes such as cardiovascular disease (Kaplan & Frishman, 2001). Conflictual relationships are a type of psychosocial stress that has been linked with elevated inflammatory markers that indicate chronic, low-grade inflammation in adults (Kiecolt-Glaser et al., 2005). In contrast, supportive relationships have been linked with lower levels of inflammatory markers such as C-reactive protein (CRP) and interleukin (IL)-6 (Lutgendorf, Anderson, Sorosky, Buller, & Lubaroff, 2000; Shen, Farrell, Penedo, Schneiderman, & Orth-Gomer, 2010). Further, negative early childhood experiences are associated with elevated inflammation measured up to one and a half years later (Miller and Chen 2010). Because inflammatory processes react to psychosocial stress, it would be expected that stressful environments would be associated with increased levels of inflammatory markers such as CRP, while supportive environments may be associated with lower levels of inflammatory markers.

To date, the majority of research linking social relationships to inflammation has been with adults. However, adolescence is a time of profound psychological and biological change and an opportune time in which to examine family relationships and immune functioning.
stress may be especially deleterious for the health of adolescents; it could derail the normal development of neurobiology and physiology during this period (Gunnar & Quevedo, 2007) such that they are more vulnerable to exaggerated inflammatory responses (Miller et al. 2011).

Substantial research has indicated that family processes are particularly salient predictors of adolescent emotional (e.g., Schwartz et al. 2014), behavioral (e.g., Stocker et al. 2007), and health functioning (for review, see Repetti et al. 2002). Together with the adult literature described above, showing that stressful relationships are associated with immune processes, this research suggests the likelihood that family processes will be predictive of inflammatory processes, especially because the family is a group in the child’s microsystem that most directly influences the child’s development (Bronfenbrenner, 1992).

Most research that has examined the link between family environments and inflammation in adolescence has measured environments through self-report methods. For example, adolescents who reported greater frequency of interpersonal stress had higher levels of CRP eight months later (Fuligni et al., 2009). Another study found that harsher family climate was associated with greater inflammation in girls (Miller and Chen 2010). Similarly, in one recent study from our lab, we showed that parents’ own self-report on parenting behavior, particularly poor supervision and monitoring, is associated with their children’s levels of inflammation and immune activation (Byrne et al., In Press). These studies indicate that both adolescents’ impressions of stress and parents’ self-evaluations of parenting behavior are associated with poorer immune function. However, given that there may be discrepancies between reports of parenting and actual parenting behavior, it is notable that there have not been many research studies examining the association between immunity and observed parenting behavior. Factors such as depressive symptomatology, which may influence how adolescents interpret interpersonal situations (Gotlib
& Hammen, 1992), are also associated with elevated inflammation (Howren, Lamkin, & Suls, 2009) and decreased immune cell function (Zorrilla et al., 2001). It is possible that response bias is present in these studies because adolescents who recall or focus on more negative experiences may also have more pro-inflammatory profiles. Similarly, in our study, social desirability may have influenced parents’ reports. Two studies have examined observed parenting behavior and adolescent immune functioning. The first (Danese et al., 2008) used observational and questionnaire indices of parenting, and showed that childhood maltreatment was associated with inflammation and depression in adulthood. The second (O’Connor et al., 2015) examined emotional behavior in parents while interacting with their children (rather than parenting style or actions), which is known to be especially salient in the emergence of mental health problems in adolescents (Schwartz et al., 2012; Schwartz et al., 2014). That study found that parental negative behavior in a conflictual task predicted lower antibody concentrations in 11-year-old children post-vaccine. This suggests that how parents emotionally interact with their children may have an effect not only on their psychological but also their physical health.

A promising and feasible way to explore this issue in children is to measure inflammation in saliva, to see if oral inflammation is associated with measures of parenting behavior. Compared with blood, saliva is safer and easier to collect in research studies, and with the correct protocol, can ease burden for both participants and researchers (Granger et al., 2007). In particular, saliva has been found to be correlated with systemic or major sources of the general inflammatory marker, CRP (Byrne et al., 2013; Ouellet-Morin, Danese, Williams, & Arseneault, 2011; Out, Hall, Granger, Page, & Woods, 2012). Those studies showed medium to large effect sizes for correlations between serum or plasma and salivary CRP ($rs = 0.53, 0.38, 0.49, 0.72, 0.53$), although another study found that salivary and plasma CRP were not significantly correlated
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(Dillon et al., 2010). CRP is an acute phase protein that activates the complement system, which assists the immune system in killing and clearing pathogens from the body. It has been recognized as an inflammatory marker that increases rapidly after infection or tissue damage and is part of the body’s systemic inflammatory response (Black, Kushner, & Samols, 2004). CRP is, therefore, a particularly good general marker of inflammation. Higher levels of CRP have been shown to be related to various indicators of stress and poor mental health such as depression (see Howren, Lamkin, & Suls, 2009) and retrospective reports of risky early family environments (Taylor, Lehman, Kiefe, & Seeman, 2006). In particular, studies with children have shown that salivary levels of CRP (sCRP) are associated with various measures of physical (Goodson et al., 2014; Naidoo, Konkol, Biccard, Dudose, & McKune, 2012) and psychological (Cicchetti, Handley, & Rogosch, 2015) health, and have also been associated with the stress response in adults (Laurent, Lucas, Pierce, Goetz, & Granger, 2016; Lucas et al., 2016). On balance, the extant literature suggests that salivary measures of CRP are convenient, cost effective, associated with physical and psychological health measures, and may be associated with systemic levels of inflammation.

To date, no study with adolescents has examined observed parental emotional behavior and inflammation. More research is needed to objectively determine the aspects of parental behavior that may be associated with chronic and systemic inflammation. Observational methods of parenting behavior offer several advantages over self-report assessments and are considered the gold standard for measuring the objective quality of emotional and interactional behaviors between parent-child dyads (Zeman, Klimes-Dougan, Cassano, & Adrian, 2007). Observing parenting behavior eliminates response- or interpretation-bias from self-report questionnaires completed by the adolescent or the parent. Observational indices of parent behavior have been
shown to be strong predictors of adolescent mental health and neurobiological development (e.g., Schwartz et al., In Press; Whittle et al., 2014). This type of research can examine more direct associations between parenting behavior and adolescent physical health. Moreover, observational indices of parental behavior, derived from semi-naturalistic settings, have the potential to provide direct recommendations for inclusion in parenting interventions designed to promote adolescent health outcomes.

A further limitation of existing research is that almost all previous studies with adolescents have focused on adverse outcomes of negative environments rather than examining possible protective effects of specifically positive behavior. Previous work from our lab has shown that positive and negative parenting behavior both influence adolescent mental health, with higher levels of aggressive behavior and lower levels of positive behavior prospectively associated with higher levels of depressive symptoms and the onset of major depressive disorder (Schwartz et al. 2012; Schwartz et al. 2014). However, as of yet, little is known about the independent effect of both negative and positive observed parenting behaviors on immune system functioning in adolescence. The recent study by O’Connor and colleagues (2015) measured positive parenting behavior in a conflictual task, but it was not associated with immune function. Further research is needed to determine whether positive behavior is associated with levels of inflammatory markers.

Most observational studies of parenting behavior only use one type of context (usually one designed to elicit conflict) and therefore cannot determine if it is overall rates of behaviors across context or if it is rates of behaviors within certain types of contexts that are more predictive of adolescent health. While O’Connor and colleagues (2015) found that parental negative behavior in a conflictual task was associated with the child’s immune function, they did not examine
negative behavior in a task designed to elicit positive behavior. Our previous work using positive and negative interaction tasks suggests that negative parental behaviors during the positive task predict adolescent depression (Schwartz et al. 2011) and depressive and anxiety symptoms (Schwartz et al. 2012), possibly because they are not elicited by task demands. Also, positive behavior by parents during negative tasks has been shown to be protective against developing depression in adolescents (Schwartz et al. 2014). In the marital literature, positive behavior during negative tasks is predictive of relationship stability and satisfaction (Gottman, Coan, Carrere, & Swanson, 1998). Therefore, emotional behavior that is incongruent with context (i.e., negative behavior in a positive task or positive behavior in a negative task) may show particular associations with biological factors, as well.

Finally, the Miller and Chen (2010) study found that self-reported harsh family environments predicted levels of inflammation over the course of one and a half years, suggesting that family environments may be a distal and pervasive risk factor for immune health across adolescence. This is further supported by data showing that observed parental emotional behavior is stable over a period of one year during adolescence (Sheeber, Hops, Alpert, Davis, & Andrews, 1997). Therefore, further longitudinal data is needed to determine if observed parental behavior is associated with adolescent inflammation measured later, in order to establish this type of family variable as a possible distal risk factor for poorer immune health in adolescence.

The current study examined whether positive and negative parental emotional behaviors observed during family interaction tasks at approximately age 12 were associated with inflammation measured approximately 2.5 years later in adolescents, using a community cohort. We predicted that greater frequency of negative parental emotional behaviors would be associated with greater levels of sCRP, while greater frequency of positive parental emotional
behaviors would be associated with lower levels of sCRP. Also, in this study, we conducted observational assessments during interaction tasks designed to differentially elicit positive and negative affect, to explore whether the effect of parent behavior on adolescent inflammation differed across interactional context.

**Method**

The current study included data from the Adolescent Development Study (ADS) – a large-scale longitudinal research project conducted from 2004 to 2012 at the Orygen Research Centre at the University of Melbourne, Australia. Family data were collected during the first assessment at T1 when participants were approximately 12 years old, whilst adolescent immune data from a subgroup of participants were obtained two to three years later at T2.

**Recruitment and screening of participants**

The recruitment and screening of participants has been reported in detail previously (Yap, Allen, & Ladouceur, 2008). Screening (done approximately one year prior to T1) was conducted to identify a community sample of 10- to 12-year-old primary school students representing the full spectrum of temperament, as measured by the Early Adolescent Temperament Questionnaire–Revised (EATQ-R; Ellis and Rothbart 2001). Students (N = 2453) in the final year of primary school were recruited from schools across metropolitan Melbourne to participate in an initial school-screening phase, which involved completion of the EATQ-R. Based on their scores on this measure, a smaller sample of 415 students was selected to be part of the study. Adolescents at the extreme ends of the temperamental distributions of effortful control (EC) and negative emotionality (NE) were oversampled to maximize inter-individual differences. Of the
selected adolescents, 245 students participated in the T1 data collection phase (at approximately 12 years of age), which included a clinical assessment described below. A subset of 89 participants was randomly selected to participate in the immune analyses two to three years later at T2. Informed consent was obtained from the participants, as well as a legal guardian. This study was approved by the Human Research Ethics Committee at the University of Melbourne, Australia. Participants and their parents were informed that they could cease participation at any time.

**Procedure and measures**

**Clinical assessment at T1 and T2.** Participants completed a battery of psychosocial interviews and self-report questionnaires, including the Center for Epidemiological Studies Depression (CES-D; Radloff, 1991) scale for current depressive symptoms at T1 and T2, the “Stressful Life Events” Questionnaire (SLE; Lewinsohn et al. 1994) for total number of lifetime stressful events that happened to family members, friends, or self at T2, and Tanner stage line drawings (Morris & Udry, 1980) to assess pubertal stage at T2. One participant was at Tanner pubertal stage II, three at stage III, 36 at stage IV, 16 at stage V, and five did not report pubertal stage (see missing data analysis below). These questionnaires were included as covariates. Cronbach’s alpha coefficients of reliability for the CES-D at T1 (0.896) and T2 (0.841) were in the “good” internal consistency range and are similar to or better than those from other samples of adolescents in the same age range (Chabrol, Montovany, Chouicha, & Duconge, 2002; Manson et al., 1990).

**Family-interaction assessment at T1.** Parent-adolescent dyads completed two lab-based interaction tasks of 20 minutes each, designed to differentially elicit positive (Event-Planning
Interaction) and conflictual/aggressive (Problem-Solving Interaction) behavior. The Event-Planning Interaction was always performed first to prevent negative emotions elicited during the Problem-Solving Interaction from bleeding into the Event-Planning Interaction, as negative affect generally takes longer to extinguish (Gilboa & Revelle, 1994). For the Event-Planning Interaction, parents and adolescents were instructed to plan one or more enjoyable events based on topics they both agreed were ‘very pleasant’ (out of ‘not pleasant’, ‘somewhat pleasant’, and ‘very pleasant’) on the Pleasant Events Schedule (MacPhillamy, 1976), and for the Problem-Solving Interaction, the interviewer selected up to five topics that both the parent and adolescent endorsed as occurring the most frequently (participants were asked how many times they had discussed the issue in the last two weeks) and had the highest intensity of anger (participants filled out a Likert scale of 1 – 5 where 1 is ‘calm’, 3 is ‘a little angry’ and 5 is ‘angry’ for each issue) on the Issues Checklist (Prinz, Foster, Kent, & O’Leary, 1979), consisting of items representing common topics of conflict between parents and adolescents. Interactions were video-recorded with separate cameras focused on each participant.

Affective and verbal behavior were coded using the Living in Familial Environments (LIFE) event-based observational coding system that records micro changes in social behaviors, (Hyman Hops, Davis, & Longoria, 1995). The LIFE system consists of 10 nonverbal affect codes (e.g., anger, dysphoria, happy) and 27 verbal content codes (e.g., validation, complaint, provoke), coded within an event-based protocol in which new codes are entered each time the affect or content of one of the participants changes (i.e., coding is done in real time using an event-based protocol). Composite Aggressive and Positive behavior constructs were derived from the affect and content codes. Aggressive behavior includes all codes with contemptuous, angry, or belligerent affect, as well as cruel, provocative, annoying/disruptive, or argumentative verbal
statements made with neutral affect. Positive behavior consisted of all codes with happy, pleasant, and caring affect, as well as approving, validating, affectionate, or humorous comments made with neutral affect. We focused on the differential contributions of Aggressive and Positive behavior constructs (rather than a ratio) because they measure qualitatively different behaviors that could contribute separately to health outcomes. Trained observers were blind to psychosocial and demographic information about participants and a second observer coded 20% of interactions to provide estimates of inter-observer consistency. Training of observers is a rigorous process in which coders memorize code definitions and become skilled in differentiating between codes before they begin viewing videotapes. During training, observers code in pairs and discuss coding disagreements, which are resolved in supervision meetings. Training requires approximately 3 months of half-time work. Random pairs of observers were assigned to the interactions to minimize observer ‘drift’. Kappa coefficients for aggressive constructs were 0.70 for mothers and 0.77 for fathers, and positive constructs were 0.86 for mothers, and 0.84 for fathers, reflecting good levels of agreement. Consistent with our prior research (e.g., Schwartz et al. 2012), rate per minute (RPM) of each behavior construct was calculated, and represented the average number of times per minute that each type of behavior was displayed during interactions. The average pairwise intra-class correlations among four raters for the Aggressive and Positive rate per minute variables (over the Event-Planning Interaction and Problem-Solving Interaction and parents) were 0.81 and 0.78, respectively. The validity of the LIFE coding system as a measure of family processes has been established in numerous studies of adolescents (Katz and Hunter 2007; Schwartz et al. 2011; Sheeber et al. 2007). For example, observational indices of parent behavior, derived from the LIFE system, first, have been shown to vary between different family environments, such as mothers with
depression and families with marital distress (Hops et al., 1987), and second, as noted earlier, have been shown to be strong predictors of adolescent depression (for review, see Schwartz et al., In Press) and neurobiological development (Whittle et al., 2014).

**Immune Assessment at T2.** Two mL of whole, unstimulated saliva was collected from 89 participants at T2 (mean age = 15.49 years, SD = 0.50) using the passive drool method to analyze peripheral concentrations of CRP. As discussed above, CRP can be detected in saliva as well as in blood, and the measures correlate with medium to large effect sizes ($r = 0.38 - 0.72$) in adults (Ouellet-Morin et al., 2011; Out et al., 2012), and in adolescents ($r = 0.42$; Byrne et al., 2013). Previous work from our lab also shows that many inflammatory markers have higher detection rates in saliva compared to blood (Michelle L. Byrne et al., 2013).

Collection time varied by participant. However, research suggests that CRP does not have a diurnal variation (Meier-Ewert et al., 2001). 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. Though PIC is usually added to samples at the time of collection, this was not convenient to do in participants’ homes and personal freezers where the samples were collected. We also did not want to thaw the samples from the -20°C only to add the PIC and then re-freeze (which would have resulted in an additional freeze/thaw cycle). Therefore, we chose to add the PIC on the day of centrifugation. However, we did not test differences between including and not including the PIC, so it is not yet clear if the addition of PIC is a necessary step for future research. 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. Samples in this study therefore had a total of two freeze/thaw cycles. 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.

Concentrations of sCRP were analyzed according to manufacturer’s instructions, described elsewhere (Byrne et al., 2013), by the Bio-Plex multiplex bead array immunoassay system of human cytokine panel and plates read on Bio-Plex Array Reader (Bio-Plex 200 System and Bio-Plex Manager Version 4.0, Bio-Rad Laboratories, Inc., New South Wales, Australia). Multiplex platforms have been used to measure sCRP in previous studies (Byrne et al., 2013; Lee et al., 2012). 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; Ouellet-Morin et al., 2011). For the assays, the test volume was 50 μL, with a range of standards from 10 – 79560 pg/mL. The mean of recovery percentages \( \frac{\text{Observed Concentration}}{\text{Expected Concentration}} \) from standards was 99.42%, S.D. = 11.31%, range: 75% - 116%.

**Other Covariates.** A measure of socio-economic status (SES) was calculated for participants by using the Australian National University-4 (ANU4) scale for occupations (Jones & McMillan, 2001). Parents (both mother and father) were asked about their occupation and education during the T2 interview assessment. The ANU4 occupational scale is the Australian version of the International Socio-Economic Index of Occupational Status, a measure of SES based on the International Standard Classification of Occupations (Ganzeboom, De Graaf, & Treiman, 1992). Using similar methods, the ANU4 uses Australian Census data from 1996 to determine scores.
for 117 occupation categories. 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. ANU4 scores were averaged across the mother and father scores for each family (if applicable). This method of measuring SES has been recommended in Australia to the National Education Performance Monitoring Taskforce (Marks, McMillan, Jones, & Ainley, 2000).

BMI was measured at T2 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.

**Data Processing and Analysis**

Analyses were performed using IBM SPSS statistical software, version 21.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at p < .05.

Saliva from participants that had reported taking any medication (N=14) in the 24 hours prior to saliva collection was excluded. These medications included antihistamines, ibuprofen, and cold and flu tablets, which can affect inflammatory processes. Furthermore, one participant’s inflammatory marker was considered to be an outlier as it was 8.08 SD above the mean and was excluded, leaving 74 useable saliva samples. All sCRP levels were detectable. sCRP data was not normally distributed, so these values were log transformed (i.e., log(sCRP)).

The overlap between participants who completed the family interaction tasks with their parent at T1 (n=195), and participants who had useable saliva samples at T2 (n=74) was 61 participants (37 male adolescents and 15 male parents), and this comprised the final sample. This included eight male parent/male adolescent dyads, 29 female parent/male adolescent dyads,
seven male parent/female adolescent dyads, and 17 female parent/female adolescent dyads. Table 1 lists the percentages of ethnicity (identified by the adolescent) and household composition (identified by the parent). One-way ANOVAs showed that the subgroup of 61 dyads did not differ significantly from the larger group of 195 dyads that completed the family interaction tasks on measures of SES, adolescent depressive symptoms, BMI, age, or any of the parenting behavior variables.

Some data was missing for pubertal stage only from five participants (8.2%). 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 cannot be rejected, $\chi^2 = 24.730$ (df = 27; $p = 0.590$), i.e., that the data was missing completely at random.

**Covariates.** Given previously identified associations between inflammation and low SES (Appleton et al., 2012; Pollitt et al., 2007), biological sex, (Bouman, Heineman, & Faas, 2005), body-mass index (BMI) (Gillum, 2003), depression (for review, see Howren et al., 2009), stressful life events (Miller and Blackwell 2006), and puberty (Delany et al., 2016) these variables were tested for possible inclusion as covariates, and if significantly associated with inflammation, included in our models as covariates. Also, fathers have a significant role in emotion socialization in their children, but this influence may differ between mothers and fathers (Cassano, Adrian, Veits, & Zeman, 2006; L. B. Sheeber et al., 2007) as adolescents respond to mothers and fathers affective behavior differentially (Allen, Kuppens, & Sheeber, 2012), therefore, the sex of the parent participating in the interaction tasks was also tested as a possible covariate. Finally, although there is evidence that personality factors, such as conscientiousness (Luchetti, Barkley, Stephan, Terracciano, & Sutin, 2014), are associated with lower CRP, no
research has examined associations between inflammation and more stable, temperament factors. However, adolescent temperament has been shown to be associated with observed parental behavior (Davenport, Yap, Simmons, Sheeber, & Allen, 2011). Furthermore, studies have shown associations between children and adolescents’ other physiological stress systems and the two temperament factors from which we selected the current sample, EC (Oldehinkel, Hartman, Nederhof, Riese, & Ormel, 2011) and NE (Blandon, Calkins, Keane, & O’Brien, 2010); therefore, these were also tested as possible covariates. A series of bivariate two-tailed correlations between log(sCRP) and adolescent sex, parent sex, SES, BMI, CES-D at T1, CES-D at T2, number of SLE, Tanner pubertal stage, EC, and NE were conducted. Only BMI ($r = 0.298, p = 0.020$) and CES-D at T2 ($r = 0.343, p = 0.007$) were significantly associated with log(sCRP), therefore, these were included in the final models as covariates.

Four linear hierarchical regressions (one for each parental behavior in each task) were performed to assess associations between parental behaviors and adolescent inflammation. The dependent variable was log(sCRP). BMI and CES-D at T2 were entered as covariates in the first block. In the second block, a single parental behavior variable (e.g., aggressive behavior rate-per-minute in the Event-Planning Interaction task) was entered to test main effects.

**Results**

**Descriptive Statistics**

Means and standard deviations for all variables are presented in Table 2. Paired sample t-tests showed that parental aggressive behavior occurred less frequently in the Event-Planning Interaction compared to the Problem-Solving Interaction [$t(60) = -11.325, p = 0.000$], and positive behavior occurred more frequently in the Event-Planning Interaction [$t(60) = 7.843, p$]
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=0.000], in line with task demands. Correlations between variables (two-tailed) are presented in Table 3. Inflammation was positively correlated with BMI, CES-D at T2, and parental aggressive behavior in the Problem-Solving Interaction, and negatively correlated with parental positive behavior in the Problem-Solving Interaction.

**Hypothesis Testing**

Table 4 shows associations between parental behaviors and adolescent CRP, controlling for adolescent BMI and depressive symptoms ($R^2$s = 0.169 - 0.281), as well as the effect of the covariates in each model. Results showed that, as hypothesized, greater frequency of positive parental behavior and less frequency of aggressive behavior in the negative (problem-solving) interaction task were both associated with lower inflammation in adolescents (final results in Table 4). Contrary to hypotheses, no significant associations between behavior and inflammation were found during the positive (event-planning) task.

**Discussion**

The aim of this research was to examine relationships between observed parental emotional behaviors across positive and conflictual contexts, and levels of inflammation (as measured by sCRP) in adolescents. The hypotheses that more frequent parental expressions of positive and negative emotional behaviors would predict lower and higher levels of inflammation, respectively, were supported. Relationships between lower inflammation and both greater frequency of positive parental behavior and less frequency of aggressive parent behavior were significant in the conflictual task when controlling for covariates of adolescent depressive symptoms and BMI. Associations with positive and negative behavior during the positive task were not significant, suggesting that behavior in the context of a negative task is most important
for direct effects on adolescents’ immune health. Future research with larger sample sizes would be able to examine interactional effects of different types of task and adolescent physical health.

In particular, the strongest effect from these results was the positive parental behavior during an interaction task designed to elicit conflictual (i.e., stressful) behavior. This positive emotional behavior in a negative or stressful context may represent family processes that have a protective effect on inflammatory processes and the immune response to stress in adolescents, and this hypothesis is supported by previous work. For example, the Miller & Chen (2010) study mentioned previously showed that adolescent girls with self-reported harsh family environments had elevated production of inflammatory markers after a stressful life event, while girls from less harsh environments had lower inflammation. Harshness of family environment did not make a difference to inflammatory processes if the girls had not experienced any stressful life event recently. Therefore, positive family environments may have a buffering effect that safeguards adolescents’ physical health from stress. Negative family processes during a potentially stressful interactional task (i.e., the problem-solving interaction) may be associated with increased inflammation, but the opposite may be true if family processes remain supportive and positive during the task. In other words, in a supportive family environment with positive parental behavior, adolescents may not find the conflictual interactional task (and other life events) as stressful as adolescents who experience negative or harsh family environments, and therefore may have lower levels of inflammation. These results may have implications for family interventions designed to promote adolescent physical as well as mental health. Conflict is an unavoidable component of parent-adolescent interactions, and the results indicate that the nature of emotional behaviors during conflict has implications for immune functioning, as it does for psychological functioning (Schwartz et al., 2011; Sheeber et al., 2007). Interventions in which
parents are coached to increase positive affective behavior and reduce aggressive behavior, may have beneficial effects. Nonetheless, further research that measures actual changes in inflammation during observed tasks or as a result of randomized controlled trials of parenting interventions is needed to support this hypothesis.

It should be noted that our finding that positive parental behavior was associated with lower inflammation was inconsistent with findings from a recent study (O’Connor et al., 2015) that found no association between positive parental behavior and the child’s antibody response when vaccinated. However, while increased inflammation and decreased antibody response both indicate immune dysfunction, family environments may affect diverse aspects of a child’s immune system differently. For example, one study found that increased parental psychiatric symptoms were associated with a higher frequency of illness and enhanced natural killer cell function in children (Caserta et al., 2008). While adult studies have found inverse relationships between NK cell function and psychiatric symptoms, other studies of adolescents have also found positive correlations between these two aspects of immunity (Schleifer et al., 2002). Future studies should comprehensively characterize adolescent immune health, which may be different to adult immune health.

Additionally, we found an initial significant correlation between adolescent BMI and inflammation \((r = 0.298, p = 0.020)\), and when included as a covariate in our models, it was still significantly associated with inflammation in the model with positive behavior during the conflictual task \((t = 2.287, p = 0.026)\). This is consistent with much other research in children and adolescents (Breslin et al., 2012; Gillum, 2003; Głowińska & Urban, 2003; Lambert et al., 2004; Naidoo et al., 2012). However, our models showed that parental behavior better predicted
inflammation than BMI did. Nevertheless, future research should continue testing BMI as a possible covariate in studies of risk factors for elevated inflammation.

There are several limitations of the current study to note. First, we only measured inflammation once, so further research is needed to determine if parenting behavior is a protective factor for inflammation in adolescents, or simply a marker. Also, given that 2-3 years passed between the family interactions and collection of immune function data, other intervening stressors could have accounted for the changes in inflammation over time. Although we tested number of stressful life events as a possible covariate in our model (but did not include it), prospective research utilizing multiple waves of immunological measures is needed to truly determine the temporal relationships. It should be noted, however, that other studies found that psychosocial factors were associated with differences in CRP levels in adolescents eight months after stressful events were measured (Fuligni et al., 2009), suggesting that stressful interpersonal environments may have chronic effects on inflammation, and, as noted earlier, Miller & Chen (2010) found that self-reported harsh family environments were associate with a proinflammatory profile up to 1.5 years later. Furthermore, observed parental emotional behavior during interactions with their adolescents has shown to be stable over a period of one year (Sheeber et al., 1997).

Second, while our study excluded participants taking medication, we did not measure temperature or assess illness, or dental hygiene, which could be especially relevant for inflammatory markers in saliva. Physical illness and dental hygiene should be carefully assessed in future studies.

Third, our small sample size (N= 61) did not give us enough power to conduct analyses that would provide more detailed information about specificity of type of behavior and context.
Issues of multicollinearity prevented us from examining one type of behavior (e.g., positive) while controlling for the other type of behavior (e.g., negative). Also, our results differed depending on the type of context/task (positive vs. negative). However, while positive behavior and inflammation were only significantly associated in the negative task, they were associated in the positive task at a trend level. Therefore, it is not entirely clear yet if the inverse association between positive parental behavior and adolescent inflammation is context-specific. Our study did not measure inflammation during each type of task. It was only measured two years after the family interactions. Future studies could explore this difference by conducting repeated-measures analyses across contexts and measuring inflammation during each type of task. We also lacked the power to include child behavior as a variable to assess if conditional probabilities of behavior within the dyad during interactions would have an effect on the association between parental behavior and adolescent immune health. Previous research has shown that elicitation of certain parental behaviors in response to adolescent behaviors predicts the onset of depression (Schwartz et al., 2011) and this may also be relevant for increased inflammation.

Furthermore, due to the small number of father-adolescent dyads in the study, we lacked the power to examine mothers and fathers separately. Although bivariate associations between parent sex and adolescent inflammation were not significant, future research with larger numbers of participating fathers could determine the role that different parents play in the development of their adolescents’ immune health.

Fourth, our study used observed parental emotional behavior as a measure of family environment and did not have enough power to control for self-reported parental mental health symptoms. Future studies should also assess parental mental health symptoms, as these are known to be associated with immune functioning and illness in children (Caserta et al., 2008).
Finally, concentrations of CRP in the saliva samples were low compared not only to those normally found in blood (as would be expected), but also to those identified in saliva with adult samples (Laurent et al., 2016; Lucas et al., 2016; Mohamed, Campbell, Cooper-White, Dimeski, & Punyadeera, 2012; Ouellet-Morin et al., 2011; Out et al., 2012). While our samples were stored in -20°C for most of the time and degradation at this temperature may have been possible, previous research has shown that CRP is stable in saliva (e.g., at room temperature up to eight hours after collection; Ouellet-Morin et al., 2011) and it is recommended to store sCRP at or below -20°C (Salimetrics, Inc.; http://www.salimetrics.com). Therefore, this is unlikely to be the reason for our lower values and it is more likely due to the younger age of our sample. The values of salivary CRP in our sample (mean = 39 pg/ml, median = 20 pg/ml) were lower compared to one other study of children 11 years of age (Goodson et al., 2014), but higher than another study of children 9 years of age (Naidoo et al., 2012) and one other study of adolescents 13-18 years of age (Byrne et al., 2013). To the best of our knowledge, there are no other studies of salivary CRP in physically healthy children in the age range of our sample that have reported raw values of CRP. Currently, we are unable to interpret the values in our sample compared to samples in other studies because there are no validated norms by age for this measure yet. Therefore, more research on sCRP norms, which also account for flow rate, is needed for children and adolescents.

Importantly, these levels likely do not yet indicate the presence of physical illness. It is possible that higher, yet still subclinical levels, of inflammation in adolescence may be a risk factor for developing inflammatory diseases later in life if levels are chronic or increasing. However, as of yet, no research has followed children and adolescents longitudinally to determine how salivary markers of inflammation change over time. Although one study has
suggested clinical levels of sCRP may indicate risk for cardiovascular disease in adults (Out et al., 2012), no studies have examined if there are specific threshold levels of inflammation in children and adolescents that infer risk for any type of medical illness later in life. However, variations in subclinical levels of inflammation have been associated with mental health problems, especially depression, in children (Delany et al., 2016), adolescents (Copeland, Shanahan, Worthman, Angold, & Costello, 2012) and adults (Howren et al., 2009). Therefore, more longitudinal, prospective research is needed to determine if different social and parenting contexts have chronic effects on immune-related physical health outcomes. This study provides initial evidence identifying certain parental emotional behaviors that may be especially relevant for further investigation into inflammatory processes.

Furthermore, it is important to note that only CRP was measured as a marker of inflammation. Although CRP is a stable measure of inflammation that is related to depression, and a single measure such as this may be appropriate for small sample sizes, other cytokines that are involved in the early stages of the acute phase response, such as IL-6, IL-1, TNF-α, and IFN-α, may also be associated with family environment factors identified in this study and other mental health factors. Our data indicate that further studies broadening the inflammation monitoring to include the above and other cytokines would increase our understanding of the relationship between the immune system and mental health.

These results are an early step in a growing field of research that has implications for intervention strategies with parents. Historically, health promotion and disease preventative interventions targeting risk for inflammatory-related disorders have either focused on adults or on lifestyle factors during childhood (Shonkoff, Boyce, & McEwen, 2009), rather than on improving stressful family environments. Interventions to promote health outcomes may be
improved by attending to these psychosocial mechanisms. Additionally, although parents remain a significant influence on development across adolescence (Stocker et al., 2007), the importance of peer relationships increases over time (Steinberg & Morris, 2001). Therefore, future research, especially with older adolescents, should assess social environments across multiple contexts.

Current findings show that parental behavior is associated with inflammation measured 2.5 years later in adolescents. This study adds to a growing body of literature on family environment and immune health, which increasingly suggests a role for family-oriented intervention strategies in health research, especially those targeting prevention of inflammatory-related disorders such as obesity or cardiovascular disease. Family-focused or parenting interventions that assist parents in developing skills to engage in warm and supportive ways and reduce harsh and conflictual behavior could have lifelong implications for the health of their children.

Acknowledgements: The authors would like to thank all of the families involved in the study for their ongoing participation.
References


Association, 95(7), 545–552.


POSITIVE PARENTING AND INFLAMMATION


## Table 1 - Demographics

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>N</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>White/Caucasian</td>
<td>53</td>
<td>86.9%</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>4.9%</td>
</tr>
<tr>
<td>Aboriginal or Torres Strait Islander</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Arab or Middle Eastern</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Black or African</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Maori, Polynesian, Melanesian, or other Pacific Islander</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>More than one race</td>
<td>5</td>
<td>8.2%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Household composition</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-parent households with siblings and/or other relatives</td>
<td>44</td>
<td>72.1%</td>
</tr>
<tr>
<td>Two-parent households with no siblings or other relatives</td>
<td>5</td>
<td>8.2%</td>
</tr>
<tr>
<td>Single-parent (mother) households with siblings and/or other relatives</td>
<td>8</td>
<td>13.1%</td>
</tr>
<tr>
<td>Single-parent (mother) households with no other siblings or other relatives</td>
<td>2</td>
<td>3.3%</td>
</tr>
<tr>
<td>Single parent (father) households with siblings</td>
<td>1</td>
<td>1.6%</td>
</tr>
<tr>
<td>Relatives other than biological parents, stepparents, adoptive parents, or grandparents (e.g., aunts or uncles as parental figures)</td>
<td>1</td>
<td>1.6%</td>
</tr>
</tbody>
</table>
### Table 2 - Descriptive Statistics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± S.D. (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw sCRP (pg/ml)</td>
<td>39 ± 42 (0 – 190)</td>
</tr>
<tr>
<td>Log(CRP)</td>
<td>-3.76 ± 1.10 (-6.91 – -1.66)</td>
</tr>
<tr>
<td>SES (ANU4)</td>
<td>56.71 ± 20.65 (14.00 – 91.10)</td>
</tr>
<tr>
<td>BMI</td>
<td>22.80 ± 4.32 (15.92 – 32.61)</td>
</tr>
<tr>
<td>CES-D at T1</td>
<td>11.61 ± 10.14 (0.00 – 51.00)</td>
</tr>
<tr>
<td>CES-D at T2</td>
<td>9.24 ± 6.92 (0.00 – 27.00)</td>
</tr>
<tr>
<td>Number of SLE</td>
<td>12.56 ± 9.15 (0.00 – 50.00)</td>
</tr>
<tr>
<td>EC</td>
<td>3.43 ± 0.68 (1.71 – 4.92)</td>
</tr>
<tr>
<td>NE</td>
<td>3.34 ± 0.54 (2.23 – 4.77)</td>
</tr>
<tr>
<td>Parent behavior</td>
<td></td>
</tr>
<tr>
<td>Positive RPM</td>
<td>2.343 ± 0.479 (1.34 – 4.03)</td>
</tr>
<tr>
<td>Aggressive RPM</td>
<td>0.572 ± 0.421 (0.00 – 1.68)</td>
</tr>
</tbody>
</table>

sCRP = salivary C-reactive protein. SES = Socio-economic status. ANU4 = Australian National University-4 scale. CES-D = Center for Epidemiological Studies Depression scale. RPM = Rate per minute. EC = Effortful Control. EPI = Event Planning Interaction. NE = Negative Emotionality. PSI = Problem Solving Interaction. SLE = stressful life events
Table 3 – Pearson correlations between variables (two-tailed)

<table>
<thead>
<tr>
<th></th>
<th>Log (sCRP)</th>
<th>BMI</th>
<th>CES-D at T2</th>
<th>Positive behavior (EPI)</th>
<th>Aggressive behavior (EPI)</th>
<th>Positive behavior (PSI)</th>
<th>Aggressive behavior (PSI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log (sCRP)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>.298*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CES-D at T2</td>
<td>.343**</td>
<td>.287*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive behavior (EPI)</td>
<td>- .250</td>
<td>- .064</td>
<td>- .042</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggressive behavior (EPI)</td>
<td>.143</td>
<td>- .049</td>
<td>.247</td>
<td>- .135</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive behavior (PSI)</td>
<td>- .350**</td>
<td>.106</td>
<td>-.115</td>
<td>.401**</td>
<td>-.463**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Aggressive behavior (PSI)</td>
<td>.286**</td>
<td>.039</td>
<td>.127</td>
<td>-.187</td>
<td>.570**</td>
<td>-.452**</td>
<td>1</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01. sCRP = salivary C-reactive protein. CES-D = Center for Epidemiological Studies Depression scale. RPM = Rate per minute. EPI = Event Planning Interaction. PSI = Problem Solving Interaction.
Table 4 - Associations of parent emotional behavior (IV) with sCRP (DV), controlling for adolescent BMI and CES-D at T2

<table>
<thead>
<tr>
<th>Parent behavior</th>
<th>EPI</th>
<th>PSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-value</td>
<td>t-value</td>
</tr>
<tr>
<td>Positive RPM</td>
<td>-0.226</td>
<td>-1.917</td>
</tr>
<tr>
<td><strong>Covariates:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.205</td>
<td>1.665</td>
</tr>
<tr>
<td>CES-D at T2</td>
<td>0.275</td>
<td>2.238</td>
</tr>
<tr>
<td>Aggressive RPM</td>
<td>0.092</td>
<td>0.729</td>
</tr>
<tr>
<td><strong>Covariates:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.230</td>
<td>1.805</td>
</tr>
<tr>
<td>CES-D at T2</td>
<td>0.255</td>
<td>1.942</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01. IV = Independent variable. DV = Dependent variable. sCRP = salivary C-reactive protein. CES-D = Center for Epidemiological Studies Depression scale. RPM = Rate per minute. EPI = Event Planning Interaction (positive task). PSI = Problem Solving Interaction (negative task).
Author/s:
Byrne, ML; Horne, S; O'Brien-Simpson, NM; Walsh, KA; Reynolds, EC; Schwartz, OS; Whittle, S; Simmons, JG; Sheeber, L; Allen, NB

Title:
Associations Between Observed Parenting Behavior and Adolescent Inflammation Two and a Half Years Later in a Community Sample

Date:
2017-07-01

Citation:
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