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Longitudinal associations between attachment quality in infancy, C-reactive protein in early childhood, and BMI in middle childhood: preliminary evidence from a CPS-referred sample

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ABSTRACT

In the current pilot study, we examined whether insecure or disorganized attachment was associated with elevated inflammation (i.e. C-reactive protein [CRP]) in children with histories of child protective services (CPS) involvement, and whether early childhood CRP predicted body mass index (BMI) in middle childhood. Participants included 45 CPS-referred children and 39 low-risk comparison children, for whom we assessed levels of CRP in early childhood (Mean age = 4.9 years). For the CPS-referred children, who were drawn from an ongoing longitudinal study, we had attachment classifications (assessed during infancy with the Strange Situation) and BMI data (assessed during early and middle childhood); these data were not available for the low-risk comparison group. CPS-referred children who had insecure or disorganized attachments during infancy had higher levels of CRP in early childhood than CPS-referred children who had secure attachments, who had similar levels of CRP to low-risk comparison children. Among CPS-referred children, early childhood CRP predicted age 8 BMI, controlling for BMI at age 4. Findings offer preliminary support for the association between attachment quality and inflammation in early childhood, which may have implications for later physical health.

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Childhood adversity, such as exposure to maltreatment and poverty, is associated with increased risk for physical health problems across the lifespan, such as obesity and cardiovascular disease (Anderson et al., 2018; Ehrlich, Miller, & Chen, 2016; Felitti et al., 1998; Melchior, Moffitt, Milne, Poulton, & Caspi, 2007). Chronic low-grade inflammation is thought to be one mechanism by which childhood adversity “gets under the skin” to influence adulthood health outcomes (Miller, Chen, & Parker, 2011). Insecure or disorganized attachment, which occurs at elevated rates among children exposed to maltreatment and high sociodemographic risk (Cyr, Euser, Bakermans-Kranenburg, & Van Ijzendoorn, 2010), has been proposed as a possible risk factor for chronic inflammation.

KEYWORDS

Insecure attachment; maltreatment; C-reactive protein; body mass index; inflammation
The current study leveraged pilot data collected within the context of an ongoing longitudinal study of children with histories of child protective services (CPS) involvement to examine whether insecure and disorganized attachment quality during infancy predicted elevated C-reactive protein (CRP) levels in early childhood, and whether CRP levels, in turn, were associated with body mass index (BMI) in middle childhood.

**Childhood adversity and chronic inflammation**

Acute inflammatory responses are initiated in response to damage to healthy tissue resulting from injury or infection (Netea et al., 2017). Immune cells involved in the inflammatory response (e.g. white blood cells, T-cells) gather at the site to remove the pathogen and repair damaged tissue. Cytokines (e.g. interleukin-6, TNF-α), which serve a signaling role in regulating this inflammatory response, induce the synthesis of CRP. CRP is a molecule released by the liver, which further supports the repair and removal of affected tissue. Whereas these acute inflammatory responses are critical for survival, chronic inflammation poses significant threats to health (Hänsel, Hong, Cámara, & von Känel, 2010). Chronic inflammation may result from dysregulation of other closely integrated stress response systems (Danese & McEwen, 2012). Specifically, exposure to prolonged or intense psychosocial or environmental stressors may result in the overactivation of the sympathetic nervous system (Bierhaus et al., 2003; Kuras et al., 2017; Rohleder, 2014). In addition, the hypothalamic pituitary adrenal (HPA) axis and the parasympathetic nervous system, both of which serve anti-inflammatory functions, may down-regulate following exposure to chronic stress (Bernard, Butzin-Dozier, Rittenhouse, & Dozier, 2010; McLaughlin et al., 2015). The resulting low-grade chronic inflammation can be examined by measuring the concentration of inflammatory markers, including cytokines and CRP. CRP has been suggested as a good measure of chronic inflammation because it is more stable than cytokines when measured in blood and is a predictor of risk for age-related diseases, such as cardiovascular disease (Ehrlich, Miller, Jones, et al., 2016).

Elevated levels of CRP have been found in adults who grew up in low socioeconomic status (SES) households or experienced maltreatment during childhood (Baumeister, Akhtar, Ciufolini, Pariante, & Mondelli, 2016; Coelho, Viola, Walss-Bass, Brietzke, & Grassi-Oliveira, 2014; Danese, Pariante, Caspi, Taylor, & Poulton, 2007; Pollitt et al., 2007; Slopen et al., 2015; Taylor, Lehman, Kiefe, & Seeman, 2006). Emerging evidence also suggests that elevated CRP can be observed during childhood, following exposure to adversity. For example, in a nationally representative sample of approximately 13,000 children (between 2 and 18 years old), poverty and low parental education were associated with elevated low-grade inflammation, with effects stronger in early versus late childhood (Schmeer & Yoon, 2016). Similarly, Broyles et al. (2012) found that children (between 5 and 18 years old) living in risky neighborhoods, characterized by high poverty and crime, had 2.7 times the odds of having elevated CRP compared to children from low-risk neighborhoods. Further, in the Environmental Risk (E-Risk) Longitudinal Twin Study, child victimization (i.e. domestic violence exposure, physical abuse and neglect, sexual abuse, emotional abuse and neglect, and peer bullying), measured prospectively from birth to age 12 years, was associated with elevated CRP at age 12
among children with concurrent depression (Danese et al., 2011), and with elevated CRP at age 18 among females (Baldwin et al., 2018).

Although these studies provide strong support for the biological embedding model of early adversity (Miller et al., 2011), few studies have examined whether chronic inflammation is evident in very early childhood (i.e. before age 5) following exposure to early adversity. One exception is a recent study of 49 low-income mother-infant dyads recruited from a Women, Infants, and Children (WIC) program (David, Measelle, Ostlund, & Ablow, 2017). CRP was assessed from baseline saliva samples taken during a laboratory visit conducted when infants were 17 months old. Family socioeconomic disadvantage (a composite of low household income and maternal report of limited resources) and maternal psychosocial stress (a composite of parenting stress and daily hassles) independently predicted infants’ CRP levels. Thus, this study provides preliminary support that environmental adversity may be associated with chronic inflammation even very early in life.

**Childhood obesity and inflammation**

Childhood obesity is associated with negative long-term outcomes in adulthood, including diabetes, cancer, hypertension, heart disease, asthma, metabolic syndrome, and premature mortality (Baker, Olsen, & Sørensen, 2007; Bjørge, Engeland, Tverdal, & Smith, 2008; Franks et al., 2010; Okasha, Davey, McCarron, & McEwen, 2002). Emerging evidence suggests that risk for later obesity may be evident within the first few years of life. For example, Pryor et al. (2011) showed that BMI in early childhood was predictive of being overweight or obese later on; toddlers with a higher BMI were more likely to be overweight or obese at 8 years old, whereas toddlers with a lower BMI were more likely to have normal weight throughout childhood. Children who are overweight (BMI >85th percentile) in early childhood are five times more likely to remain overweight during adolescence than typical-weight peers (Nader et al., 2006). In addition, in a study of approximately 1700 children, 10.9% of individuals showed an “early onset” overweight trajectory, characterized by becoming overweight by 2 years of age and remaining so until 12 years of age (Li, Goran, Kaur, Nollen, & Ahluwalia, 2007). This trajectory was more than twice as common as the “late onset” trajectory, characterized by becoming overweight at 6 years of age. Taken together, these studies suggest that early childhood is a critical period for examining the onset of obesity-related problems.

Chronic inflammation, as assessed via CRP, has been associated with obesity across developmental periods. In the National Health and Nutrition Examination Survey, cross-sectional analyses of over 16,000 children demonstrated an association between obesity risk and CRP levels from ages 3 to 17 years (Skinner, Steiner, Henderson, & Perrin, 2010). Longitudinal studies further suggest that elevated CRP in childhood predicts obesity in adulthood. For example, in the Cardiovascular Risk in Young Finns Study, children with elevated levels of CRP were at increased risk for obesity in adulthood 21–27 years later (Juonala et al., 2011). Although it is unclear to what extent CRP plays a causal role in obesity, it is likely that there are bidirectional associations at play, such that inflammation both results from obesity (Das, 2001; Selvin, Paynter, & Erlinger, 2007; Timpson et al., 2011) and contributes to obesity (Bochud et al., 2009; Leibowitz et al., 2012). In support of the role of CRP as a precursor of obesity, a study of approximately 1,000 children living in the Brazilian Amazon showed that high CRP
concentrations predicted greater increases in BMI over time (Lourenço, Cardoso, & Study Team, 2014).

Insecure attachment as a risk factor for inflammation and obesity

There are a number of possible socioemotional and physiological mechanisms by which attachment patterns may influence trajectories towards physical health risk among children who experience early adversity. Secure attachment reflects the capacity of children to effectively seek and obtain comfort from their parent in times of distress, typically resulting from a history of interactions characterized by sensitive and responsive care (Ainsworth, Blehar, Waters, & Wall, 1978). When children develop insecure or disorganized attachments, presumably following insensitive, inconsistent care, or frightening care, they are unable to rely on their parents in times of distress. These differences in attachment quality also shape children’s emerging emotion regulation strategies (Halligan et al., 2013; Qu, Leerkes, & King, 2016). Children’s capacities to access support or effectively cope with stressors on their own may influence the extent to which physiological systems are activated in response to challenge, as well as their regulation over time (Hostinar, Johnson, & Gunnar, 2015; Hostinar, Sullivan, & Gunnar, 2014). Children with insecure or disorganized attachments, for example, have been found to show dysregulated functioning of the HPA axis, characterized by heightened physiological responses to acute stress (Bernard & Dozier, 2010; Hertsgaard, Gunnar, Erickson, & Nachmias, 1995), and low maternal sensitivity has been linked with blunted diurnal rhythms of cortisol (Ben-Dat Fisher et al., 2007; Roisman et al., 2009). Combined with the dysregulation of other stress response systems, these maladaptive physiological responses may contribute to chronic inflammation (Danese & McEwen, 2012).

To date, the empirical evidence for the association between insecure attachment and elevated CRP is limited to a few studies. In a longitudinal study from adolescence to adulthood, Jones et al. (2017) found that adolescents’ perceptions of their parents as a secure base prospectively predicted levels of CRP in adulthood. Specifically, adolescents (approximately 12 years old) who endorsed that they could consistently depend on their parents for support when faced with a social/personal problem had lower levels of CRP at age 32 than adolescents who lacked such expectations of parental secure base support. This effect was specific to secure base support (not general support) during adolescence (not early adulthood), suggesting that expectations about attachment relationships specifically, perhaps during sensitive periods of development, may have particular relevance for inflammatory processes. Perhaps of most relevance to the current study, in a cross-sectional study of low-income mother-infant dyads, Measelle, David, and Ablow (2017) found that 17-month-old infants with disorganized attachments had higher levels of salivary CRP than infants with secure or insecure attachments. This study is the only one to date examining the link between attachment quality and inflammation during infancy.

Meta-analytic findings have also supported a link, albeit small ($r = .08, p = .06$), between attachment security and BMI in children (Diener et al., 2016). Of the five studies in this meta-analysis that examined this link in children, only two of them
assessed attachment using the Strange Situation (Ainsworth et al., 1978). In the first, Anderson and Whitaker (2011) found that insecure attachment during infancy was linked with increased risk of obesity at preschool age. In the second, Anderson, Gooze, Lemeshow, and Whitaker (2012) found that poor-quality maternal-child relationships (quantified as an aggregate measure of insecure attachment and low maternal sensitivity assessed across three time-points) were associated with obesity in adolescence. Of note, both of these studies examined the link between attachment and obesity in large community samples; thus, little is known about the association between attachment quality and obesity in predominantly high-risk samples. It is possible that the effect of attachment quality on obesity is different in high-risk samples, when attachment insecurity may be only one of many risk factors linked to obesity, such as poverty (Lee, Andrew, Gebremariam, Lumeng, & Lee, 2014), maltreatment (Danese & Tan, 2014), and exposure to stress prenatally (Reynolds, Labad, Buss, Ghaemmaghami, & Raikkonen, 2013; Stout, Espel, Sandman, Glynn, & Davis, 2015). Further, no studies have explored whether inflammation mediates the association between attachment quality in infancy and childhood obesity.

The current study: linking attachment, inflammation, and obesity in high-risk CPS-referred children

CPS-referred children represent a vulnerable group typically exposed to multiple forms of early adversity (e.g. poverty, abuse/neglect, insensitive care, parental mental health problems; Campbell, Cook, LaFleur, & Keenan, 2010; Connell et al., 2007; Jonson-Reid, Drake, & Zhou, 2013). Children exposed to maltreatment and/or elevated sociodemographic risk are at an elevated risk for insecure and disorganized attachment (Cyr et al., 2010), as well as physical health problems across the lifespan, such as obesity (Danese & Tan, 2014; Helton & Liechty, 2014; Mason et al., 2016). Given that CPS-referred children represent a population with elevated exposure to these types of early adversity, we were interested in examining links between attachment quality, inflammation, and obesity risk in such a sample.

For the current pilot study, we collected CRP samples during early childhood (4–6 years old) from a subsample of high-risk CPS-referred children who were enrolled in an ongoing longitudinal study, as well as from children in a low-risk comparison group. For the CPS-referred sample, we had attachment classifications (assessed during infancy with the Strange Situation) and BMI data (assessed during early and middle childhood) from the ongoing longitudinal study; these data were not available for the low-risk comparison group. We tested three principal hypotheses. First, we predicted that CPS-referred children would have higher CRP levels than low-risk comparison children. Second, among CPS-referred children (the only children for whom we had attachment assessments), we predicted that children with insecure or disorganized attachments would have higher CRP levels than children with secure attachments. We further examined whether CPS-referred children with secure attachments and those with insecure attachments differed in CRP levels from low-risk comparison children. Finally, in the CPS-referred children, we explored the associations among attachment quality, CRP, and BMI, predicting that CRP in early
childhood would be associated with BMI in early childhood and middle childhood, and that there would be an indirect effect of attachment quality in infancy on BMI in middle childhood via early childhood CRP.

Method

Participants

One hundred children and their mothers were recruited for the present study: 55 dyads with histories of referral to CPS and 45 dyads recruited from the community without histories of CPS involvement. The final analytic sample included 84 participants (reasons for the reduced sample size are described below). Descriptive statistics for demographic data for each group are presented in Table 1.

Procedure

Participant recruitment

CPS-referred mothers and children were drawn from an ongoing longitudinal study examining the effectiveness of a parenting intervention for infants. All CPS-referred mothers were originally referred to the study when children were infants (under 24 months old) following involvement with CPS; after consenting, dyads were randomly assigned to participate in one of two 10-session parenting interventions: Attachment

Table 1. Demographic Information and Descriptive Statistics for CPS-referred and Low-risk Samples.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CPS-referred (n = 45)</th>
<th>Low-risk (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child ethnicity</td>
<td>n %</td>
<td>n %</td>
</tr>
<tr>
<td>African American</td>
<td>35 (77.8)</td>
<td>29 (74.4)</td>
</tr>
<tr>
<td>White</td>
<td>4 (8.9)</td>
<td>8 (20.5)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3 (6.7)</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Biracial</td>
<td>3 (6.7)</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Male</td>
<td>26 (57.8)</td>
<td>16 (41.0)</td>
</tr>
<tr>
<td>Female</td>
<td>19 (42.2)</td>
<td>23 (59.0)</td>
</tr>
<tr>
<td>Dichotomized Parent Risk Factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low income</td>
<td>39 (86.7)</td>
<td>15 (38.5)</td>
</tr>
<tr>
<td>Low education</td>
<td>18 (40.0)</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>Single mother</td>
<td>30 (66.7)</td>
<td>21 (53.8)</td>
</tr>
<tr>
<td>Young mother</td>
<td>15 (33.3)</td>
<td>9 (23.7)</td>
</tr>
<tr>
<td>Attachment Classifications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secure</td>
<td>19 (46.3)</td>
<td>–</td>
</tr>
<tr>
<td>Avoidant</td>
<td>4 (9.8)</td>
<td>–</td>
</tr>
<tr>
<td>Resistant</td>
<td>2 (4.9)</td>
<td>–</td>
</tr>
<tr>
<td>Disorganized</td>
<td>16 (39.0)</td>
<td>–</td>
</tr>
<tr>
<td>M (SD)</td>
<td>31.6 (8.0)</td>
<td>31.6 (6.2)</td>
</tr>
<tr>
<td>Mother age</td>
<td>19.8–49.1</td>
<td>22.8–45.5</td>
</tr>
<tr>
<td>Child age</td>
<td>4.9 (0.5)</td>
<td>5.0 (0.6)</td>
</tr>
<tr>
<td>Household income</td>
<td>$15,574 (13,058)</td>
<td>$33,718 (54,051)</td>
</tr>
<tr>
<td>Risk composite</td>
<td>2.18 (0.96)</td>
<td>1.69 (0.92)</td>
</tr>
<tr>
<td>CRP level (mg/L)</td>
<td>0.43 (0.38)</td>
<td>0.37 (0.31)</td>
</tr>
<tr>
<td>Log-transformed CRP</td>
<td>−0.47 (0.29)</td>
<td>−0.53 (0.27)</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.88 (0.04)</td>
<td>0.89 (0.04)</td>
</tr>
<tr>
<td>BMI at age 4 (Z score)</td>
<td>0.19 (1.48)</td>
<td>−3.39–3.39</td>
</tr>
<tr>
<td>BMI at age 8 (Z score)</td>
<td>0.38 (1.43)</td>
<td>−2.20–2.71</td>
</tr>
</tbody>
</table>
and Biobehavioral Catch-up (ABC; Dozier & Bernard, 2017), which aimed to enhance parental sensitivity, or Developmental Education for Families (DEF), which targeted children’s language and motor development. Following completion of the interventions, dyads continued to participate in annual follow-up visits. For the current study, a subset of CPS-referred children (n = 55: 27 ABC, 28 DEF) from the ongoing longitudinal study were recruited for the collection of blood spot samples when children were between 4 and 6 years old. Low-risk comparison children and mothers were recruited from community day-care centers, local parent groups, announcements on a university website, and previous studies.

Research assessments
Both CPS-referred and low-risk comparison children and mothers participated in an identical home visit for the collection of blood spot samples when children were approximately 4 to 6 years old (Mean age = 4.9 years, SD = 0.6). Also during this visit, key covariates were assessed, including demographic and risk information (e.g. income-to-needs ratio) and children’s waist-to-hip ratio. For the CPS-referred group only, additional data (i.e. attachment classifications, BMI) were drawn from other research assessments completed as part of the ongoing longitudinal study, including: the Strange Situation (Ainsworth et al., 1978), which was conducted at a laboratory visit following completion of either intervention; and weight and height measurements, which were collected when children were 4 years old and 8 years old at annual home visits. Given that the low-risk comparison group was only recruited to provide a comparison for the pilot study of CRP in early childhood, longitudinal data on attachment during infancy and BMI during middle childhood were not available for these children.

Measures

C-reactive protein
At the home visit, the child’s finger was cleaned with an alcohol swab and incised with a pressure-activated, retractable-tip, disposable lancet. Five drops of whole blood were collected on filter paper (Whatman 903 Protein Saver Card; GE Healthcare). Cards were allowed to dry completely, then transferred into small Ziploc bags and stored in a −20°C freezer until being shipped for assay. Samples were shipped overnight with icepacks to the Laboratory for Human Biology Research at Northwestern University, Evanston, IL, and assayed for CRP levels using a high-sensitivity immunoassay (ELISA) protocol developed by McDade, Burhop, and Dohnal (2004). Following this protocol, validation studies have shown high correlations between CRP assayed from dried blood spot samples and plasma. All samples were analyzed by the same technician, using a single lot of capture antibody, detection antibody, and calibration material, to minimize between-assay variation (McDade, Borja, Largado, Adair, & Kuzawa, 2016). Between-assay coefficients of variation for low, mid, and high control samples were 7.81%, 12.21%, and 7.82%, respectively.

Values were converted to mg/L and an equation was used to convert blood spot concentration of CRP to serum concentration (Serum CRP [mg/L] = 1.15 × blood spot CRP [mg/L] + 0.13, McDade et al., 2004), following studies of similar samples (Danese et al., 2011). This conversion facilitates comparisons with clinically relevant cut-off values. Although this formula was developed based on matched samples of blood spot and
serum, caution is warranted given that we did not collect serum data for the participants in the current study.

Four CRP values (two CPS-referred, two low-risk) were excluded from analyses due to having a CRP concentration >10 mg/L, which is the recommended cut-off value for identifying elevations in CRP that may reflect acute infection, rather than chronic inflammation (Pearson et al., 2003). Additional outliers, defined as >2 SD above the mean group CRP level were removed (three CPS-referred, two low-risk) to prevent extreme values from influencing the results. Finally, CRP values were log-transformed to normalize data due to a positive skew, following procedures used in other studies (Danese et al., 2011; Guan et al., 2016). Descriptive statistics for CRP data are provided in Table 1.

**Attachment**

For children in the CPS-referred group, attachment was assessed using the Strange Situation (Ainsworth et al., 1978). The Strange Situation is a laboratory procedure that involves two brief separations and reunions between an infant and the parent. The Strange Situation was administered during a post-intervention laboratory visit, when children were between 12.4 and 29.5 months old ($M_{age}$ 19.0, $SD = 4.7$). Children were classified as secure, avoidant, or resistant; in addition to a primary classification, children could be coded as disorganized, following criteria specified by Main and Solomon (1990). A primary coder scored all of the videos (third author, who was trained by Alan Sroufe and Elizabeth Carlson at University of Minnesota and met certification criteria on their reliability set), with 36% of the videos (randomly selected) scored by a second expert coder (Elizabeth Carlson). The coders agreed on 93% of videos for the three-way classification ($k = 0.64$) and 87% of videos for the four-way classification ($k = 0.87$). Conferred classifications were used in analyses. For analyses, we compared children with secure attachments ($n = 19, 46\%$) to children with insecure or disorganized attachments ($n = 22, 54\%$); four children did not complete the laboratory research visit at which the Strange Situation was conducted.

**Body mass index**

CPS-referred children’s weight and height were measured at annual follow-up visits when children were approximately 4 years old ($n = 45$, Mean age = 4.0 years, $SD = 0.1$) and 8 years old ($n = 37$, Mean age = 8.3 years, $SD = 0.3$). Missing data at age 8 were due to dyads not completing the research visit. BMI was calculated using the Center for Disease Control and Prevention (CDC) formula: weight in kilograms divided by height in meters squared, and converted to z-scores based on children’s age and gender. Raw BMI scores were converted to BMI z-scores (standardized by age and gender) based on the CDC growth charts; BMI z-scores were used in analyses (See Table 1 for descriptive statistics).

**Covariates**

Following procedures used in other papers (e.g. Baldwin et al., 2018), children waist-to-hip ratio (ratio of waist circumference to hip circumference measuring using a flexible measuring tape) was assessed as a covariate. Children’s temperature (in °F, measured using an oral thermometer) was also assessed to ensure that children did not have a fever at the time of blood spot sample collection; all children had temperatures within a typical range (97.1–99.6 °F).
In addition, we computed a composite sociodemographic risk score, following recommendations of previous studies (e.g. Appleyard, Egeland, van Dulmen, & Sroufe, 2005), to control for risk factors beyond CPS involvement that may have differed between groups and affected outcomes of interest. Specifically, the following risk indicators were coded dichotomously and summed: low income (income-to-needs ratio <1), low education (less than high school education), single parenthood, and being a young mother (defined as <21 years old at the time of the child’s birth). See Table 1 for descriptive information about risk indicators and the composite risk score across groups.

**Analytic approach**

First, we conducted preliminary analyses to examine bivariate associations between primary outcomes of interest and possible covariates (e.g. intervention group, demographic and risk variables, waist-to-hip ratio). Second, we conducted an analysis of covariance with group (CPS-referred vs. low-risk) predicting log-transformed CRP level, including waist-to-hip ratio and composite risk as covariates. The remaining analyses were conducted using only the CPS-referred group; intervention group was included as an additional covariate in all analyses of the CPS-referred children. Third, we conducted a hierarchical multiple regression to examine whether attachment quality predicted children’s CRP levels in early childhood. Log-transformed CRP was entered as the dependent variable, with attachment classification (coded 0 for secure, 1 for insecure/disorganized) as the predictor in Model 1, and covariates (i.e. waist-to-hip ratio, intervention group, composite risk) included as covariates in Model 2. Fourth, in another hierarchical multiple regression, we examined whether CRP levels predicted children’s BMI at age 8 (Model 1), controlling for age 4 BMI, composite risk, and intervention group (Model 2). Finally, we examined whether secure attachment had a significant indirect effect on BMI at age 8 via early childhood CRP levels, using a mediation model in PROCESS 2.16.3 (Hayes, 2013).

**Results**

**Preliminary analyses**

There were no significant differences between children whose mothers participated in ABC and those whose mothers participated in DEF in their likelihood of having a secure attachment for the sub-sample included in the current study (although there was for the full sample (Bernard et al., 2012). There were also no significant intervention group differences in CPS-referred children’s CRP levels or BMI at either time point. Thus, we collapsed ABC children ($n = 21$) and DEF children ($n = 24$) into the CPS-referred group for primary analyses.

There were no significant differences between CPS-referred and low-risk groups in mother age, child age, child minority status, child gender, or child waist-to-hip ratio. CPS-referred and low-risk groups significantly differed in composite risk score, $t(82) = 4.26, p < .001$, with CPS-referred children having on average one more risk factor than low-risk children. Bivariate correlations among covariates and main study outcomes are reported in Table 2.
Principal analyses

CPS-referred versus low-risk group differences in early childhood CRP

In an analysis of covariance controlling for waist-to-hip ratio and composite risk, the effect of risk group on log-transformed CRP was non-significant, $F(1, 80) = 1.48, p > .05)$, suggesting no overall difference in log-transformed CRP between CPS-referred children ($M = -.47, SD = .29$) and low-risk children ($M = -.53, SD = .27$) in early childhood.

Effect of attachment quality on CRP among CPS-referred children

Hierarchical multiple regression results demonstrated that attachment quality significantly predicted early childhood CRP among CPS-referred children (See Table 3). Children who had insecure or disorganized attachments in infancy had higher CRP levels in early childhood than children who had secure attachments in infancy, controlling for covariates. To compare CPS-referred children with insecure or disorganized attachments and CPS-referred children with secure attachments and to low-risk children, we conducted an analysis of covariance with log-transformed CRP level as the dependent variable, group as the predictor, and waist-to-hip ratio and composite risk as covariates. There was a significant effect of group on CRP, $F(1, 75) = 3.98, p = 0.023$ (See Figure 1). Pairwise comparisons demonstrated that CPS-referred children with insecure or disorganized attachments had significantly higher CRP levels than both low-risk children ($p = 0.010$) and CPS-referred children with secure attachments ($p = 0.026$); CPS-referred children with secure attachments did not differ significantly from low-risk children ($p = 0.705$).

Table 2. Bivariate Correlations Among Covariates and Primary Variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Risk composite</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Waist-to-hip ratio</td>
<td>−0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. BMI at age 4</td>
<td>−0.05</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. BMI at age 8</td>
<td>0.05</td>
<td>0.03</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>5. Log-transformed CRP level</td>
<td>−0.04</td>
<td>0.13</td>
<td>0.26</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Note: †$p < .10$, **$p < .01$. Correlations with BMI are based on reduced samples of only CPS-referred children: at age 4 ($n = 45$), and at age 8 ($n = 37$).

Table 3. Hierarchical Regression Analysis Predicting Levels of Early Childhood C-reactive Protein.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Model 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecure/disorganized attachment</td>
<td>0.19</td>
<td>0.08</td>
<td>2.35</td>
<td>0.024</td>
<td>0.19</td>
<td>0.09</td>
<td>2.21</td>
</tr>
<tr>
<td>Composite risk</td>
<td>−0.02</td>
<td>0.05</td>
<td>−0.39</td>
<td>0.702</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention group</td>
<td>−0.09</td>
<td>0.09</td>
<td>−1.06</td>
<td>0.294</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.03</td>
<td>1.13</td>
<td>0.03</td>
<td>0.977</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Insecure or disorganized attachment: 0 = Secure, 1 = Insecure or disorganized; Intervention group: 0 = DEF, 1 = ABC
Effect of early childhood CRP on age 8 BMI among CPS-referred children

In bivariate correlations, early childhood CRP was associated with BMI at age 4, \( r(45) = 0.26, p = .08 \), and BMI at age 8, \( r(37) = .52, p = .001 \). Hierarchical multiple regression demonstrated that early childhood CRP levels significantly predicted BMI at age 8, controlling for BMI at age 4 (See Table 4), suggesting that elevated CRP in early childhood was associated with increases in BMI over time. Mediation analyses demonstrated a significant indirect effect [95% CI: 0.04, 1.11] from insecure/disorganized attachment to age 8 BMI via early childhood CRP, controlling for composite risk, intervention group, and age 4 BMI (See Table 5).

**Table 4. Hierarchical Regression Analysis Predicting Body Mass Index at Age 8.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>t</td>
<td>p</td>
<td>B</td>
<td>SE</td>
<td>t</td>
<td>p</td>
</tr>
<tr>
<td><strong>Predictor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP level</td>
<td>2.46</td>
<td>0.69</td>
<td>3.57</td>
<td>0.001</td>
<td>2.00</td>
<td>0.66</td>
<td>3.03</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Covariates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite risk</td>
<td>0.05</td>
<td>0.21</td>
<td>0.22</td>
<td>0.825</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention group</td>
<td>0.65</td>
<td>0.37</td>
<td>1.74</td>
<td>0.091</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI at age 4</td>
<td>0.42</td>
<td>0.14</td>
<td>3.10</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. CRP level is log-transformed CRP value; Intervention group: 0 = DEF, 1 = ABC; BMI at age 4 and at age 8 are z-scores calculated based on norms for child gender and age.
Findings demonstrated that attachment quality was associated with CRP levels in early childhood among CPS-referred children. Specifically, CPS-referred children with insecure or disorganized attachments had higher levels of CRP than CPS-referred children with secure attachments and low-risk comparison children. In contrast, CPS-referred children with secure attachments had similar levels of CRP to low-risk comparison children. Further, CRP in early childhood predicted children’s BMI in middle childhood, controlling for early childhood BMI, suggesting that elevated low-grade inflammation may predict weight gain during childhood among CPS-referred children. In mediation analyses, the indirect effect of insecure/disorganized attachment on middle childhood BMI via early childhood CRP level was significant. Given the small sample size, we consider our findings to be preliminary; similar models should be tested using larger samples and longitudinal designs. Nevertheless, our findings offer novel support for an association between attachment quality and early chronic inflammation, which in turn may have implications for later physical health.

An important direction for future research will be to examine the developmental timing of when early experiences (e.g. exposure to adversity, sensitive parenting, attachment quality) begin to show associations with CRP levels, or other indicators of inflammation. As described earlier, the only other study to examine these links in early childhood demonstrated cross-sectional associations between early life adversity and disorganized attachment and salivary CRP in 17-month-old low-income children (David et al., 2017; Measelle et al., 2017). Our findings add longitudinal evidence by showing that attachment quality in infancy predicted CRP levels several years later in early childhood. Of note, in addition to using small sample sizes (<50 children), both studies only measured CRP at one time point, limiting our ability to understand how inflammation may change longitudinally. Previous research has found that CRP levels during childhood are associated with CRP levels during adulthood, although only moderately. In a sample of 1617 participants in the Cardiovascular Risk in Young Finns Study, CRP levels in childhood (assessed for 6 age cohorts: 3, 6, 9, 12, 15, 18 years) were correlated with CRP levels in adulthood (21 years later, at ages 24 to 39 years) at \( r = .29 \) (Juonala et al., 2006). The association was significant in every age group included, except for children who were 3 years old at the time of the childhood assessment, raising the question of whether early childhood CRP levels are meaningful predictors of later inflammation. Thus, it will be important in future research to

<table>
<thead>
<tr>
<th>Effect</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>p</th>
<th>95% CI</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome: CRP (log-transformed), ( R = 0.35, R^2 = .12, F(1, 31) = 4.30, p = .047 )</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>−0.56</td>
<td>0.06</td>
<td>−8.90</td>
<td>0.000</td>
<td>−0.69 to −0.43</td>
<td>−0.69</td>
<td>−0.43</td>
</tr>
<tr>
<td>Insecure/disorg attachment</td>
<td>0.20</td>
<td>0.09</td>
<td>2.07</td>
<td>0.047</td>
<td>0.003 to 0.39</td>
<td>0.003</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Outcome: Age 8 BMI (z-score), ( R = 0.71, R^2 = 0.50, F(5, 27) = 5.46, p = .001 )</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>0.61</td>
<td>0.75</td>
<td>0.81</td>
<td>0.427</td>
<td>−0.94 to 2.15</td>
<td>−0.94</td>
<td>2.15</td>
</tr>
<tr>
<td>Composite risk</td>
<td>0.15</td>
<td>0.24</td>
<td>0.62</td>
<td>0.542</td>
<td>−0.35 to 0.65</td>
<td>−0.35</td>
<td>0.65</td>
</tr>
<tr>
<td>Intervention group</td>
<td>0.55</td>
<td>0.40</td>
<td>1.40</td>
<td>0.174</td>
<td>−0.26 to 1.36</td>
<td>−0.26</td>
<td>1.36</td>
</tr>
<tr>
<td>Age 4 BMI (z-score)</td>
<td>0.43</td>
<td>0.14</td>
<td>2.99</td>
<td>0.006</td>
<td>0.14 to 0.73</td>
<td>0.14</td>
<td>0.73</td>
</tr>
<tr>
<td>Insecure/disorg attachment</td>
<td>0.03</td>
<td>0.44</td>
<td>0.06</td>
<td>0.955</td>
<td>−0.87 to 0.93</td>
<td>−0.87</td>
<td>0.93</td>
</tr>
<tr>
<td>CRP (log-transformed)</td>
<td>2.18</td>
<td>0.77</td>
<td>2.85</td>
<td>0.008</td>
<td>0.61 to 3.78</td>
<td>0.61</td>
<td>3.78</td>
</tr>
<tr>
<td><strong>Indirect effect: Attachment on age 8 BMI via CRP</strong></td>
<td>0.43</td>
<td>0.26</td>
<td>–</td>
<td>–</td>
<td>0.04 to 1.11</td>
<td>0.04</td>
<td>1.11</td>
</tr>
</tbody>
</table>
examine to what extent early childhood CRP levels predict CRP levels later in life. Further, few studies that link CRP to obesity have measured CRP levels in early childhood. Although our findings suggest that CRP levels in early childhood may be useful as a biomarker of risk for later obesity risk, we were unable to test the opposite direction of effect given that we only assessed CRP levels once in early childhood. Larger samples with repeated assessments of CRP and BMI are needed to further test the strength and directionality of these associations over time, as it is possible that BMI at earlier ages may predict elevated CRP at later ages.

We only had attachment data available for the CPS-referred sample and not the comparison group, and thus were only able to test the main effect of attachment quality on CRP within the high-risk sample. In future research, it will be interesting to examine alternative models that take into account the additive and/or interactive effects of multiple risk factors. In line with a dual-risk (or cumulative risk) model, it is possible that the added stress indicators of CPS involvement (which may reflect multiple environmental and familial risk factors, including poverty and maltreatment) and insecure attachment contribute to increased low-grade inflammation. Alternatively, stress buffering models offer a framework for testing the moderating role of attachment relationships. Such models propose that sensitive parenting and/or secure attachment relationships may protect children in the face of early adversity by reducing the negative effects of stressors on physiological processes implicated in physical health problems (Chen, Brody, & Miller, 2017). As an example, Chen, Miller, Kobar, and Cole (2011) found that, in a sample of adults who were raised in low SES households, retrospectively-reported maternal warmth during childhood was associated with reduced pro-inflammatory responses. Such studies that examine whether attachment-related constructs protect children in the face of stress may offer potential targets for intervention for vulnerable populations.

Several limitations should be considered, in addition to those already noted (i.e. small sample size, only one assessment of CRP). First, inflammatory activity was assessed only in peripheral blood; it is unclear to what extent our findings would generalize to immune system activity in other tissues and organs. Second, we were unable to characterize the nature of maltreatment exposure due to limited access to CPS records. It is possible that CPS-referred children with secure attachments and CPS-referred children with insecure or disorganized attachments differed in the type, quantity, severity, and chronicity of early experiences of neglect or abuse, such that children’s attachment classifications were confounded with previous experiences of maltreatment. The lack of CPS records also limits our ability to know whether our CPS-referred sample should be considered a “maltreated” sample. That said, children with unsubstantiated cases of maltreatment following referral to CPS have similar levels of developmental and behavioral problems (Hussey et al., 2005) and risk for re-referral to CPS (Drake, Jonson-Reid, Way, & Chung, 2003; Kohl, Jonson-Reid, & Drake, 2009) to children with substantiated cases of maltreatment; thus, referral to CPS may reflect a general indicator of risk, regardless of substantiation status. Third, all CPS-referred children were exposed to an early intervention (approximately half to an attachment-based intervention); although we did not find differences between the intervention groups on variables of interest, results from this atypical sample may not generalize to other high-risk samples. Finally, in addition to preventing us from testing stress buffering models with attachment as a moderator, the lack of attachment data and BMI data on the low-risk
comparison group further raises the question of whether findings of a main effect of attachment on inflammation would replicate in low-risk samples.

In conclusion, findings from the current study add to research on the biological embedding model of early adversity, offering preliminary support that insecure and disorganized attachment is associated with chronic low-grade inflammation in early childhood among high-risk children with histories of CPS involvement. Future research using longitudinal studies should explore issues related to developmental timing (e.g. How early does chronic inflammation emerge following exposure to adversity? At what age is CRP a valid biomarker for later health risk?), identify socioemotional and physiological mechanisms linking stress to chronic inflammation in childhood, examine the extent to which insecure attachment itself is a risk factor for chronic inflammation in low-risk samples, and test the buffering role of secure attachment in the association between stress and inflammation. Such research into the developmental origins of disease vulnerability may identify novel opportunities for the prevention of pervasive physical health conditions later in life.

Acknowledgments

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References


