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Internalizing and externalizing behaviors predict elevated inflammatory markers in childhood

Natalie Slopen [a,b], Laura D. Kubzansky [b], Karestan C. Koenen [c,*]

[a] Center on the Developing Child, Harvard University, Cambridge, MA, USA
[b] Department of Social and Behavioral Sciences, Harvard School of Public Health, Boston, MA, USA
[c] Department of Epidemiology, Columbia University, Mailman School of Public Health, New York, NY, USA

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Summary
Background: Children with behavior problems, such as internalizing or externalizing disorders, are at increased risk for poorer physical health in adulthood. Inflammation has been posited as a potential biological mediator underlying this association. However, it is unclear how early in development associations between behavior problems and inflammation may be detected, and whether associations are present for both internalizing and externalizing behaviors in pre-pubertal children.
Methods: Using data from children in the Avon Longitudinal Study of Parents and Children, we examined associations between behavior problems at age 8 (assessed via the parent-report Strengths and Difficulties Questionnaire) and inflammatory markers assessed at age 10. Inflammatory markers included C-reactive protein (CRP; $n = 4069$) and interleukin-6 (IL-6; $n = 4061$). We further evaluated whether body mass index (BMI) mediated associations, and tested for potential reverse causality by considering whether age 10 inflammation was associated with changes from initial levels to age 12 behavior problems.
Results: After adjusting for relevant covariates, age 8 externalizing behaviors were associated with elevated CRP at age 10, and age 8 internalizing and externalizing behaviors were associated with elevated IL-6 at age 10 (p's < 0.05). We found no evidence that observed associations were mediated by BMI or that inflammatory markers at age 10 were associated with increased internalizing or externalizing behavior problems at age 12.
Conclusions: These findings document an association between behavior problems and elevated concentrations of CRP and IL-6 at 10 years. Heightened inflammation in childhood may be a pathway through which early behavior problems increase risk for adult chronic diseases.

* Corresponding author at: Mailman School of Public Health, Columbia University, 722 West 168th Street, Room 720G, New York, NY 10032, USA. Tel.: +1 212 305 7482; fax: +1 646 317 0013.
E-mail address: kck5@cumc.columbia.edu (K.C. Koenen).

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The childhood origins of adult chronic disease are well-established (Garner et al., 2012), with childhood behavior problems, such as internalizing and externalizing disorders, emerging as potential risk factors for poor adult physical health (Odgers et al., 2007; Jokela et al., 2009; Appleton et al., 2011). However, we have a limited understanding of how early in life childhood behavior problems may become linked to pathophysiological mechanisms that produce excess risk for poor adult physical health. Both internalizing and externalizing behaviors are associated with dysregulated hypothalamic–pituitary–adrenal axis (HPA) (Cappadocia et al., 2009; Lopez-Duran et al., 2009) and sympathetic nervous system (SNS) (Matthys et al., 2013; Obradović and Boyce, 2012) function, which could lead to the initiation and/or sustained release of pro-inflammatory cytokines (Miller and Cole, 2012). The resulting inflammation may serve as one biological mechanism by which childhood behavior problems result in excess disease risk decades later. The aim of this study was to investigate whether childhood internalizing and externalizing behaviors predict elevated chronic inflammation two years later, as indicated by C-reactive protein (CRP) and interleukin-6 (IL-6) levels, and focused on whether such effects may be evident even in pre-pubertal children.

CRP and IL-6 are well-established markers of systemic inflammation (Danesh et al., 2004, 2008). CRP is the principal downstream mediator of the acute phase response to inflammatory processes occurring in the body (e.g., a rise in IL-6). Prospective studies show that elevated CRP and IL-6 are associated with greater atherosclerosis and incident coronary events in adults (Danesh et al., 2008; Kaptoge et al., 2010). Thus, elevated levels of these markers can provide an early indicator of disease risk even if causal associations with disease outcomes are unclear (C Reactive Protein Coronary Heart Disease Genetics Collaboration, 2011). Prior research supports a relationship between childhood CRP and IL-6 levels with other well-established cardiovascular and atherosclerotic risk factors in children (Cook et al., 2000; Jarvisalo et al., 2002); and, elevated CRP in childhood tracks into adulthood (Juonala et al., 2005).

Internalizing behavior problems refer to symptoms of anxiety, depression, or withdrawal, while externalizing behavior problems refer to symptoms of hyperactivity, conduct problems, or aggression (Goodman et al., 2010). Several epidemiologic studies suggest a long-term influence of childhood internalizing and externalizing behaviors on inflammation in adulthood (Odgers et al., 2007; Appleton et al., 2011, 2012); however, few studies have examined these behavior problems in relation to inflammation among children prior to puberty (Bartlett et al., 1995; Brambilla et al., 2004; Caserta et al., 2011). Notably, all previous studies using pre-pubescent samples examined major depression or depressive symptoms, but not other forms of internalizing or externalizing behaviors. Most studies using adolescent samples also focused on depression (Targum et al., 1990; Shain et al., 1991; Birmaher et al., 1994; Schleifer et al., 2002; Gabbay et al., 2009; Copeland et al., 2012; Henje Blom et al., 2012; Miller and Cole, 2012). Evidence on the relation between depression and immune parameters in children or adolescents is inconsistent. Some studies show no differences in immune parameters by depressive status (Targum et al., 1990; Shain et al., 1991; Birmaher et al., 1994), while others indicate at least modest evidence of immune-related abnormalities associated with depressive symptoms (Bartlett et al., 1995; Schleifer et al., 2002; Brambilla et al., 2004; Gabbay et al., 2009; Caserta et al., 2011; Copeland et al., 2012; Henje Blom et al., 2012; Miller and Cole, 2012). Only a few studies have examined externalizing behavior problems in relation to immune parameters among adolescents (Targum et al., 1990; Birmaher et al., 1994; Pajer et al., 2002). Similar to studies examining depression, findings for externalizing behavior are inconsistent. Some evidence suggests no association between externalizing behavior and immunological abnormalities (Targum et al., 1990; Birmaher et al., 1994), while some evidence supports a positive association (Pajer et al., 2002).

Understanding the association between behavior problems and inflammation in children is constrained by six limitations in extant research. First, most prior studies used small convenience samples. Selection bias and limited power may contribute to discrepant results. Second, few existing studies have compared effects of a range of both internalizing and externalizing behaviors on inflammation. Thus, it is unknown whether adverse effects of early behavior problems on health are general or specific. Third, across the prior literature on behavior problems or depression in youth and immune parameters, most studies examining multiple outcomes have reported differing associations across immune markers. This makes it challenging to interpret overall findings, and to draw conclusions about the reliability of reported associations. Fourth, few existing studies examined potential differences by sex, even though some evidence suggests this may be important among preadolescents (Caserta et al., 2011). Fifth, body mass index (BMI) has not been examined as a mediator of observed associations. Childhood internalizing and externalizing behaviors are associated with elevated body mass in adolescents (Goodman and Whitaker, 2002; Cortese et al., 2008), and BMI is associated with inflammation (Lambert et al., 2004). Although some studies in youth have included BMI as a covariate, BMI has rarely been examined as a pathway. Finally, most prior studies collected information about behavior and inflammation at a single time-point and thus could not explore directionality. Studies of adults have detected a bi-directional association between inflammation and depression (Howren et al., 2009), however it is less clear whether a bidirectional association occurs in younger populations (Copeland et al., 2012; Miller and Cole, 2012).

In the present study, we capitalized on data from a large population-based cohort to test the hypothesis that higher internalizing and externalizing behaviors are associated with higher levels of CRP and IL-6 among children prior to adolescence. We examined behavioral problems at age 8 in relation to inflammatory outcomes at age 10 to evaluate these associations prior to puberty, and to ensure our predictor variable was measured temporally prior, but in close proximity, to our main outcome measure. We also hypothesized that positive associations would be mediated by elevated BMI, for reasons described above. Finally, as an exploratory analysis, we tested the possibility of reverse causality by evaluating whether inflammation at age 10 could predict increased internalizing or externalizing behaviors among children at age 12, taking account of initial levels of behavior problems. Consistent with prior research, we included relevant covariates in our models to reduce potential confounding, including

sex, ethnicity, age, current medication use, maternal education at gestation, and family income at age 8.

1. Materials and methods

We performed a secondary analysis of existing data from the Avon Longitudinal Study of Parents and Children (ALSPAC), a population-based longitudinal study of children born to mothers who resided in Avon County, England while they were pregnant (Golding and the ALSPAC Study Team, 2004; Boyd et al., 2012). ALSPAC was initiated to study mechanisms by which social, biological, and environmental factors influence pregnancy outcomes and child mental and physical health. 14,541 pregnant women with estimated delivery dates between April 1991 and December 1992 agreed to participate; this included approximately 85% of eligible participants. A total of 13,988 children were still alive at 12 months, and the study aimed to follow these children. Questionnaires were periodically mailed to parents during the pregnancy period and beyond; beginning at age 7, the sample was invited for bi-annual clinic visits. The ALSPAC Law and Ethics Committee and Local Research Ethics Committee approved the study. In-depth methodological details are described on the study website (www.alspac.bris.ac.uk) and have been reported in other publications (Golding and the ALSPAC Study Team, 2004; Boyd et al., 2012). Children who dropped out of the sample were more likely to have behavior disorders and to have mothers with more financial difficulties and lower education (Wolke et al., 2009).

Surviving children whose parents provided consent for continuing participation and with valid contact information were recruited for biological assessments at the Age 9 Clinic (mean age at visit = 9.8 years; hereafter we refer to the outcomes as an age 10 measure). A total of 7725 children attended the clinic (62% of invited participants), and 5086 of these children were willing to participate in the venipuncture protocol. Active infection can cause elevations in inflammation unrelated to behavior problems, therefore we excluded individuals who reported infection in the 7 days prior to blood collection (431 children). Children with a valid measure of CRP or IL-6 were eligible for this analysis (N = 4655 and 4647, respectively). The sample for the main analyses included 4069 children who met the following inclusion criteria: data on behavior problems at age 8, data on all relevant covariates, and a valid assessment of CRP or IL-6 at age 10 (models of CRP, n = 4069; models of IL-6, n = 4061).

In a sub-analysis, we examined if inflammation at age 10 was associated with increased behavior problems at age 12. This sub-analysis included 3634 individuals with assessments of CRP or IL-6 at age 10, complete data on control covariates, and data on behavior problems for the age 8 and age 12 measures; models of CRP, n = 3634; models of IL-6, n = 3627). In Appendix 1, we summarize the timing of assessment for the exposures and outcomes in our analyses.

1.1. Measures

1.1.1. Inflammatory outcomes

Blood samples were collected using standard methods. Concentration of CRP (mg/L) was measured by automated particle-enhanced immunoturbidimetric assay (Roche, UK), and concentration of high sensitivity IL-6 (pg/mL) was measured by enzyme-linked immunosorbent assay (R&D systems, Abingdon, UK). Assay coefficients of variation for both outcomes were $<$5%. Both CRP and IL-6 were log-transformed for regression analyses. Following standard practices, observations greater than three standard deviations above or below the sample mean were winsorized to prevent bias due to outliers in linear models (IL-6, N = 44; CRP, N = 39) (Wilcox and Keselman, 2003).

1.1.2. Behavior problems

Behavior problems were assessed via mail questionnaire using the parent-rated Strengths and Difficulties Questionnaire (SDQ) (Goodman, 2001), a validated assessment of behavioral symptomatology. Mothers were asked to rate 25 items describing their children's behavior (in the past 6 months) as ''not true'', ''somewhat true'', or ''certainly true''. The SDQ includes one subscale to assess prosocial behavior, and four subscales to assess problematic emotions and behaviors, including conduct, hyperactivity, peer, and emotional problems (Goodman, 2001). The reliability of the SDQ is considered satisfactory (Goodman, 2001), according to measures of internal consistency (mean Chronbach's alpha across subscales = 0.73), cross-informant correlation (mean across subscales = 0.34) and test–retest stability after 4–6 months (mean across subscales = 0.62). For analyses in epidemiological samples within the general population, there is theoretical and empirical evidence to support two broad categorizations of problems, an ''internalizing'' subscale which combines the emotional and peer problems subscales (10 items; e.g., often seems worried; often unhappy, depressed or tearful; rather solitary, prefers to play alone), and an ''externalizing'' subscale which combines the conduct and hyperactivity problems subscales (10 items; e.g., restlessness; often loses temper; often lies or cheats) (Goodman et al., 2010); therefore, we used the internalizing and externalizing subscales in our main analyses (possible range for both scales: 0–20).The full scale is available at http://www.sdqinfo.com. The internalizing and externalizing scales were transformed into z-scores for analysis to improve interpretability.

Primary analyses utilized SDQ symptoms reported in the Age 8 questionnaire. For participants missing SDQ data from the Age 8 questionnaire, we created scores from identical questions in the Age 7 questionnaire (n = 392, 9.6%). The overall mean age at the time of report was 8.1 years. In additional analyses, SDQ scores from the Age 11 questionnaire were used in models to examine whether inflammation was associated with subsequent increases in internalizing or externalizing behaviors. The mean age at the Age 11 questionnaire completion was 11.7 years, thus we refer to these assessments as age 12 behavior problems (see Appendix 1).

1.1.3. BMI

At the Age 9 clinic (mean age of respondent = 9.81 years, hereafter referred to as age 10 BMI), staff measured height using the Harpenden Stadiometer to the last complete millimeter, and unclothed weight to 0.1 kg. Child BMI was calculated as weight (kg)/length $(m)^2$. BMI was transformed to BMI z-scores following UK 1990 BMI population reference data (Cole et al., 1995).

1.1.4. Covariates

We included a number of covariates known to be associated with variation in inflammation (O'Connor et al., 2009) or that have been used as covariates other studies of inflammation in youth (Caserta et al., 2008; Copeland et al., 2012; Fuligni et al., 2009b; Murasko, 2008; Slopen et al., 2012). During pregnancy, mothers reported their highest level of education (below O-level, O-level only, A-level only, university degree or more, missing). After the child was born, mother's reported the child's sex (male, female), and ethnicity (white, non-white; based on mother's report for herself and her partner). In the Age 8 questionnaire, mothers reported the weekly household income (<£100, £100–£199, £200–£299, £300–£399, >£400, unknown). And, at the time of the blood draw, children reported their age (recorded in months) and parents reported if the child was taking any medication (yes/no).

1.2. Analyses

First, we used linear regression models to examine associations of internalizing and externalizing behaviors assessed at 8 years with CRP and IL-6 assessed at age 10. To show any change in the main associations of interest after standard control covariates were introduced, we used a two-step model building approach. We started with a simple model that included controls for sex and age at blood collection. We then fit models additionally adjusted for ethnicity, current medication use, maternal education at gestation, and family income at age 8. Second, we considered potential sex differences in associations by testing interaction terms for sex by internalizing or externalizing behaviors. Third, we used a bootstrapping approach to examine the extent to which observed associations between behavior problems and inflammation were mediated by BMI (Preacher and Hayes, 2008). This method provided an estimate of the *indirect effect* of BMI, which describes the proportion of the association between behavior problems and inflammation that is explained by BMI (i.e., the degree of mediation that occurred) (Preacher and Hayes, 2008). See Appendix 2 for a diagram and further explanation of the mediation analyses. If the confidence interval for an indirect effect does not cross zero, this indicates that significant mediation occurred.

Finally, to examine if inflammation was associated with an increase in later behavior problems, we estimated linear regression models with CRP and IL-6 at age 10 as the independent variables, and behavior problems at age 12 as the dependent variables (in separate models). These models adjusted for internalizing or externalizing behaviors at age 8, in addition to the same covariates used in the primary analyses. By adjusting for baseline problems, we obtained an estimate of the change in behavior from initial assessment to age 12 assessment associated with age 10 levels of CRP and IL-6. All analyses were conducted in SAS v.9.2, and statistical significance was established at $p < 0.05$.

2. Results

Table 1 presents a description of the sample. Approximately half of the sample was female, and the mean age was 9.8 years at the time of blood collection. Consistent with the region, 95% of the sample was White. There was heterogeneity by socioeconomic status. At the time of gestation, 19% of children had mothers who had not completed an O-level education; at 8 years, 46% of children resided in households that earned £400 or more per week.

In initial analyses we did not find evidence for significant differences by sex. Thus, in all further analyses, we present results combined across girls and boys.

Table 1 Descriptive information on primary variables and covariates ($N = 4069$).

	Median (SD), mean (SD), or % (*n*)
Age 10 CRP (median, mg/L)	0.20 (1.07)
Age 10 IL-6 (median, mg/L)	0.77 (1.38)
Age 8 behavior	
Internalizing score (mean)	2.77 (2.66)
Externalizing score (mean)	4.69 (3.38)
Age 12 behavior[a]	
Internalizing score (raw mean)	2.37 (2.57)
Externalizing score (raw mean)	3.83 (3.12)
Covariates	
Sex (% female)	48.51 (1974)
Ethnicity (% white)	95.40 (3882)
Mean age at clinic visit (years)	9.84 (0.30)
Mean BMI at clinic visit	17.53 (2.72)
Medication use reported at clinic visit (%)	10.84 (441)
Maternal education at gestation[a] (%)	
Missing	4.92 (200)
Below O-level	18.55 (755)
O-level only	33.50 (1363)
A-level	26.39 (1074)
University degree+	16.64 (677)
Weekly family income, 8 years (%)	
Unknown	16.69 (679)
<£100	1.33 (54)
£100–£199	6.37 (259)
£200–£299	13.20 (537)
£300–£399	17.38 (707)
£400+	45.05 (1833)

Medians or means and standard deviations (SD) are reported for continuous variables and percentages are reported for dichotomous variables. Median values of CRP and IL-6 are derived from raw (non-transformed) data. CRP, C-reactive protein; IL-6, interleukin-6; BMI, body mass index.

[a] O-level ("Ordinary" level) is an exam taken at the end of the 11th year of school; this exam is not sufficient for university entrance. A-level ("Advanced" level) is an examination taken at the end of secondary school that is used to qualify for entrance into a university.

2.1. Associations between behavior problems and CRP and IL-6

Table 2 presents parameter estimates for the associations between internalizing and externalizing behavior at age 8

Table 2 Associations between age 8 internalizing and externalizing behaviors and C-reactive protein and Interleukin-6 levels at age 10.

	Simple model[a]		Adjusted for covariates[b]	
	β (SE)	p-Value	β (SE)	p-Value
Outcome: log C-reactive protein				
Internalizing z-score	0.04 (0.02)	0.02	0.03 (0.02)	0.06
Externalizing z-score	0.05 (0.02)	0.01	0.04 (0.02)	0.04
Outcome: log interleukin-6				
Internalizing z-score	0.06 (0.01)	<0.0001	0.05 (0.01)	<0.01
Externalizing z-score	0.05 (0.01)	<0.01	0.04 (0.01)	<0.01

[a] Simple linear regression models are adjusted for age and sex.
[b] Covariates include sex, ethnicity, age, current medication use, maternal education at gestation, family income at age 8. Sample sizes vary slightly due to missing information on inflammatory markers (n = 4069 for CRP and n = 4061 for IL-6). SE, standard error.

and CRP and IL-6 at age 10. The pattern of significant associations for minimally adjusted models and fully adjusted models are similar, although the coefficients are slightly attenuated in the fully adjusted models. Considering fully-adjusted models with log-CRP as the outcome, externalizing behavior was associated with elevated CRP (β = 0.04, p = 0.04, model R^2 = 0.05), while the association between internalizing behavior and CRP was marginally statistically significant (β = 0.03, p = 0.06, model R^2 = 0.05). In the fully adjusted models with log IL-6 as the outcome, both internalizing and externalizing behaviors were associated with elevated levels of IL-6 (i.e., internalizing: β = 0.05, $p \leq 0.01$, model R^2 = 0.03; externalizing: β = 0.04, $p \leq 0.01$, model R^2 = 0.03).

2.2. BMI as a potential mechanism

For the significant associations described above, we evaluated BMI z-score as a potential mediator. Age 8 externalizing behavior was associated with BMI z-score (β = 0.04, p = 0.04), but there was no association for age 8 internalizing behavior (β = 0.01, p = 0.76). BMI z-score was positively associated with CRP (β = 0.39, $p < 0.0001$) and IL-6 (β = 0.15, $p < 0.0001$). However, the indirect effect (i.e., the proportion of the association that can be explained by BMI) in each of the mediation models was not significant, thereby failing to provide evidence that the observed associations were mediated by BMI (for table of coefficients, see Appendix 3).

2.3. Associations between CRP and IL-6 and later behavior problems

Table 3 presents associations between CRP and IL-6 at age 10 and change in internalizing and externalizing behaviors between the earlier assessment and the age 12 assessment. Neither CRP nor IL-6 was associated with change in internalizing or externalizing behaviors over time.

3. Discussion

In a large cohort of pre-adolescent children, we found behavior problems were associated with elevation in inflammatory biomarkers, and associations were maintained after taking account of a range of relevant covariates. To our knowledge, this is the first large population-based study to examine internalizing and externalizing behaviors together in relation to inflammation in a pre-adolescent sample. Elevated CRP levels were strongly associated with externalizing behavior and more weakly associated with internalizing behavior, while both internalizing and externalizing behaviors were associated with elevated IL-6 levels. We found no evidence for differential associations by sex, or that observed associations were mediated by BMI. While a true prospective examination was not possible in the primary

Table 3 Associations between age 10 C-reactive protein and interleukin-6 and change in internalizing and externalizing behaviors between initial assessment and 12 years.[a]

	β (SE)	p-Value
Outcome: internalizing behavior (z-score)		
Log C-reactive protein	0.00 (0.01)	0.81
Log interleukin-6	0.01 (0.02)	0.57
Outcome: externalizing behavior (z-score)		
Log C-reactive protein	−0.02 (0.01)	0.13
Log interleukin-6	−0.02 (0.02)	0.24

Sample sizes vary slightly due to missing information on inflammatory markers (n = 3634 for CRP and n = 3627 for IL-6). BMI, body mass index; SE, standard error.
[a] Models adjusted for age, sex, ethnicity, current medication use, maternal education at gestation, family income at age 8, age 10 BMI z-score, and age 8 internalizing/externalizing behavior.

analysis due to lack of available information on initial levels of inflammation, the finding that elevated inflammation was not associated with subsequent increases in internalizing or externalizing behaviors over time reduces concerns about reverse causality. Taken together, our findings suggest that inflammation may be a pathway through which childhood behavior problems increase later risk for chronic diseases in adulthood.

Our findings extend a small set of studies that have examined depression or other behavior problems in relation to CRP or IL-6 in pre-adolescent or adolescent samples (Gabbay et al., 2009; Caserta et al., 2011; Copeland et al., 2012; Henje Blom et al., 2012; Miller and Cole, 2012). Two recent studies have found associations between depression and elevated CRP among adolescents (Copeland et al., 2012; Miller and Cole, 2012); while the present study demonstrated an association between internalizing behavior and elevated CRP (after adjustment for family socioeconomic status) that was somewhat weaker than expected, it is consistent with findings from these studies. Our results build on the prior literature by also examining externalizing behavior in relation to CRP and IL-6 in pre-adolescent children, and documenting a strong association. Our findings further support a uni-directional relationship between behavior problems and later inflammation, similar to results from a prospective cohort study that tested bi-directional longitudinal associations between CRP and depression in a slightly older sample over a longer period of time (Copeland et al., 2012). The alternative pathway (i.e., inflammation to later depression) has been supported in older age groups (Gimeno et al., 2009); it is possible that this pathway is slower to develop or simply less common so that preadolescents and adolescents do not show the same pattern reported among adults.

Studies on behavior problems in relation to inflammation in pre-pubertal children make an important contribution to our understanding of the relationship between psychopathology and inflammation and the likely direction of effects for two reasons. First, identifying the developmental period at which the association between behavior problems and inflammation becomes apparent has implications for chronic disease prevention strategies. If child psychopathology is truly a risk factor for adult chronic disease via impact on inflammation, interventions aimed at treating or preventing psychopathology in children may reduce risk of chronic disease later in life. Future research is needed to test this hypothesis. For example, child psychopathology interventions could measure inflammatory markers before and after treatment to determine if effective reduction of behavior problems is associated with reducing inflammation. Second, pre-adolescent children tend to have fewer physical health conditions than adults and have not yet initiated many of risk behaviors (i.e., smoking, alcohol consumption) that may influence inflammatory markers. Thus, estimated associations between behavior problems and inflammation in child samples can be expected to have less error, because younger samples have less heterogeneity with respect to other factors that introduce variation into inflammatory markers. Notably, the models had modest R^2 values in that we accounted for 3–5% of the variance in inflammation, thus suggesting that other factors also contribute to individual variation in CRP and IL-6 levels in children. However, it is important to keep in mind that relatively modest effects can have a meaningful impact when applied to the population-level (Rosenthal and Rubin, 1982). We are unable to compare the size of the R^2 values to prior studies of behavior problems in relation to inflammation among children prior to puberty (Bartlett et al., 1995; Brambilla et al., 2004; Caserta et al., 2011), because these studies did not present R^2 values. However, the magnitude of our findings are comparable in size to those obtained in research (using similar models) considering potential effects of childhood adversity on inflammatory markers in youth (Murasko, 2008; Fuligni et al., 2009a).

Our results point to several directions for future research. First, research is needed to identify mechanisms that underlie the observed associations. We did not find evidence for elevated BMI as a pathway and potential behavioral pathways such as smoking or high alcohol consumption are less relevant to pre-adolescent samples. Studies show that behavioral/emotional problems are associated with atypical HPA axis activity in youth (Lopez-Duran et al., 2009; Matthys et al., 2013), and that HPA dsyregulation has a broad influence on the immune system (Sternberg, 2001). Therefore, physiological correlates of HPA dysregulation (e.g., abnormal diurnal cortisol profile) may function as potential mediators. Other potential mediators include sleep duration and quality, given that sleep problems are common in children with behavior problems (Alfano et al., 2007; Sung et al., 2008) and poorer sleep predicts inflammation (Simpson and Dinges, 2007). Second, research is needed to examine whether changes in behavior problems are related to changes in inflammatory markers. By incorporating pre-and post-treatment assessments of CRP and IL-6 into trials of behavioral and pharmaceutical interventions for children with behavior problems, investigators could test whether reductions in behavior problems produce reductions in inflammatory marker levels. Such data would provide further support for the hypothesis that behavior problems increase risk for elevated inflammation in youth. Third, studies are needed to clarify whether the timing of onset or duration of behavior problems have implications for later inflammation and long-term disease risk. For example, some research suggests that chronicity of depression (Copeland et al., 2012) or conduct disorder (Odgers et al., 2007) in childhood or adolescence has a marked influence on later CRP. Finally, research is needed to characterize the long-term health risks associated with elevated inflammation in childhood.

Our findings should be considered in the context of several limitations. This study is limited by attrition and incomplete data, and consistent participation was socially patterned such that individuals from more disadvantaged households were less likely to participate. Children with behavior problems were more likely to drop out of the sample (Wolke et al., 2009), therefore this sample underestimates the prevalence of behavior problems in the target population. Other researchers have carried out simulation analyses using the ALSPAC cohort to examine the impact of selective dropout on the strength of prediction, and have indicated that the validity of regression models to predict behavior disorder is only slightly affected (Wolke et al., 2009). Our models controlled for variables associated with attrition (e.g., household income, maternal education) which may attenuate potential bias (Kleinbaum et al., 1982). However, in light of non-differential missingness, our results cannot be

generalized to the original population-representative cohort, and may underestimate the associations. In addition, our study only had one assessment of inflammation prior to adolescence; therefore, we were unable to examine bi-directional associations. Furthermore, although this study relies on temporally-ordered data, we cannot conclude that behavior problems preceded increased inflammatory markers, as inflammation may have already been up-regulated at the time that the behavior problems were assessed. This limitation also has implications for the tests of mediation; if inflammation was elevated at the time that behavior problems were assessed, paths in the mediation models may be over- or underestimated relative to their true values. Finally, information on asthma or other inflammatory health conditions was not collected at the clinic visit when blood samples were provided. However, we note that results were unchanged when we adjust for past-year reports of asthma or eczema at age 7 (data not shown), suggesting that the observed associations are not confounded by these inflammation-related conditions.

In conclusion, this study provides evidence for associations between behavior problems and elevated CRP and IL-6 in a large sample of children suggesting that associations are already evident prior to adolescence. Specifically, externalizing and somewhat less strongly, internalizing behavior at age 8 were associated with higher levels of CRP assessed two years later, and internalizing and externalizing behavior at age 8 were associated with elevated levels of IL-6 two years later. These associations did not differ for boys and girls, and somewhat surprisingly were not explained by elevated BMI. The findings suggest an etiological pathway that may connect childhood behavior problems to elevated chronic disease risk beginning early in life. Additional research on the connection between childhood behavior problems and inflammation is needed to determine whether behavior problems are causal for elevated inflammation and whether interventions aimed at behavior problems reduce risk of elevated inflammation and, potentially, long term health risks in children.

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None.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2013.07.012.

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