Do upsetting life events explain the relationship between low socioeconomic status and systemic inflammation in childhood? Results from a longitudinal study

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Abstract

Background: Children from families of low socioeconomic status (SES) are more likely to be exposed to upsetting situations and stressors. Such exposures have, in turn, been linked to inflammation in some studies. In this study we explore if low SES is related to inflammation in children via such stressful life events. **Methods:** Data on 4,525 children of the Avon Longitudinal Study of Parents and Children, a general population birth cohort, were used to explore associations between SES at ages 0-3 years, upsetting life events at ages 3-9 years and inflammatory markers [interleukin 6 (IL-6) and C-reactive protein (CRP)] at age 9 years. Confounders included body mass index, gender, financial problems, and upsetting life events at ages 0-3 years. **Results:** Using Structural Equation Modelling, we found that early socioeconomic disadvantage predicted higher levels of IL-6 (β=0.034, 95% CI=0.005, 0.063) even after adjusting for confounders. This association was partially mediated by upsetting life events (β=0.003, 95% CI=0.001, 0.006). **Conclusions:** In the general child population, low SES is associated with increased exposure to stressful life events, in turn associated with later inflammation. These findings highlight the role of stressors associated with poverty and disadvantage in the development of inflammation among children in the general population.

Keywords: ALSPAC; SES; inflammation; life events; childhood, IL-6

Introduction

Low socioeconomic status (SES) in childhood has been extensively associated with chronic diseases in the adult population (Adler & Rehkopf, 2008; Calixto & Anaya, 2014). Many studies now suggest that adults who were exposed to low SES as children are at higher risk of developing various chronic diseases later in life such as cardiovascular disease, diabetes and cancer, regardless of their adult SES (Hart et al., 1998; Kittleson et al., 2006; Ljung & Hallqvist, 2006). There are several mechanisms that explain the relationship between SES and disease. For example, high body mass index (BMI) and unhealthy lifestyle choices partially account for this association (Miller et al., 2011). More recent literature suggests that another, in many ways related, pathway may be inflammation. Inflammation is the body's primary response to injury or infection but also stress (Fagundes & Way, 2014; Kuhlman et al., 2017; Minihane et al., 2015). The inflammatory response is an effort of the immune system to repair tissue and eliminate the risk of infection, but inflammation can become maladaptive if it remains after the infection is cleared. Linking low SES to inflammation is in line with the biological embedding hypothesis that low SES and poverty in childhood, as early stressful experiences, can impact on the immune system. Chronic exposure to such experiences leads to elevation in inflammation levels, due to the chronic stimulation of the sympathetic nervous system, as well as to the progressive down-regulation of key anti-inflammatory pathways, such as the Hypothalamic-Pituitary-Adrenal (HPA) axis and the parasympathetic nervous system. The HPA axis plays a major role in the perception of and response to adverse experiences (Hertzman, 1999; Shonkoff et al., 2009). One product of the stimulation of the HPA axis is cortisol secretion which has effects on body organs. These help in body adaptation (allostasis). However, overstimulation and chronic exposure to such experiences can lead to maladaptive wear-and-tear on the body and the brain (allostatic load) (McEwen & Gianaros, 2010; Yehuda et al., 1991). This, in turn, can damage selected neurons in the hippocampus which consequently causes not only cognitive deterioration but also organ damage (Hertzman, 1999; Meany, 1999; Sapolsky Robert, 1992).

Several studies to date have examined the relationship between childhood SES and inflammation in adulthood. However, results are mixed partly due to the use of different measures of SES, inflammatory markers, methodological approaches and life stages (Milaniak & Jaffee, 2019; Muscatell et al., 2018). In some studies no significant relationships were found while in others the effect of low SES on inflammation remained even after

controlling for demographic but also health-related characteristics such as BMI (Carroll et al., 2011; Park et al., 2005; Rexrode et al., 2003) and smoking (Malfertheiner & Schütte, 2006; Ohsawa et al., 2005). However, since not all adults who lived in low SES conditions as children go on to develop inflammation and as health and lifestyle seem to explain only part of the association between childhood SES and adult inflammation, other mechanisms could be at play (Friedman & Herd, 2010; Loucks et al., 2010).

Researchers have mainly focused the search for such mechanisms or mediators in childhood. One possible mediator is adverse or stressful life events. Low SES is linked to greater exposure to stressful life events throughout childhood and adolescence (Chandler et al., 1985; Gad & Johnson, 1980; Gillum et al., 1984). This is probably because children from disadvantaged families are more likely to grow up in environments that are uncontrollable and unpredictable (Evans, 2004). As for the link between adverse life events or experiences in childhood and adult inflammation, maltreatment especially has been associated with higher levels of inflammation in adults (Danese et al., 2007; Slopen et al., 2015) with a recent meta-analysis showing that traumatic experiences, in general, during childhood contribute to a pro-inflammatory state in adulthood (Milaniak & Jaffee, 2019). Researchers exploring this link usually draw on Miller, Chen and Parker's (2011) biological embedding model of early adversity, according to which stressors and adversity during early life, a critical and sensitive period of development, may become embedded within immune cells programmed to have a proinflammatory phenotype. This model draws, in turn, on the Barker hypothesis about the foetal origins of adult disease (Barker, 1992), life-course epidemiology (Lynch & Smith, 2005), stress physiology (McEwen, 1998), and behavioural immunology (Coe & Lubach, 2005; Raison & Miller, 2003). Simply put, stressors during early sensitive periods "activate" proinflammatory tendencies in cells which are intensified by behavioural patterns and hormonal dysregulation throughout life. Early exposure to stressors for example gives rise to behaviours that accentuate inflammation such as reading threat into situations, reacting with anger, mistrusting others, and making poor life choices (Chen et al., 2004; Chen & Matthews, 2003; Kiecolt-Glaser, 2010; Pollak, 2005; Pollak et al., 2005; Yanbaeva et al., 2007). In addition, these behaviours also lead to elevated reactivity of the HPA axis, related to inflammation as discussed earlier.

A recent study showed that the link between early SES and adult inflammation can be related to recent life events, too (John-Henderson et al., 2016). In that study, participants had to give retrospective information on

their parents' as well as their own current (objective and subjective) SES. They also provided information on current negative events such as divorce and financial loss. The study showed that adults with a low childhood SES and a high number of recent negative events were more likely to develop inflammation. However, the role of adverse life events in explaining the SES-inflammation link in children has not been explored yet. Among children, both the association between inflammation and SES (Dowd et al., 2010; Howe et al., 2010; Murasko, 2008; Pietras & Goodman, 2013; Schmeer & Yoon, 2016) and the one between inflammation and SES-adjusted adverse life events (Flouri et al., 2019) have been investigated, but we still do not know if poverty and low SES bring about inflammation in children via increasing exposure to such psychosocial stressors. We carried out this study to explore this. In particular, we used data from the Avon Longitudinal Study of Parents and Children (ALSPAC) which includes information on inflammation [interleukin 6 (IL-6) and C-reactive protein (CRP)] for 4,525 children at age 9 years to explore if the association between early SES (at ages 0-3 years) and inflammation (at age 9 years) is via adverse life events (at ages 3-9 years).

2. Methods

2.1 Sample

The Avon Longitudinal Study of Parents and Children (ALSPAC) is an ongoing birth cohort study that recruited 14,541 pregnant women resident in Avon, UK, with expected delivery dates from April 1st 1991 to December 31st 1992 (http://www.bristol.ac.uk/alspac/researchers/our-data/; the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool). ALSPAC is a transgenerational prospective observational study that investigates influences on health and development across the life span. It takes into account multiple genetic, epigenetic, biological, psychological, social and other developmental exposures in relation to a similar diverse range of health, social, and developmental outcomes (Boyd et al., 2013). Ethical approval of the ALSPAC cohort was obtained from the ALSPAC Ethics and Law Committee and local research ethics committees. Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time and no financial compensation was given (more details at www.alspac.bris.ac.uk).

The study initially recruited 14,541 pregnant women which represented about 85% of the eligible population. Of those, there was a total of 14,676 foetuses, resulting in 14,062 live births and 13,988 children were still alive at the age 12 months and have been followed up since then (Golding & Team, 2004). Additional children were recruited using the original enrolment definition from the participating children's age 7 years onwards, allowing us to have available study data for 15,445 foetuses. Of those, 14,684 were alive at 1 year of age. In attempt to bolster the initial sample size, new pregnancies have been enrolled since then resulting in additional children being enrolled as well. To date, the total sample size for analyses using any data collected after the age of seven is therefore 15,454 pregnancies, resulting in 15,589 foetuses. Of these 14,901 were alive at 1 year of age (Fraser et al., 2013). Children whose parents provided consent were eligible to continue and were invited to participate in the biological assessments. A total of 7,725 participated in the clinic assessments at age 9 (62% of those invited). Assessments of the ALSPAC Cohort Profile showed that attrition and non-response was higher among mothers who were younger, were from lower socioeconomic backgrounds, did not have a university degree, had already two or more children, had higher pre-pregnancy BMI, and experienced hypertensive disorder of pregnancy. Our study's analytic sample (n = 4,525) comprised children who had valid data on inflammatory markers at age 9 years, were singletons or first-born twins, and did not have an infection at the time the blood samples were taken.

2.2 Measures

2.2.1 Inflammatory markers

In ALSPAC, inflammation in childhood was measured with CRP and IL-6 at age 9. Blood samples were collected using standard methods. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Concentration of CRP (mg/L) was measured by automated particle-enhanced immunoturbidimetric assay (Roche, UK). Concentration of high sensitivity IL-6 (pg/mL) was measured by enzyme-linked immunosorbent assay (R&D systems, Abingdon, UK). All interassay coefficients of variation were less than 5%. Both CRP and IL-6 were log-transformed for the regression analyses.

2.2.2 Socioeconomic status

Socioeconomic status was measured as a latent variable using information from five observed variables during the first 3 years of the child's life: maternal education, paternal social class, overcrowding, housing tenure and financial difficulties. As the observed variables were measured on different scales, we recoded them into binary variables prior to including them in the measurement model as follows:

Maternal education: Children's mothers reported their highest educational qualification at 32 weeks of gestation. There were 5 categories (1=CSE/none to 5=Degree) and a binary variable was then created as 1=degree, 0=otherwise.

Paternal social class: This was derived using information about the father's occupation, measured also at 32 weeks of gestation, using information on job codes from the Office of Population Censuses and Surveys. The initial categories were 6 [1=I to 6=V (Armed forces)]. 'Armed forces' was considered missing, and the paternal social class variable was then recoded into 1=non-manual, 0=manual.

Overcrowding: This was calculated using information obtained from the mother's questionnaire when the child was 33 months old (2.8 years). A crowding index was calculated by dividing the number of people living in the home by the number of rooms (including kitchen/diner) in the home, ranging 1 to 19. The index was then recoded into a binary variable using the <1 cut-off to define the less crowded homes (1=non-crowded, 0=overcrowded).

Housing tenure: Home ownership status was reported by the mothers when they were 8 weeks pregnant. The question invited 7 possible answers (0=mortgaged to 6=other), then recoded into 1=owns the home, 0=does not own the home.

Financial difficulties: An index of financial difficulties was created using the following variables from the mother's questionnaire when the child was 8 months, 21 months, and 33 months old. Mothers were asked, "How difficult at the moment do you find it to afford these items?" a) food, b) clothing, c) heating, d) rent or mortgage, and e) things you need for the baby. Responses were recorded on a 4-point scale ranging from 1="Very difficult" to 4="Not difficult". After reverse coding, the total score (ranging 0-15) was calculated for each timepoint. We then summed the three scores into a continuous variable ranging from 0 to 45. Following

visual inspection of its distribution, we dummy-coded it to differentiate the bottom third (1=no or little difficulty affording items) from the top two-thirds (0=difficulty affording items).

2.2.3 Upsetting events

Mothers were asked at 7 timepoints in the child's life until age 9 years (18 months, 30 months, 42 months, 57 months, 69 months, 81 months and 103 months) whether a list of 15 upsetting events had been experienced by the child since the previous timepoint, starting from when the child was 6 months old (e.g. child was taken into care, moved home, a pet died, was physically hurt by someone, was sexually abused, was separated from mother/or father for at least a week, acquired a new parent, changed carer). (Full list of events in Table S1 in the Supplementary Material.) Each question was answered on a 5-point scale (1=event happened and child was very upset to 5=event did not happen). We first reverse-coded the responses and then calculated a within-timepoint upsetting events score for each of the 7 timepoints by summing the items at each timepoint together. Participants with missing values on all 15 items were considered as missing (around 10-19% of missingness across the 7 timepoints). Then, a total (across-timepoint) upsetting events score was calculated by summing the 7 (within-timepoint) scores. For analysis purposes, two indicators of upsetting events were created. The first captured the upsetting events that occurred from 3 to 9 years and was used as the mediator of the study. The second captured the upsetting events that occurred in the early years (0-3 years) and at the period SES was measured in the study, and was used as a covariate.

2.2.4 Covariates

We included several individual and family covariates that are known to be associated with the predictor and the outcome of the study, including early upsetting events (as described above), gender, money problems, and BMI. *Money problems*[1] was measured using two questions from 9 timepoints (18 weeks of pregnancy to 9 years of

[1] We used 'money problems' as an additional covariate in the analysis because it captures subjective financial difficulties from the prenatal period until age 9 years, when inflammation was measured. Although this variable is conceptually similar to the 'financial difficulties' variable that was used to create the latent construct of early SES, it was not included in the calculation of the latent SES

age) that were asked of the mothers: whether a) their income was reduced and b) they had a major financial problem since the previous timepoint. Answers were on a 5-point scale from "Affected a lot" to "Did not happen". Answers were subsequently recoded into 1="happened" (irrespective of impact) and 0="did not happen". A final variable was then created such that respondents who reported income reduction or major financial difficulties in any of the 9 timepoints would get a value of 1, indicating money problems since the previous timepoint. Those who reported, across all timepoints, that they did not have their income reduced or any major financial difficulty as well as those who had missing data in at least 6 out of the 9 timepoints would get a value of 0. *BMI (*weight (kg)/height $(m)^2$) was measured using information from the clinic assessments at age 9 years.

2.2.5 Analytic strategy

Analyses were performed in STATA 15.0 (Stata Corporation, College Station, TX, 1997) and Mplus statistical package (version 8) (Muthén et al., 2017). To create the latent SES factor, first we conducted a principal components analysis which showed that all five SES indices loaded onto a single factor. Then we tested the construct validity of the SES factor by conducting a Confirmatory Factor Analysis (CFA) in Mplus for the five categorical indicators. The CFA was performed using the weighted least squares mean and variance adjusted estimator for categorical variables and, again, one factor was extracted (factor loadings in Table S2 in the Supplementary Material). We used the following indices of fit: 1) Comparative Fit Index (CFI), 2) Standardized Root Mean squared Residual (SRMR) and 3) Root Mean Square Error of Approximation (RMSEA). According to the recommended cut-offs of CFI (≥ 0.95), SRMR (≤ 0.08), and RMSEA (≤ 0.06) (Hu & Bentler, 1999), the fit to the data was excellent (CFI=0.95, SRMR=0.06, RMSEA=0.05, 90% CI=0.000, 0.000). Factor scores were then saved and used as a continuous variable in the analysis, allowing us to handle missing data using full information maximum likelihood. In addition, to avoid listwise deletion of cases with missing on the covariates, we specified their variances which allowed them to covary and therefore, full information maximum likelihood was applied for the covariates too. Following this, we extended the CFA to a Structural Equation Model (SEM) to test the relationships between SES and the two inflammatory markers separately, adjusting for the covariates.

construct as we wanted that to measure 'objective' early SES. Furthermore, its addition to the confirmatory factor analysis (see section 2.2.5) worsened model fit.

Finally, we examined the extent to which the observed associations between SES and inflammatory markers were mediated by upsetting events. We also carried out a sensitivity analysis to explore if results change when we exclude participants with CRP>10 mg/L, as those CRP levels likely indicate infection.

3. Results

3.1 Descriptive analysis

Table 1 shows the descriptive statistics (Supplementary Table S3 for the amount of missingness in each study variable). As can be seen, children in the analytic sample had average BMI. The impact of upsetting events was approximately the same for both early and later measurements (1.40 and 1.04, respectively[2]). Regarding the observed SES variables, most of the mothers in the analytic sample did not have a university degree (83%). More than half of the fathers belonged to non-manual social classes (62%), and the majority of the children lived in homes which their parents owned outright or were buying with a mortgage (83%) and which were not overcrowded (94%). Finally, around 85% of the mothers reported that they had not experienced income reduction or had a major financial problem. However, 73% of them reported that at some point they had difficulties affording items such as food, clothing, or things for the baby. We also ran a bias analysis (Supplementary Table S4) to explore any differences in the study variables for those in and out of the analytic sample. Those in the analytic sample had lower levels of IL6 and CRP, had experienced fewer upsetting events between ages 3 and 9 and had lower BMI. Furthermore, their parents were more likely to have a university degree, be from higher socioeconomic backgrounds, live in less overcrowded places, own their home, and have less difficulties in affording things.

The correlations among the main study variables are shown in Table 2. The two inflammatory markers were moderately related to each other. IL-6 was negatively related to SES, was positively associated with most of the covariates (adversity at ages 3-9 years, BMI, money problems and female gender) and was unrelated to

[2] The early and later upsetting events specifications capture time periods of different length. Hence, to make mean scores comparable, we divided the sum of the events for each period by the months each period covered.

adversity at ages 0-3 years. Similarly, CRP was negatively related to SES and positively related to later adversity, BMI and female gender, but was not related to early adversity. It was also unrelated to money problems. SES was inversely associated with later adversity, BMI, and money problems. A moderate relationship was also found between early and later adversity. However, early adversity was only related to money problems while later adversity was positively associated with BMI, money problems and female gender. Finally, being female was positively related to having a higher BMI.

Table 1. Descriptive statistics of the main variables of the study (N=4,525)

	Continuous	
	n	**M(SD)**
IL-6 (pg/mL)	4,525	1.21(1.48)
CRP (mg/L)	4,525	0.63 (1.94)
Upsetting events (early)	3,776	33.71(3.95)
Upsetting events (later)	3,050	91.16 (7.80)
BMI	4,474	17.58 (2.77)
	Categorical	
	N	**%**
Gender		
Male	2,296	50.79
Female	2,225	49.21
Maternal education		
Degree	703	16.91
Other	3,454	83.09
Paternal social class		
Non-manual	2,358	61.55
Manual	1,473	38.45

Overcrowding		
Not overcrowded	3,489	93.77
Overcrowded	232	6.23
Housing tenure		
Owning the home	3,474	83.35
Not owning the home	694	16.65
Financial difficulties		
No difficulties affording things	1,211	26.76
Difficulties affording things	3,314	73.24
Money problems		
No money problems	3,639	85.06
Money problems	639	14.94

Note. IL-6=interleukin 6; CRP=C-reactive protein; Upsetting events (early)=measured at 0-3 years; Upsetting events (later)=measured at 3-9 years.

Table 2. Correlations of the main variables of the study

	1.	2.	3.	4.	5.	6.	7.	8.
1. IL6	1							
2. CRP	0.44***	1						
3. SES	-0.06***	-0.04*	1					
4. Early upsetting events	-0.00	-0.01	0.03	1				
5. Later upsetting events	0.07***	0.04*	-0.05*	0.29***	1			
6. BMI	0.24***	0.43***	-0.07***	-0.00	0.04*	1		
7. Money problems	0.05**	-0.02	-0.15***	0.11***	0.14***	0.02	1	
8. Female	0.14***	0.20***	-0.01	-0.01	0.05*	0.09***	-0.01	1

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Note. IL-6=interleukin 6 (log-transformed); CRP=C-reactive protein (log-transformed)

3.2 CFA

As explained, we assessed the construct validity of the SES factor by conducting a CFA. Model fit was excellent as all fit indices were within the recommended cut-offs (CFI=0.95, SRMR=0.06, RMSEA=0.05). All factor loadings were satisfactory (≥ 0.40), as shown in Table S2 in the Supplementary Material.

3.3 SEM

Results from the SEM (Table 3) showed that SES at ages 0-3 years was a significant predictor of IL-6, independently of other covariates, but not of CRP at age 9 years. Adverse life events at ages 3-9 years were positively associated with levels of IL-6 at age 9. Regarding the other covariates, BMI and being female were also related to higher levels of IL-6. However, adverse life events at ages 0-3 years or income reduction/financial problems were not significant predictors of IL-6 at age 9. We also adjusted for the impact of early adversity on later adversity to estimate the net effect of SES on subsequent adversity independently of its continuation. Results revealed that early upsetting events predicted later upsetting events, and also that lower SES at an early age still predicted higher inflammation several years later. As discussed, CRP was not related to SES but, for completeness, we present the model results for CRP in Supplementary Table S5.

3.3.1 Mediation analysis

Later adversity mediated part of the effect of early SES on later levels of IL-6 (indirect effect: b=-0.005, SE=0.002, $p<0.05$, 95% CI=-0.011, -0.001, β=-0.003; total effect: b=-0.068, SE=0.028, $p<0.05$, 95% CI=-0.123, -0.015, β=-0.036; direct effect: b=-0.063, SE=0.028, $p<0.05$, 95% CI=-0.063, -0.005, β=-0.034). Figure 1 shows all paths in this final model tested.

Table 3. Fully-adjusted mediation model for IL-6 (N=4,525)

Direct paths to IL-6			
	b	**SE**	**95% CI**
1. SES -> IL-6	-0.06*	0.03	-0.118, -0.010
2.Later upsetting events -> IL-6	0.01**	0.00	0.002, 0.009
3.Early upsetting events -> IL-6	-0.00	0.00	-0.009, 0.005
4. Money problems -> IL-6	0.06	0.04	-0.014, 0.125
5. BMI-> IL-6	0.07***	0.00	0.061, 0.076
6. Female -> IL-6	0.19***	0.03	0.138, 0.234

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Note: b=Unstandardised regression coefficient; SE=Standard error; CI=Confidence interval; IL-6=Interleukin 6

3.4 Sensitivity analysis

The SEM showed that SES was not a significant predictor of CRP. Only having a higher BMI (b=0.174, SE=0.004, p<.001, β=0.42) and being female (b=0.368, SE=0.028, p<.001, β=0.16) were associated with higher levels of CRP. Although in our analyses throughout we excluded children with a reported infection at the time the blood measures were taken, we also carried out a sensitivity analysis where we refitted our SEM for CRP

after we excluded participants with CRP>10 mg/L. Results from the sensitivity analysis showed no differences before and after excluding those participants. SES was still not significantly associated with CRP, and having a higher BMI and being female remained significant predictors of higher levels of CRP (b=0.169, SE=0.004, p<.001, β=0.42; b=0.373, SE=0.028, p<0.001, β=0.17, respectively).

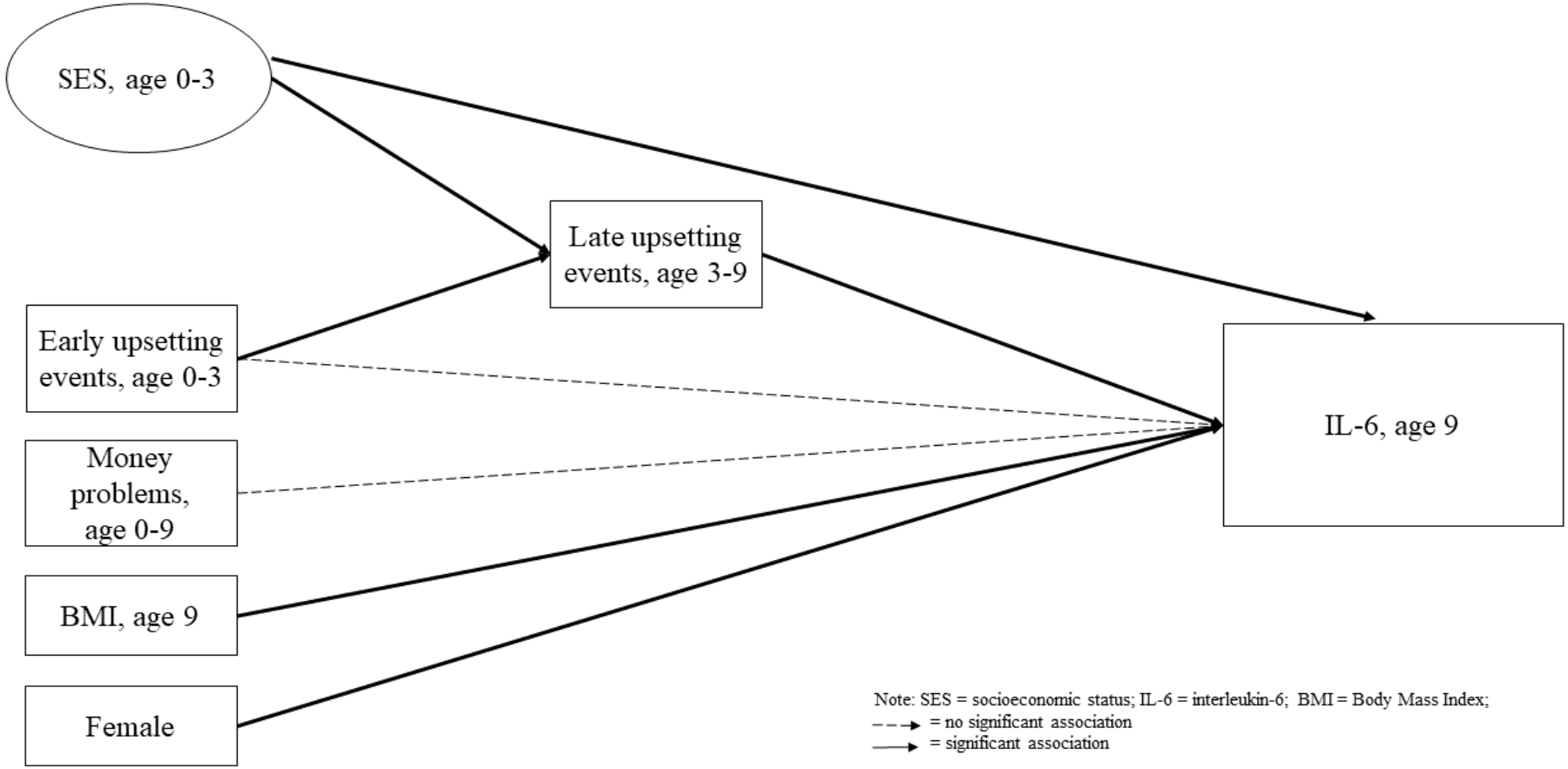

Figure 1. SEM and path analysis results

4. Discussion

In a large sample of children from the general population, we found that low SES early in life was related to increased levels of inflammation in middle childhood (age 9 years) as measured by IL-6. We did not find similar effects for CRP, the other inflammatory marker available in our dataset, at this age. The association between SES and IL-6 in children is an important finding. Despite much evidence for an association between low SES in childhood and increased levels of inflammation in adulthood (Muscatell et al., 2018), only few studies have examined the effect of SES on inflammation in childhood (Dowd et al., 2010; Howe et al., 2010; Kuhlman et al., 2019; Murasko, 2008; Pietras & Goodman, 2013; Schmeer & Yoon, 2016). In our study, the effect of SES on IL-6 in middle childhood was small, but in line with the evidence produced by a recent meta-analysis by Kuhlman and colleagues (2019) that the association between early life disadvantage and both CRP and IL-6 across childhood was very modest, with particularly small effect sizes for links in middle childhood compared to infancy and adolescence.

Our study however went beyond exploring the effect of SES on inflammation in childhood in the general population. It also tested if stressful and adverse life events and experiences explain the two, by drawing on the well-established association between childhood poverty and adversity (Evans, 2004) as well as on Miller and colleagues' (2011) biological embedding model of early adversity, which suggests that stress during childhood activate proinflammatory tendencies. To date, this has not been explored in children. A recent study, with adults, by John-Henderson et al. (2016) showed that individuals with a relatively low childhood SES exhibit an inflammatory phenotype in the context of a high number of recent negative life events. Our study showed that upsetting events from early to middle childhood explained part of the association between early SES and inflammation in middle childhood. Therefore, this is the first study to provide evidence suggesting that upsetting life events may be a reason for the link between early SES and inflammation in childhood. Our study has another two important strengths. First, the data we had available allowed us to create a latent factor of parental SES that took into account several related but distinct aspects of SES including education, social position, income and material conditions. Second, by adjusting for adversity at the time of measurement of SES we could show that early SES increased adversity, in turn associated with inflammation in middle childhood.

However, our study has several weaknesses, too. First, we had only one measurement of inflammatory markers in childhood (at age 9 years) which does not allow us to examine associations longitudinally or in younger ages. Second, the small indirect effect of SES on IL-6 in children via upsetting events suggests that other mechanisms are at play. Some of them can relate to the type of family and area environments that children in poverty are exposed to (Evans, 2004). For example, poor nutrition and lack of exercise but also environmental factors such as exposure to air pollution and "wear and tear" of the immune system caused by extended cortisol elevation (Danese & McEwen, 2012; Janicki-Deverts et al., 2009; Juster & McEwen, 2015), are all associated with both low SES and inflammation. Related to this, we did not control for cortisol. As described earlier, one of the end products of the HPA axis activation by stressful life events is cortisol. The role of cortisol is to inhibit the production of inflammatory cytokines by binding to glucocorticoid receptors in healthy immune cells. Thus, increases of the HPA axis activity could possibly conceal the pro-inflammatory phenotype in children as measured by circulating cytokines due to cortisol secretion. Consequently, children exposed to adversity may display lower levels of inflammation. Inflammation is carefully regulated throughout childhood and adolescence and accumulates with age through biological ageing and via behavioural, psychosocial and environmental pathways. Therefore, it is possible that the association between inflammation and SES-related stressors does not emerge before adulthood or may be masked by regulatory processes (Franceschi & Campisi, 2014; Miller et al., 2011). Finally, the upsetting events checklist was completed by the mother, who may have over or under-estimated the impact these events truly had on the child. A self-completed checklist would capture the direct perceived impact of life events, however, given the children's very young age in our sample this was not possible.

Despite these limitations, our study has significant strengths. Understanding the mechanisms that likely underlie the SES effect on inflammation is important for both theory and policy (Hertzman, 1999). In turn, if inflammation is a pathway through which early life adversity determines health outcomes across the life course, then identifying the determinants of inflammation that are amenable to early intervention will have much public health benefit (Kuhlman et al., 2019; McEwen & Gianaros, 2010). Further research is needed however to elucidate some of the relationships we identified. For example, since different types of adversity can affect the psychoneuroimmune development differently (Kuhlman et al., 2017), it is important to explore the role of

different types of adversity, their timing and chronicity. In addition, in light of the evidence that individuals with moderately elevated IL-6 and/or CRP do not necessarily suffer from chronic low-grade inflammation (Del & Gangestad, 2018), a clear understanding of the complex interactions between biological systems is needed before interpreting causal processes involving biomarkers. As already discussed, it is also important to consider the role of cortisol especially in such processes in childhood.

4.1 Conclusions

Our findings suggest that upsetting events and experiences mediate, at least partly, the relationship between low SES and inflammation in the general child population. As such, our study is novel in that it tested a mediating mechanism that had not been tested before in children. Future research should explore why adversity likely increases inflammation among children in the general population.

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