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Research Report

Socioeconomic disadvantage, chronic stress, and proinflammatory phenotype: an integrative data analysis across the lifecourse

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Abstract

Socioeconomic disadvantage confers risk for many chronic illnesses, and theories have highlighted chronic psychological stress and alterations to inflammatory processes as key pathways. Specifically, disadvantage can heighten chronic stress, which may promote a proinflammatory phenotype characterized by immune cells mounting exaggerated cytokine responses to challenge and being less sensitive to inhibitory signals. Importantly, lifecourse perspectives emphasize that such immune alterations should be more potent earlier in life during a sensitive period when bodily tissues are highly plastic to environmental inputs. However, examining these propositions is resource intensive, as they require cell-culturing approaches to model functional inflammatory activities, a wide age range, and longitudinal data. Here, we integrated data from five independent studies to create a diverse sample of 1,607 individuals (960 with longitudinal data; 8 to 64 years old; 359 Asian, 205 Black, and 151 Latino/a). Leveraging the resulting lifecourse data, rich interview assessments of disadvantage and stress, and ex vivo assessments of inflammation, we examined two questions: (1) Does chronic stress account for the link between disadvantage and proinflammatory phenotype? (2) Is there a developmental period during which inflammatory responses are more sensitive to disadvantage and chronic stress? Disadvantage was associated with higher chronic stress, which was linked with a proinflammatory phenotype cross-sectionally, longitudinally, and in terms of prospective change across 1.5 to 2 years. Consistent with the sensitive period hypothesis, the magnitude of these indirect associations was strongest in earlier decades and declined across the lifecourse. These findings highlight the importance of taking a lifecourse perspective in examining health disparities.

Keywords: socioeconomic disadvantage, chronic psychological stress, inflammation, development, lifecourse

Significance Statement:

Chronic diseases are patterned by socioeconomic status (SES), but the underlying pathways and how their magnitude vary across the lifecourse are unclear. Here, in an integrated sample of 1,607 individuals aged 8 to 64 years, we found that socioeconomic disadvantage was associated with higher chronic stress, which in turn predicted a proinflammatory phenotype characterized by immune cells mounting aggressive responses to challenges and being less sensitive to inhibition signals. Importantly, the magnitude of these links was strongest earlier in life and declined across the lifecourse, supporting the notion of a sensitive developmental period during which environmental input may have stronger impacts. If replicated, these findings suggest that efforts to mitigate health disparities may be most efficacious when implemented during childhood.

Introduction

Socioeconomic disadvantage—typically defined by low income, savings, or education level—is associated with physical health problems throughout the lifecourse. In childhood and adolescence, disadvantage is associated with indicators of cardiovascular risk, such as higher blood pressure and fasting glucose; in adulthood, it is linked with morbidity and mortality from various chronic illnesses, including cardiovascular, respiratory, autoimmune diseases, and some cancers (1–6). These findings have spurred conceptual models attempting to explain how socioeconomic disadvantage contributes to such a wide range of health problems across the lifecourse, and excessive inflammation has been highlighted as one key pathway.

Inflammation is one of the body's primary defense mechanisms against invading pathogens and tissue damage. When such infections or injuries are detected, cells of the innate immune system—monocytes, dendritic cells, and macrophages—mount an acute inflammatory response, which is coordinated by proinflammatory cytokines, such as Interleukin (IL)-6, IL-1ß, and Tumor Necrosis Factor (TNF)-α. These proinflammatory cytokines attract other

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immune cells to the site, and cause those cells to proliferate, release antimicrobial signals, and mature into phenotypes best suited to countering the threat. The acute inflammatory response is critical for survival; however, it needs to be tightly regulated because sustained "nonresolving" inflammation can damage tissue and organs in ways that increase risk for a range of chronic diseases (7). This regulation is mediated via both local (e.g. IL-10) and systemic (e.g. cortisol) signals that, at sufficiently high concentrations, suppress the acute inflammatory response by inhibiting cell recruitment and cytokine production.

In the health disparities literature, there has been considerable interest in nonresolving inflammation as a mechanistic pathway. Typically, studies have measured inflammation by quantifying protein biomarkers like C-Reactive Protein (CRP), IL-6, and TNF-α in circulating blood. This approach is motivated by longitudinal studies indicating that circulating biomarkers forecast risks of morbidity and mortality from diabetes, heart attack, and stroke, as well as functional decline and premature death (8–10). Over the past two decades, numerous studies have considered whether concentrations of these biomarkers are heightened in socially disadvantaged individuals. Several meta-analyses have synthesized these results, concluding that lower socioeconomic status (SES) is associated with higher levels of circulating protein biomarkers, which are thought to reflect nonresolving inflammation (11–13). This association was observed in children, adolescents, and adults (11–13).

Despite the consistency of these results, they do not enhance scientific understanding of the mechanistic basis of the association between disadvantage and health. Cytokines such as IL-6 and TNF-α are released not only by innate immune cells, but also by endothelial, skeletal, and adipose cells, for purposes that do not always involve defending against infection or repairing tissue injuries (14–16). Similarly, CRP functions as an antimicrobial peptide, but the liver releases it when IL-6 is high, irrespective of the precipitating stimulus (17). Thus, these biomarkers do not necessarily reflect underlying inflammation. A more direct approach to measuring inflammatory processes is to utilize ex vivo methods that simulate an immune challenge. Specifically, studies have cultured immune cells with microbial stimuli, such as the bacterial product lipopolysaccharide (LPS), and then quantified the ensuing production of proinflammatory cytokines. Conceptually, this measure provides a proxy for the magnitude of the inflammatory response to challenges. In addition, cells can be simultaneously exposed to a microbial stimulus and a compound that exerts anti-inflammatory effects, such as cortisol or IL-10. The anti-inflammatory compound partially inhibits cytokine production, but the magnitude of this inhibition varies from person to person. As such, the extent of inhibition can be used as a proxy for cellular sensitivity to inhibitory signals. Conceptually, this measure represents the capacity of cells to respond to "stop" signals.

Some studies have considered whether these functional indicators of inflammatory activity vary as a function of SES. For example, individuals with low SES had immune cells that mounted larger proinflammatory responses following stimulation and had lower capacity to inhibit inflammatory responses (18, 19). However, these studies have relatively smaller sample sizes (N's = 37 to 150) and included few participants of color, limiting broad generalization of the results. Most of these studies also utilized cross-sectional designs, so that inferences about directionality could not be made. The present study aimed to address these limitations by combining data from five independent studies, three of which utilized longitudinal designs, to carry out an integrative data analysis. Specifically, we extracted and harmonized measures of chronic stressors and ex vivo assessment of inflammatory processes to create a sample of over 1,600 individuals (over 900 with longitudinal data). The resulting sample spans a wide age range from 8 to 64 years and is diverse in race/ethnicity and SES, increasing the generalizability of results. Of note, this is achieved without the typical approach of relying on public data, for which there are increasing concerns about their overuse. Specifically, overuse of the same dataset creates dependencies among research papers that are falsely regarded as independent contributions as well as exacerbates sample-specific peculiarities and biases that may not replicate (20). Furthermore, previous studies have largely analyzed responses to stimulation and sensitivity to inhibition as independent outcomes, masking potential coupling patterns of inflammatory processes. Therefore, the current investigation estimates a latent proinflammatory phenotype profile characterized by *both* relatively higher cytokine responses to challenge and relatively lower sensitivity to inhibitory signals.

In addition, the current investigation addressed two unresolved questions. First, conceptual models have postulated that socioeconomic disadvantage may impact immune functioning through various pathways, including early infection, environmental pollution, health behaviors, and psychological stress (21–24). However, formal examinations of mediation scenarios have rarely been conducted. Leveraging the fact that each of the studies had rich interview data assessing chronic psychological stress, we focused on this potential mechanistic pathway and examined whether it could account for cross-sectional and longitudinal links between socioeconomic disadvantage and proinflammatory phenotype. We hypothesized such an indirect effect because disadvantage is known to heighten chronic stress. For example, disadvantage has been linked to exposure to violence, discrimination, and strained relationships (25–27). In turn, nascent research suggests that chronic stress may be associated with higher cytokine responses to challenge and lower sensitivity to inhibitory signals (18, 28, 29).

Second, leveraging the wide age range of the integrated data, we examined whether there may be a developmental period during which inflammatory processes are more sensitive to disadvantage and chronic stress. Numerous conceptual models postulate sensitive developmental periods in early stages of life during which bodily tissues have heightened plasticity, and thus are more sensitive to environmental input (30–33). Indeed, animal studies have found that lower maternal care during the first week of rodents' lives led to physiological alterations, which persisted in adulthood (34, 35). By contrast, lower maternal care during the second week of rodents' lives had relatively smaller effects on physiology, and lower maternal care during the third week of rodents' lives had no effects on physiology as youth and as adults (36). However, empirical tests of this proposition in humans are challenging because they require lifecourse data. Here, we leveraged the wide age range that resulted from the integrated data to address this issue directly. Based on findings from animal models, we hypothesized that the magnitude of stress-inflammation relationships would be strongest in earlier stages of life and decline with age. It is worth noting that the immune system matures and acquires memory throughout the lifespan (37, 38) and can still be modulated by environmental input at older ages (39), allowing a more thorough our hypothesis, with fewer concerns about ceiling effects.

Table 1. Descriptive statistics of sample characteristics by study (Analytical $N = 1{,}607$).

	Study A	Study B: children	Study B: parents	Study C	Study D	Study E
Sample size	277	261	261	151	349	308
Location	Chicago	Vancouver	Vancouver	Vancouver	Vancouver	Chicago
Year	2015–2017	2010–2012	2010–2012	2005–2007	2009–2012	2012–2017
Design	Longitudinal	Longitudinal	Longitudinal	Longitudinal	Cross-sectional	Cross-sectional
Age range	11–15	13–16	32–64	14–19	15–55	8–17
Age Mean (SD)	13.45 (0.62)	14.53 (1.07)	45.83 (5.50)	17.01 (1.35)	36.48 (1.78)	12.99 (2.50)
Race						
White	76 (27%)	129 (49%)	157 (60%)	76 (48%)	264 (73%)	155 (50%)
Black	106 (38%)	12 (5%)	4 (2%)	2 (1%)	2 (1%)	79 (25%)
Asian	14 (5%)	94 (36%)	83 (32%)	68 (43%)	68 (19%)	31 (10%)
Latino/a	79 (28%)	14 (5%)	11 (4%)	0 (0%)	5 (1%)	42 (14%)
POC	5 (2%)	12 (5%)	5 (2%)	11 (7%)	21 (6%)	4 (1%)
Female	175 (62%)	125 (48%)	199 (76%)	157 (100%)	198 (55%)	138 (44%)
Adiposity	23.57 (6.10)	21.37 (3.70)	25.38 (4.61)	21.70 (2.69)	25.76 (5.86)	22.43 (5.69)
SES						
Income	5.24 (2.18)	5.35 (1.83)	5.35 (1.83)	N/A	4.69 (1.80)	6.35 (2.07)
Savings	3.86 (2.55)	4.52 (2.48)	4.52 (2.48)	N/A	2.99 (1.82)	4.94 (2.74)
Education	3.53 (1.25)	3.98 (0.86)	3.98 (0.85)	3.73 (1.03)	3.36 (1.24)	3.36 (1.24)
Inflammatory parameters						
Cells	Whole blood	Whole blood	Whole blood	Whole blood	Whole blood	PBMC
Ligand for SCP	LPS, R848, HSP60	LPS	LPS	LPS	LPS	PIC, ODN
Ligand for SI	LPS	LPS	LPS	LPS	LPS	PIC
Inhibitor; dose	CRT; 2, IL-10; 3	CRT; 2	CRT; 2	CRT; 4	CRT; 3	CRT; 1
Cytokines	IL-6, IL-1β, IL-8, TNF-α in supernatant	IL-6, IL-1β, IL-8, and IL-10 in supernatant	IL-6, IL-1β, IL-8, and IL-10 in supernatant	IL-6 in supernatant	Intracellular IL-6 in CD14 + monocytes	IL-6, IL-1β, IL-8, TNF-α in supernatant
Assay method	Immunoassays	Immunoassays	Immunoassays	Immunoassays	Flow cytometry	Immunoassays

Note: Study B has relevant data for both children and parents, thus descriptive statistics are presented separately. Location refers to the city in which the study was conducted. Year refers to the years during which data was collected. Family income was coded in a 9-point scale: 1 = less than 5,000; 2 = 5,000 to 19,999; 3 = 20,000 to 34,999; 4 = 35,000 to 49,999; 5 = 50,000 to 74,999; 6 = 75,000 to 99,999; 7 = 100,000 to 149,999; 8 = 150,000 to 199,999; and 9 = 200,000 and higher. Family savings was coded in a 9-point scale: 1 = less than 500; 2 = 500 to 4,999; 3 = 5,000 to 9,999; 4 = 10,000 to 19,999; 5 = 20,000 to 49,999; 6 = 50,000 to 99,999; 7 = 100,000 to 199,999; 8 = 200,000 to 499,999; and 9 = 500,000 and higher. POC refers to other people of color. Adiposity was assessed with body mass index. PBMC refers to peripheral blood mononuclear cell. LPS refers to lipopolysaccharide. R848 refers to Resiquimod. HSP60 refers to heat shock protein-6. PIC refers to polyinosinic: polycytidylic acid. ODN refers to oligodeoxynucleotides. CRT refers to hydrocortisone. SCP refers to stimulated cytokine production, and SI refers to sensitivity to inhibition. N/A refers to not available in the dataset.

Results

Overview of integrated data

We integrated all five of our lab's studies (40–44) that included (a) relevant measures for at least one of the primary predictors—socioeconomic disadvantage or chronic psychological stress and (b) in vitro measures of stimulated cytokine production and sensitivity to glucocorticoid inhibition. Of these five studies, three utilized longitudinal designs and had two waves of data about 1.5 to 2 years apart. The integrated dataset resulted in a sample of 1,607 individuals (960 with longitudinal data), diverse in age (8 to 64 years old) and race (53% White [$n = 857$], 22% Asian [$n = 359$], 13% Black [$n = 205$], and 9% Latino/a [$n = 151$]). Table 1 summarizes sample and demographic characteristics by study. Supplementary Materials provide full descriptions of methods and statistical approach; below, we provide overall descriptions of the measures.

Socioeconomic disadvantage was assessed with family gross income, family savings, and education (parental education for youth). To harmonize family income and family savings, dollars that were reported in Canadian Dollars were first converted to US Dollars using conversion rates at the time of data collection, and then uniformly recoded into a 9-point scale. Education was similarly harmonized by recoding into a uniform 5-point scale. Finally, a composite was created by averaging the inverse of the standardized scores of these measures, such that higher values indicate greater disadvantage.

Chronic psychological stress was assessed with age-appropriate versions of the UCLA Life Stress Interview (45), which is a semistructured interview focusing on stress over the past 6 months experienced across multiple life domains, including family relationships, friendships, and either school (for youth) or work (for adults). We opted for this approach, rather than self-reported questionnaires, because trained interviewers made developmentally appropriate and contextually informed ratings of chronic stress. As such, this approach allows integration of data across studies despite the wide age range and resulting vast variations in life experiences. To simplify analyses, and capture each participant's overall chronic stress burden, we used a composite measure averaging ratings across the life domains. For both socioeconomic disadvantage and chronic stress, we used ratings made at baseline in studies with longitudinal designs to allow prospective analyses for the most rigorous examination of hypotheses.

Proinflammatory phenotype was assessed with two components of the inflammatory process: stimulated production of cytokines following a microbial challenge, and sensitivity to inhibitory signals that typically regulate this response. To assess *stimulated cytokine production*, antecubital blood was collected, and cells were dispensed into wells with one or more activating ligand(s) that stimulated the production of proinflammatory cytokines. Supernatants were harvested after incubation, and proinflammatory cytokines were measured via immunoassay or flow cytometry. Proinflammatory cytokines included IL-1β, IL-6, IL-8,

IL-10, and TNF-α, but studies varied in the specific panel of cytokines assayed, which is summarized in Table 1. Composites were formed by averaging across ligands and cytokines. In all studies, higher values indicate greater stimulated cytokine production.

To assess *sensitivity to inhibitory signals*, cells were coincubated with the activating ligand and varying doses of hydrocortisone, a molecule that provides anti-inflammatory feedback. Each study quantified the same panel of proinflammatory cytokines as above, and all coefficients of variations were less than 10%. Multilevel modeling was used to estimate and extract within-individual slopes relating dose of inhibitor and amount of cytokine production (46). These slopes had negative signs meaning that as the inhibitor dose increased, cytokine production decreased. To aid interpretation, we subsequently inversed slope values, such that higher values indicate greater sensitivity to inhibition signals. As above, a composite was created for each study by averaging the standardized slopes across cytokine. Any deviations from these general approaches are detailed in the Supplementary Materials. Immune measures were assessed both at baseline and at the follow-up visit for longitudinal studies.

To harmonize these immune measures and to define a person-focused inflammatory phenotype, latent profile analyses were conducted within each study using the readouts described above: stimulated cytokine production and sensitivity to inhibition signals. We fixed the number of profiles to two because doing so allowed us to harmonize measures across studies despite the heterogenous methods (summarized in Table 1). Moreover, across the five studies and across timepoints (for longitudinal studies), the two profiles exhibited the same hypothesized pattern of characteristics. Specifically, as summarized in Table S1 and depicted in Figure S1, one profile is characterized by relatively higher stimulated cytokine production and relatively lower sensitivity to inhibition signals (referred to as the "proinflammatory phenotype"), whereas the other profile is characterized by relatively low stimulated cytokine production and relatively high sensitivity to inhibition signals. The profiles were dummy-coded with the proinflammatory phenotype coded as 1. Some temporal stability in membership in the proinflammatory phenotype profile was demonstrated across the 1.5 to 2 years, $OR = 13.23$ (r equivalent = 0.51).

We also assessed age, sex at birth (male vs. female), race (dummy-coded with White as reference: Asian, Black, Latino/a, and other people of color), and adiposity (body mass index) as covariates, which were entered in all models. We controlled for race because there are known racial disparities in SES, chronic stress, and inflammation. We controlled for race because there are known racial disparities in SES, chronic stress, and inflammation. However, it is important to note that these racial disparities should not be interpreted as reflecting fixed or innate biological differences; rather, multiple sources indicate that they are products of experiences, policies, and practices that have systemically disadvantaged subgroups of individuals based on skin color (47–49). As controlling for these systemic influences is infeasible with our datasets, we used race as a covariate to proxy for them.

Preliminary analyses

Bivariate associations

Relative to White participants, Black and Latino/a participants had greater socioeconomic disadvantage ($r = 0.21$ and $r = 0.09$, respectively), and Asian participants had less socioeconomic disadvantage ($r = -0.08$). Black participants also had higher adiposity ($r = 0.07$) and Asian participants had lower adiposity ($r = -0.09$) compared to White participants. No other race or ethnicity differences emerged. Increased age was associated with greater adiposity ($r = 0.30$), greater socioeconomic disadvantage ($r = 0.14$), and more chronic stress ($r = 0.29$). No other age-dependent associations emerged. Female (vs. male) participants had greater socioeconomic disadvantage ($r = 0.06$). No other sex differences emerged. Finally, as would be expected, more socioeconomic disadvantage was associated with higher chronic stress ($r = 0.38$).

Main associations of socioeconomic disadvantage and chronic stress

We then conducted logistic regressions to examine the links from socioeconomic disadvantage and chronic psychological stress to proinflammatory phenotype profile. As summarized in Table S2 and depicted in Fig. 1(A), individuals experiencing greater socioeconomic disadvantage were more likely to have cells that exhibited the proinflammatory phenotype ($b = 0.18$, SE = 0.06, $P = 0.001$). Specifically, every SD increase in disadvantage was associated with 20% higher odds of having the proinflammatory phenotype, following adjustment for age, sex, race, and BMI. Similarly, as depicted in Fig. 1(B), chronic stress was also associated with the proinflammatory phenotype ($b = 0.29$, SE = 0.06, $P < 0.001$), such that every SD increase in chronic stress rating was associated with 33% higher odds of having the proinflammatory phenotype following covariate adjustment.

Next, we examined these associations longitudinally. As summarized in Table S2 and depicted in Fig. 1(C) and (D), controlling for covariates, both socioeconomic disadvantage and chronic stress were associated with proinflammatory phenotype at Time 2 ($b = 0.20$, SE = 0.08, $P = 0.009$ and $b = 0.34$, SE = 0.08, $P < 0.001$, respectively). Specifically, every SD increase in disadvantage and chronic stress were associated with 23% and 41% increased odds of exhibiting the proinflammatory phenotype 1.5 to 2 years later, following covariate adjustment. We also did change analyses, focusing on change in proinflammatory phenotype from Time 1 to Time 2. Controlling for proinflammatory phenotype at Time 1 and the same panel of covariates, chronic stress, but not disadvantage, remained associated with proinflammatory phenotype at Time 2 ($b = 0.28$, SE = 0.09, $P = 0.001$).

Hypothesis testing

Indirect effects

First, we conducted path models to examine whether chronic psychological stress accounted for the cross-sectional and longitudinal associations between socioeconomic disadvantage and proinflammatory phenotype. As depicted in Fig. 2(A), there was a significant indirect effect cross-sectionally ($b = 0.08$, SE = 0.02, CI_{95} = [.04, 0.12]), such that disadvantage was associated with higher chronic stress ($b = 0.32$, SE = 0.02, CI_{95} [.27, 0.37]), which in turn was associated with increased odds of exhibiting a proinflammatory phenotype ($OR = 1.28$, $b = 0.25$, SE = 0.06, CI_{95} [.13, 0.38]), following adjustment for age, sex at birth, race, and adiposity.

Similarly, as depicted in Fig. 2(B) and (C), there were also significant indirect effects longitudinally, ($b = 0.09$, SE = 0.03, CI_{95} = [.04, 0.15]), and in terms of prospective change from Time 1 to Time 2 ($b = 0.08$, SE = 0.03, CI_{95} = [.03, 0.15]). In both cases, controlling for covariates, more socioeconomic disadvantage at Time 1 was associated with higher chronic stress (longitudinal: $b = 0.31$, SE = 0.03, CI_{95} [.24, 0.37]; prospective change: $b = 0.30$, SE = 0.03, CI_{95} [.23, 0.36]), which in turn was associated with increased odds of exhibiting a proinflammatory phenotype at Time 2 (longitudinal: $OR = 1.35$, $b = 0.30$, SE = 0.08, CI_{95} [.14, 0.46], and with increases from Time 1 to Time 2 in the odds of exhibiting a

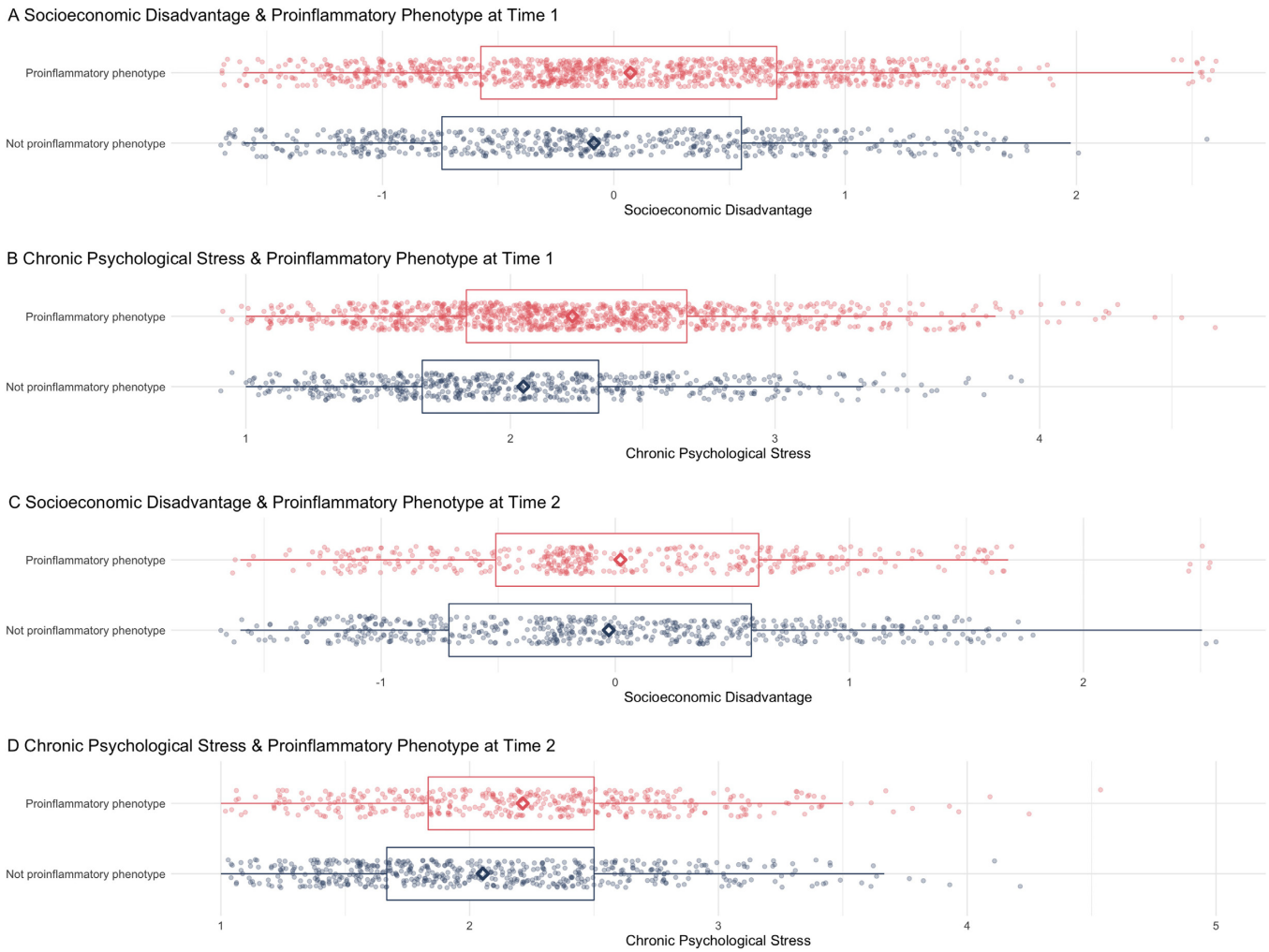

Fig. 1. Associations from socioeconomic disadvantage and chronic stress to proinflammatory phenotype cross-sectionally (Panels A and B), longitudinally (Panels C and D). Boxplots are shown with means displayed in diamonds. Raw datapoints are displayed. Note that although some datapoints appear to be outliers, they did not pass a-priori outlier threshold of +/- 3SD from the mean; nonetheless, when winsorized, all results remained the same.

proinflammatory phenotype: $OR = 1.32$, $b = 0.28$, SE = 0.09, CI_{95} [.10, 0.46]). Finally, to examine directionality, we examined an indirect effect that is temporally reversed than hypothesized; that is, whether chronic stress at Time 2 would account for the link between disadvantage at Time 2 and proinflammatory phenotype at Time 1. We found no evidence for such indirect effect ($b = 0.000$, SE = 0.032, CI_{95}[−0.06, 0.07]).

Sensitive period hypothesis

Next, we examined whether the indirect effects from disadvantage to chronic stress to proinflammatory phenotype varied by age. They did in all three cases, as reflected in significant moderated indirect effects for cross-sectional analysis ($b = -0.004$, SE = 0.001, CI_{95}[−0.007, −0.002]), longitudinal analysis ($b = -0.004$, SE = 0.002, CI_{95}[−0.008, −0.001]), and in prospective change analysis ($b = -0.004$, SE = 0.002, CI_{95}[−0.008, −0.001]). As depicted in Fig. 3(A), in all three cases, the simple indirect effects from disadvantage to proinflammatory phenotype via chronic stress were strongest at the youngest ages and linearly declined across the lifecourse.

Further decompositions revealed that these age-moderated indirect effects were driven by age moderation of the path between chronic stress and proinflammatory phenotype. Specifically, as depicted in Fig. 3(B), the magnitude of the link between chronic stress and proinflammatory phenotype was strongest at younger ages and declined across the lifecourse for cross-sectional analysis (age 10: $OR = 2.08$, age 20: $OR = 1.73$, age 30: $OR = 1.46$, age 40: OR = 1.22, age 50: $OR = 1.02$), longitudinal analysis (age 10: $OR = 2.41$, age 20: $OR = 1.93$, age 30: $OR = 1.54$, age 40: OR = 1.23, age 50 $OR = 0.98$), and prospective change analysis (age 10: $OR = 2.41$, age 20: $OR = 1.93$, age 30: $OR = 1.54$, age 40: OR = 1.23, age 50 $OR = 0.98$). Moreover, region of significance analyses suggested that these age moderations became nonsignificant at age 38 for cross-sectional analysis, age 36 for longitudinal analysis, and age 33 for prospective change analysis. In other words, the link between chronic stress and proinflammatory phenotype was observed from childhood well into early adulthood, but not after.

Finally, because there were more youth studies than adult studies, age was not evenly distributed; therefore, we conducted sensitivity analyses with age recoded into three developmental stages, each with adequate sample sizes (50, 51): childhood (under age 14; $N = 372$), adolescence (ages 14 to 18; $N = 618$), adulthood (age 19 and above; $N = 634$). Comparable results were observed such that all three moderated indirect effects remained significant.

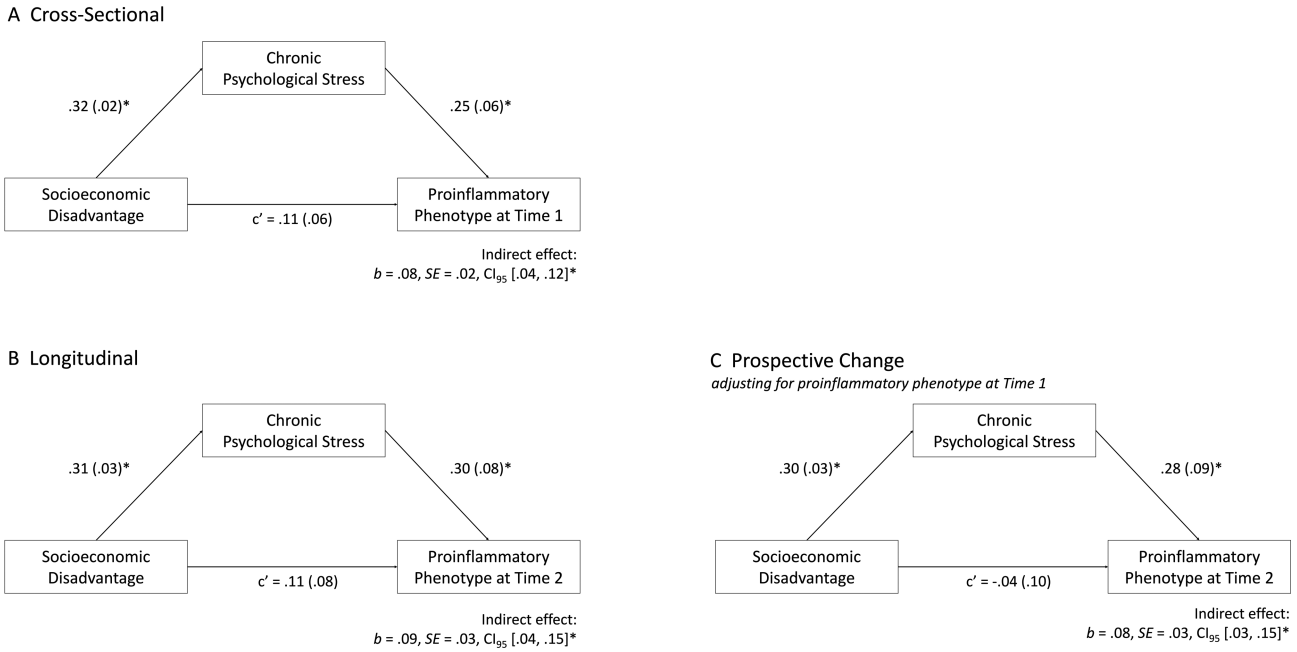

Fig. 2. Indirect effect models wherein socioeconomic disadvantage was associated with greater chronic psychological stress, which in turn was associated with proinflammatory phenotype cross-sectionally (Panel A), longitudinally (Panel B), and in terms of prospective change (Panel C). Coefficients were adjusted for race, sex at birth, and body mass index. Prospective change indirect effect model additionally controlled for proinflammatory phenotype at Time 1. In all the three panels, socioeconomic disadvantage and chronic psychological stress were assessed at Time 1.

Discussion

This integrated data analysis aimed to address two unresolved questions. First, is socioeconomic disadvantage linked to chronic psychological stress, which in turn relates to the expression of a proinflammatory phenotype? We found evidence for such indirect effects cross-sectionally, longitudinally, and in terms of prospective change over time. These findings are consistent with conceptual models specifying that disadvantage can give rise to many chronic psychological stressors, including strained relationships and academic or vocational difficulties, which in turn, can alter the operating tendencies of immune cells toward mounting more aggressive inflammatory responses upon challenge and being less sensitive to inhibition signals (31). Of note, we found no evidence for a temporally reversed scenario linking disadvantage and chronic stress at Time 2 to proinflammatory phenotypes at Time 1, shedding light on the directionality of associations.

Second, is there a developmental period during which inflammatory responses are more sensitive to environmental input? Results suggest that the magnitude of the indirect associations from socioeconomic disadvantage to proinflammatory phenotype via chronic stress were strongest in earlier decades of life and weakened across the lifecourse. These findings are consistent with the conceptual notion of a sensitive period early in life during which bodily tissues are rapidly developing and thus have heightened plasticity to environmental inputs (e.g. 30 to 33). Of note, our findings suggest that despite attenuation, plasticity is retained into early adulthood (mid-30's), suggesting a more prolonged capacity for environmental calibration of physiology than traditionally theorized (52), and highlighting the importance of a lifecourse approach in both conceptual and empirical examinations.

The current findings underscore the utility of a lifecourse perspective to conceptual models of stress. While this study begins to address lifecourse queries, it also invites additional temporal questions about whether there are conditions under which stress–inflammation links would exhibit *sensitive period patterns*, with associations larger at younger ages, versus *accumulation patterns*, with associations largest at older ages. One factor may be how inflammatory activity is assessed. Specifically, the stimulation and inhibition measures used here are functional in nature, and therefore, might show the greatest plasticity to experience during sensitive periods of development, when the immune system's operating tendencies are being calibrated. By contrast, circulating inflammatory biomarkers, such as CRP, are relatively static indicators that are thought to proxy unresolved inflammation. Elevations in these biomarkers take time to accumulate, and their association with stress might, therefore, be expected to strengthen with age. Indeed, in other investigations, we have demonstrated that socioeconomic disparities in circulating inflammation strengthens with age using an integrative data analysis approach (53) and a meta-analytical approach (54). These findings point to the importance of considering both the developmental timing of stress exposure and the nature of the inflammatory parameter being considered. These findings also have practical implications for efforts to mitigate health disparities. Specifically, both the patterns from the present study of mechanistic inflammatory processes and the patterns from our previous investigations of circulating inflammation suggest that prevention efforts may be most efficacious when implemented during childhood. However, as the current findings also observed malleability well into early adulthood, there is still utility in implementing interventions during adolescence and adulthood.

The strengths of this integrated data analysis include the use of a large, pooled sample of over 1,500 participants that is demographically diverse, increasing the generalizability of findings. This was achieved without the use of public data, for which there are increasing concerns about their overuse, creating dependencies among research papers, giving false impressions of independent contributions, and exacerbating sample-specific peculiarities that may not replicate (20). In addition to its aggregated size,

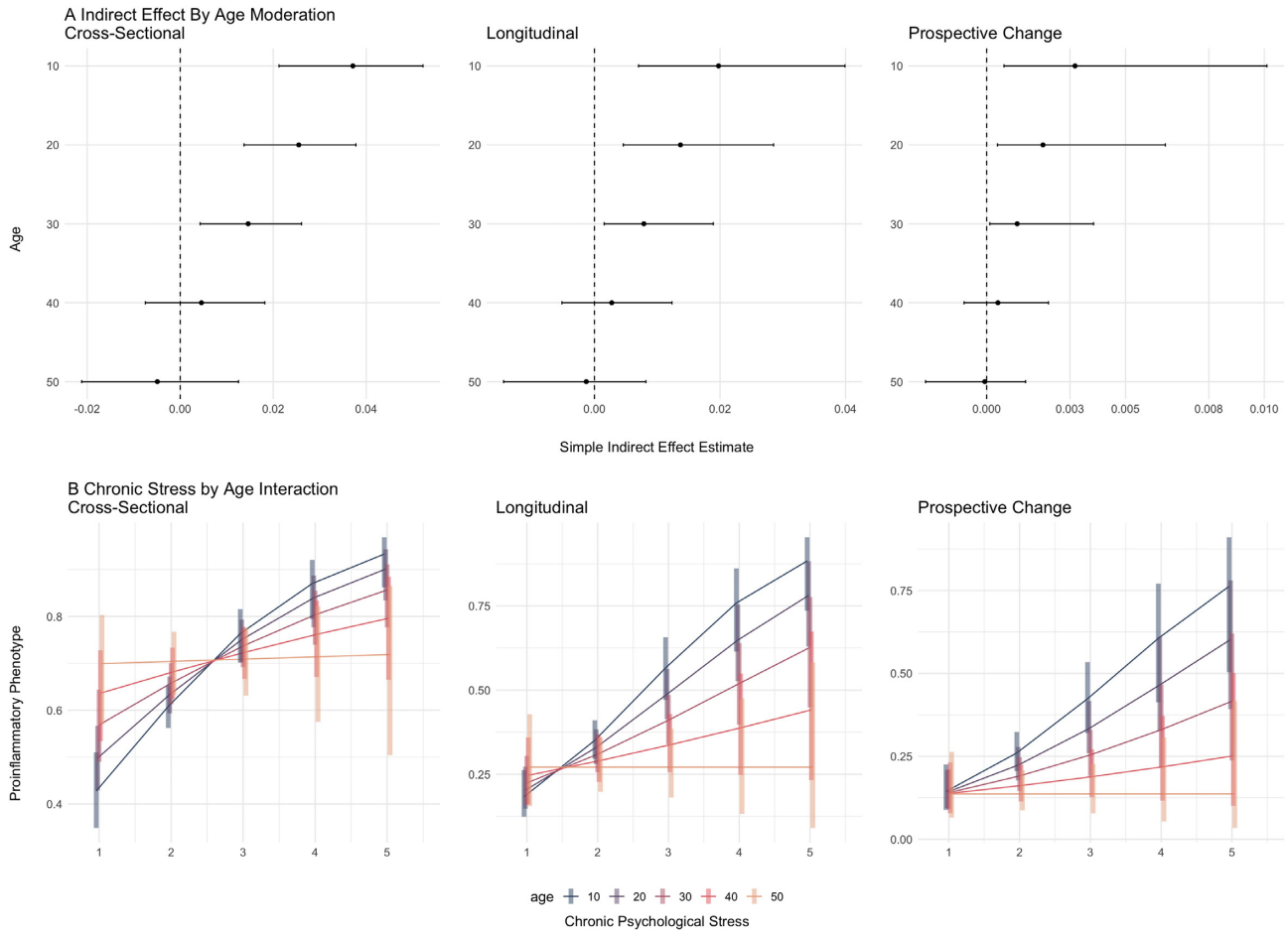

Fig. 3. Indirect effects from disadvantage to chronic stress to proinflammatory phenotype moderated by age (Panel A). Higher values on the x-axis indicate larger indirect effect estimates with horizontal bars indicating 95% CI, and higher values on the y-axis indicate older age. The link between chronic psychological stress and proinflammatory phenotype moderated by age (Panel B). Higher values on the x-axis indicate more chronic psychological stress, and higher values on the y-axis indicate higher estimated probability of being in proinflammatory phenotype with vertical bars indicating 95% CI.

the samples in this investigation are also unique in the richness of their data, including interview data on socioeconomic disadvantage and chronic psychological stress as well as in vitro cell culture data to assess functional inflammatory processes. Finally, by incorporating longitudinal data, we were also able to assess changes, shedding light on the temporal precedence of social factors.

There are also several limitations to this analysis. First, there was considerable methodological variability across studies in the cell-culture data, making it difficult to harmonize these measures across studies. We attempted to overcome this challenge via the use of latent profile analyses to estimate a proinflammatory phenotype, which provided the benefit of revealing coupling patterns of these measures. However, to harmonize across studies, we fixed the number of profiles at two. Future research is necessary to explore the optimal number of profiles. Second, relative to circulating biomarkers of inflammation, the clinical predictive value of the functional measures of inflammation used here is less well-established. Clarifying the prognostic significance of these assays will also be an important task in subsequent research. Third, an integrative analysis of longitudinal data allowed the opportunity to efficiently examine lifecourse questions; however, this approach is limited by the data available across all studies as well as the duration at which measures were repeated. Here, we did not have data on early life stress for adult participants and the duration between Time 1 and Time 2 (about 1.5 to 2 years) is relatively short for lifecourse inquiries. As such, it is possible that the observed age effects on the stress–inflammation link is due to participants having developed an adaptation to higher levels of cytokine and, thus also developed lowered sensitivity to inhibition as they aged. Indeed, we observed that older participants had decreased odds of exhibiting the proinflammatory phenotype; however, all models controlled for main effects of age, suggesting that the observed interaction between age and stress were independent of this general age trend. An ideal design would be a "cradle-to-grave" study that follows multiple cohorts from birth to death, repeating assessments of stress and proinflammatory phenotype across the lifespan, and then examining whether the within-individual link between stress and inflammation is moderated by timing of stress exposure. Fourth, whole blood, rather than isolated monocytes, were used in cell culture experiments. Previous research has compared LPS-evoked cytokine production in cultures of whole blood and monocytes, and found correspondence between these methods, concluding that whole blood culture is a valid method for assessing monocytic cytokine responsivity (55). Still, relative to isolated monocytes, the whole blood approach may have introduced additional noise into our results. Furthermore, as chronic stress has been found to promote mo-

bilization of monocytes from the spleen and bone marrow (56), higher stress-related values may partially reflect variations in the number or fraction of monocytes present in culture, rather than per-cell alterations to monocytes' operating tendencies. Future research can help clarify this by stimulating isolated monocyte populations or conducting flow cytometric analyses of monocyte-specific cytokine production.

To conclude, the link between stress and inflammation is well-established, but most extant studies have relied on circulating markers of inflammation and do not examine how associations may vary across the lifecourse. This integrated data analysis found that socioeconomic disadvantage operated through chronic psychological stress to confer risks for developing a proinflammatory phenotype characterized by cells mounting more pronounced inflammatory responses and being less sensitive to inhibition signals that typically terminate their responses. Of note, the magnitude of these associations was strongest in the early decades of life and declined with age, suggesting a sensitive developmental period during which immune functioning may be most sensitive to environmental calibration.

Methods

This integrative data analysis combined all five of our lab's studies that have relevant data (40–44). Studies were approved by the institutional review board of the university where they were conducted. Adults and a guardian of youth gave consent, and youth gave assent. Supplementary materials provide full description of the method and analytical approach. Data and analysis codes available on Github repository: https://github.com/phoebehlam/mega2.

Supplementary Material

Supplementary material is available at *PNAS Nexus* online.

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Authors' Contributions

E.C. and G.E.M. designed and collected data for all studies; P.H.L. conducted the analyses; and E.C., G.E.M., J.J.C., and P.H.L. wrote and/or revised the manuscript.

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