This paper has now been published in Clinical Psychological Science. Please cite as:

Morariu, D.P., Kautz, M., Mac Giollabhui, N., Ellman, L.M., Coe, C., Abramson, L.Y., & Alloy, L.B. (2020). Bidirectional associations between inflammatory biomarkers and depressive symptoms in adolescents: Potential causal relationships. *Clinical Psychological Science*, 8(4), 690-703. <https://doi.org/10.1177/2167702620917458>.

Bidirectional Associations Between Inflammatory Biomarkers and Depressive Symptoms in Adolescents:
Potential Causal Relationships

Daniel P. Moriarity¹, Marin M. Kautz¹, Naoise Mac Giollabui¹, Joshua Klugman¹, Christopher L. Coe²,
Lauren M. Ellman¹, Lyn Y. Abramson², Lauren B. Alloy¹

¹Department of Psychology, Temple University, Weiss Hall, 1701 N. 13th St., Philadelphia, PA 19122,
United States of America

²Department of Psychology, University of Wisconsin, 1202 West Johnson Street, Madison, WI 53706,
United States of America

Correspondence concerning this article should be addressed to Lauren B. Alloy, Department of Psychology, Temple University, Weiss Hall, 1701 N. 13th St., Philadelphia, PA 19122, United States of America. Fax: 1-215-204-5539. E-mail: lalloy@temple.edu.

Funding: This research was supported by National Institute of Mental Health grants MH079369 and MH101168 to Lauren B. Alloy. Daniel P. Moriarity was supported by National Research Service Award F31MH122116. Naoise Mac Giollabhui was supported by National Research Service Award F31MH118808. Marin Kautz was supported by National Science Foundation Graduate Research Fellowship (2018263024).

Declarations of interest: none

Abstract

There are inconsistent findings in the literature about the directionality and magnitude of the association between inflammation and depressive symptoms. This analysis separates predictors into between-person and within-person components to gain greater clarity about this relationship. Blood samples were collected and depressive symptoms assessed in 140 adolescents (54% female, 59% Black, Mage = 16.1 years) with at least three blood draws and a total of 394 follow-up observations. Multi-level modeling indicated that the within-person effect of tumor necrosis factor alpha (TNF- α) predicted change in total depressive symptoms, suggesting a potential causal relationship. There were no significant within-person effects of total depressive symptoms on change in biomarkers. Exploratory analyses examined associations between inflammatory biomarkers and subsets of depressive symptoms. These findings inform modeling decisions that may explain inconsistencies in the extant literature as well as suggest potential causal relationships between certain proteins with significant within-person effects on depressive symptoms, and vice-versa.

Introduction

Association between Inflammation and Depressive Symptoms

Growing evidence implicates inflammatory processes in the pathophysiology of depressive symptoms; however, much is still unknown about the exact role that inflammation plays in the etiology of depression. Additionally, increased levels of inflammatory proteins in the blood stream may be a downstream effect of depression. Initial evidence for the inflammation—depression relationship came from studies finding that depressive symptoms were more common in patients with medical conditions associated with increased inflammatory activity (Calder, 2006; Goodwin, Fergusson, & Horwood, 2004; Ohayon & Schatzberg, 2003; Pan et al., 2012; Whooley, 2006). In addition, many studies found higher levels of proinflammatory cytokines, including interleukin (IL)-1 β , IL-6, IL-8, and tumor necrosis factor alpha (TNF- α), and acute phase reactants, such as C-reactive protein (CRP), in individuals with clinical depression when compared to controls (Dhabhar et al., 2009; Dowlati et al., 2010; Howren, Lamkin, & Suls, 2009). Proinflammatory cytokines normally function as inter-cellular signaling proteins, and are upregulated during infection or injury, but we now know that they can act through neuroimmune pathways to stimulate regions of the brain associated with emotionality, including limbic areas and the insular cortex (Eisenberger, Moieni, Inagaki, Muscatell, & Irwin, 2017; Janeway, 1989). These findings also make sense within the larger theoretical framework of cytokines and sickness behavior, which is based on basic science studies showing that proinflammatory cytokines can evoke feelings of malaise, fatigue, anorexia and an inability to concentrate (Hart, 1988). Anti-inflammatory cytokines (e.g., IL-10), which modulate the effects of proinflammatory biomarkers, also have been associated with depressive symptoms. However, the relationship between IL-10 and depressive symptoms may be complicated, as some studies have found these proteins were associated with higher (e.g., Meyer et al., 2011), and others lower (e.g., Dhabhar et al., 2009), levels of depressive symptoms.

When inflammatory pathways are activated, there also are effects on the endocrine system, including stimulation of the hypothalamic-pituitary-adrenal axis, resulting in a downregulation of

glucocorticoid receptors (which can result in a resistance to glucocorticoid actions) and less effective hormonal regulation of inflammatory activity. The resulting proinflammatory phenotype has been posited to be a risk factor for depressive symptoms (Miller, Maletic, & Raison, 2009; Miller, Cohen, & Ritchey, 2002). This association has gained considerable support (Howren et al., 2009; Miller et al., 2009); however, the vast majority of published studies have used cross-sectional designs. More longitudinal research is needed to evaluate causality and the direction of the relationship between inflammation and depressive symptoms.

Previous prospective studies have yielded inconsistent results regarding the association between inflammatory biomarkers and depressive symptoms, illustrated here with studies testing the association between both CRP and IL-6 (two of the most commonly studied biomarkers in depression research) and depressive symptoms in adult samples. Some studies found that higher levels of CRP and IL-6 were associated with greater severity of future depressive symptoms (e.g., Gimeno et al., 2009; Zalli, Jovanova, Hoogendijk, Tiemeier, & Carvalho, 2016). Other studies found that depressive symptoms predicted changes in IL-6, but not CRP, over time (e.g., Stewart, Rand, Muldoon, & Kamarck, 2009). Importantly, both Gimeno et al. (2009) and Stewart et al. (2009) tested bidirectional associations but obtained support only for the unidirectional effects described above, which were in opposite directions. Thus, there is inconsistency in published results with respect to the presence and direction of associations, and for which biomarker the associations were observed.

Association between Inflammation and Depressive Symptoms in Adolescence

There are relatively few prospective studies on inflammation and depression in adolescent samples, a critical period for the emergence of depressive symptoms (Hankin et al., 1998). In addition to the potential for depressive symptoms to progress to clinical diagnoses, even subclinical levels of depressive symptoms can have problematic behavioral consequences, including suicide and poor school performance (Balázs et al., 2013; van Lang, Ferdinand, & Verhulst, 2007). Further, adolescent-onset depression is associated with a recurrent course of depression throughout life (Gilman, Kawachi,

Fitzmaurice, & Buka, 2003). Consequently, well-designed research on inflammatory processes that may underlie risk for depressive symptoms during this developmental period is needed.

Congruent with the adult literature, longitudinal research in adolescent samples also suggests the potential for a bidirectional association between inflammatory biomarkers and depressive symptoms. One study (Miller & Cole, 2012) reported a bidirectional relationship between inflammation and depression risk, albeit with different biomarkers, in a sample of adolescent females with a history of childhood adversity. They found that participants who experienced a depressive episode were more likely to have elevated CRP at a 6-month follow-up, and that high levels of IL-6 were associated with increased depression risk at follow-up. Another study obtained similar results, finding that high levels of IL-6 were associated with greater depression risk compared to low levels of IL-6 (Khandaker, Pearson, Zammit, Lewis, & Jones, 2014). Notably, both studies did not find associations between CRP and later depression risk. Copeland, Shanahan, Worthman, Angold, and Costello (2012) did not find a significant association between CRP and later depression; however, consistent with Miller and Cole (2012), this study found that more cumulative episodes of depression were prospectively associated with future CRP. In another study, Duivis et al. (2015) found that consistently moderate or high levels of depressive symptoms were associated with higher future CRP. These studies suggest a bidirectional relationship between inflammatory biomarkers and depressive symptoms in adolescents, highlighting the importance of collecting and analyzing longitudinal data with repeated measures of both biomarkers and symptoms.

Inflammation and Types of Depressive Symptoms

A potentially important source of the inconsistency in the research reviewed above may be differential relationships between inflammatory biomarkers and specific depressive symptoms (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Kuhlman et al., 2018). As the vast majority of studies examined diagnostic status or analyzed total depressive symptoms, differences in particular symptom endorsement could drive discrepant results. Thus, testing symptom subtypes, or specific symptoms, might provide more replicable findings. Recent commentaries have argued for the importance of investigating

inflammatory phenotypes of depression (Felger, Haroon, & Miller, 2018; Krishnadas & Harrison, 2016). This interest inspired a recent review examining experimental evidence for the association between inflammation and depression, which concluded that increased inflammatory activity likely is associated with exaggerated reactivity to negative information, altered reward reactivity, and somatic symptoms, but less likely to be associated with deficits in cognitive control (Dooley et al., 2018).

A Causal Relationship?

The majority of longitudinal studies supporting an association between inflammatory biomarkers and depressive symptoms do not allow for conclusions of causality because their analyses do not track how changes in one variable predicts changes in the other. Rather, they tested whether individuals with higher levels of X have higher levels of Y, also known as a between-person effect. Consequently, the association between inflammation and depressive symptoms might be driven by shared risk factors that contribute to between-person differences in both variables (e.g., gender). However, there is some evidence from quasi-experimental designs that supports the causal relationship between inflammation and depression.

Several studies have shown that inflammatory challenges (e.g., administering endotoxin) are associated with increases in depressive symptoms (e.g., Dantzer & Kelley, 2007; Watkins & Maier, 1999). Others have found that the use of anti-inflammatory medications is associated with reductions in depressive symptoms in some patients (e.g., Raison et al., 2013). Additionally, a recent study found that IL-6 responses to flu vaccination were associated with depressive symptoms (Kuhlman et al., 2018). Specifically, greater increases in IL-6 post-vaccine were associated significantly with increases in depressed mood, greater confusion, and lower average daily affect, but not somatic symptoms, changes in sleep, or feelings of social disconnection. Thus, there is evidence from quasi-experimental designs involving acute inflammatory challenges/interventions that changes in inflammatory physiology may cause changes in depressive symptoms. However, designs using acute inflammatory challenges may differ from natural fluctuations in basal levels of inflammatory biomarkers. Consequently, there is still a

need for longitudinal naturalistic studies that test the association between within-person changes in inflammatory biomarkers and depressive symptoms, and vice-versa.

Although experimental designs are the gold-standard for testing causal associations, this also can be done in studies with repeated measures of both predictors and outcomes by using lagged models that separate a given predictor (e.g., IL-6) into between-person and within-person variance components (Falkenström, Finkel, Sandell, Rubel, & Holmqvist, 2017). Isolating between-person variance is done by calculating the mean of IL-6 across all time points for an individual to compare differences between the participants' average levels of IL-6. Then, to isolate within-person variance over time, a second variable is created by centering the value of IL-6 at the mean for the individual at each time point. This is done by subtracting the average of IL-6 calculated in the previous step from the value at each time point to isolate changes in IL-6, controlling for a person's average levels. The resulting within-person variance component tests whether changes in the predictor are related to changes in an outcome, which tests possible causal relationships without potential confounding by unmeasured stable variables (Falkenström et al., 2017).

The Current Study

Our study examines the bidirectional longitudinal associations of five inflammatory biomarkers (CRP, IL-6, IL-8, IL-10, and TNF- α) and depressive symptoms in an ethnically diverse community sample of adolescents. The panel of inflammatory biomarkers was chosen because of their frequent use in depression research, maximizing the ability of this study to inform the interpretation and design of studies. In addition, investigating the association between inflammation and depression in a community sample is important, because most of the prior literature focused on at-risk or clinical samples (e.g. Dhabhar et al., 2009; Dowlati et al., 2010; Howren et al., 2009; Miller & Cole, 2012). The models tested in this study separated repeated-measures predictors into both between-person (average value for an individual across all time points) and within-person (person-centered, or the average value for an individual subtracted from the value at each time point) variance components to isolate the levels of a

predictor that are typical for a given participant and the effects of changes in a predictor over time, respectively. Specifically, the within-person variance components can test potential causal relationships. The *a priori* hypothesis was that within-person components of CRP and proinflammatory cytokine levels would be positively associated with subsequent change in depressive symptoms. Given mixed findings for the directionality of the association between IL-10 and depressive symptoms (Dhabhar et al., 2009; Meyer et al., 2011), no directional hypotheses were made. Additionally, we hypothesized that within-person components of depressive symptoms would predict subsequent change in biomarker levels, in the same directions described above. As exploratory analyses, we also tested the prospective relationships between the five biomarkers and subtypes of depressive symptoms. Although the within-person associations are the focus of this study because of their implications for causal relationships, between-person associations will be reported in the supplemental results to better facilitate comparison with the extant literature, which has primarily tested for between-person differences.

Methods

Participants

Participants were drawn from the Adolescent Cognition and Emotion (ACE) project at Temple University. A community sample of 641 adolescents aged 12-13 and their mothers or primary female caregivers were recruited from the greater Philadelphia area. Recruitment for this study involved a combination of mailings and follow up calls to families with children attending Philadelphia public and private middle schools (68% of the total sample) and advertisement in local newspapers (32% of the sample). Inclusion criteria for Project ACE included (1) sufficient competence with the English language to complete the assessments and (2) identification as either Caucasian or African American, as the investigation of differences in the etiology of depression comparing racial groups was one of the aims of Project ACE. Exclusion criteria for Project ACE included a history of severe psychiatric illness or developmental disorders (see Alloy et al., 2012 for further information). Informed written consent was

obtained from mothers and written assent from adolescents prior to data collection. The Temple University Institutional Review Board approved the protocol (IRB protocol #6844).

From Project ACE, a subsample of 315 adolescents volunteered to participate in an optional blood draw protocol. This analytic sample included up to seven assessments (some had more blood draws than others, as it was an optional part of annual assessments). Nineteen observations were excluded due to participants taking anti-inflammatory medications (e.g., ibuprofen) at the time of blood draw. An additional 78 observations were removed due to CRP values > 10 mg/L, which may indicate an acute infection (Bell et al., 2017; de Ferranti, Gauvreau, Ludwih, Newburger, & Rifai, 2006). After accounting for these exclusion criteria and missing data, and removing participants with fewer than three blood draws, the final analytic sample included 534 observations (394 follow-ups after losing the first observation due to lagging predictors) across 140 participants (54% female, 59% Black; mean age at first blood draw = 16.1; $SD = 1.4$ years, range = 12.1-21.1 years). Due to differential missingness in covariates between models, sample size varied slightly between models (see Tables 1 and 2). An attrition analysis was conducted to determine whether any demographic variables were associated with the number of follow-ups after the first blood draw. Demographic variables and average depressive symptoms across time were not associated with number of follow-ups (all p 's $> .05$). Across the study period, 21% of participants reported depressive symptom scores indicative of at least mild depression (Bang, Park, & Kim, 2015).

Measures

Depressive Symptoms

Symptoms of depression were measured using the Children's Depression Inventory (CDI; Kovacs, 1985). It consists of 27 items reflecting affective, behavioral, and cognitive symptoms of depression. Items are rated on a 0 to 2 scale and total scores range from 0 to 54. The CDI has been demonstrated to be a reliable and valid measure of depressive symptoms in youth samples (Klein,

Dougherty, & Olino, 2005). Internal consistency in this sample was $\alpha = 0.88$ at the first study visit. The CDI was administered at all assessments. All items in the scale were summed to create the total CDI score. Additionally, five depressive symptom subscales were used, in accordance with a meta-analytic factor structure of the CDI in adolescents (Huang & Dong, 2014). The five subscales were somatic concerns (9 items; $\alpha = 0.63$), externalizing (6 items; $\alpha = 0.69$), negative self-concept (7 items; $\alpha = 0.62$), lack of personal and social interest (5 items; $\alpha = 0.73$), and dysphoric mood (4 items; $\alpha = 0.75$).

Inflammatory physiology

Blood samples were obtained via antecubital venipuncture by a certified phlebotomist into a 10 mL vacutainer designed for freezing plasma separated from the cell fraction within the vial (BD Hemogard with K2 EDTA). Vacutainers were stored in an ultracold freezer at -80°C , and later thawed on the day of assay. At each blood draw, the time of collection and participants' body mass index (BMI) based on direct measurement of height and weight were recorded. Four cytokines were quantified by multi-cytokine array (IL-6, IL-8, IL-10, and TNF- α), and high-sensitivity CRP was determined in a singleplex assay, using an electrochemiluminescence platform and a QuickPlex SQ 120 imager for analyte detection (Meso Scale Discovery, Gaithersburg, MD). Each specimen was assayed in duplicate and the intra-assay coefficients of variation averaged 5.36 and 2.29 for the cytokines and CRP, respectively. Plasma was diluted 1:2 for the cytokine assay and 1:1000 for the CRP assay. CRP is present in blood at higher concentrations, and thus, plasma was diluted to correspond to the standard curve. Values were calculated with respect to a standard curve generated from 7 calibrators with known concentrations. The lower limit of detection (LLOD) for the cytokines was 0.1 pg/mL, with a large dynamic range up to 2000 pg/mL. The LLOD for CRP was 0.1 mg/L. Values below the LLOD were set at the LLOD. Values were converted to mg/L units to be consistent with the clinical literature (Breen et al., 2011; Dabitao, Margolick, Lopez, & Bream, 2011).

Procedure

All demographic information was self-reported during the first visit of the full Project ACE study. At each time point in the analytic sample, adolescents completed a blood draw and the CDI. All 140 participants had at least two follow-ups after the first blood draw and CDI assessment (T2: months after T1; M = 16.57 months, SD = 6.84 months; T3: months after T2; M = 13.46 months, SD = 6.72 months), 75 had a third (months after T3 M = 12.74 months, SD = 6.54 months), 30 had a fourth (months after T4 M = 10.92 months, SD = 5.13 months), 8 had a fifth (months after T5 M = 11.52 months, SD = 2.60 months), and one had a sixth (8.28 months after T6). Importantly, the average length of these time lags is consistent with recent evidence that the association between inflammatory biomarkers and change in depressive symptoms is strongest for durations > 13 months (Moriarity et al., 2019).

Data Analysis Plan

All descriptive statistics, correlations, and preliminary analyses were conducted in SPSS (IBM Corp, 2016). Primary analyses were conducted in R version 3.5.2 (R Core Team, 2013). Multilevel models with random intercepts and fixed slopes were estimated using packages lmer4 (Bates, Maechler, Bolker, & Walker, 2015) and lmerTest (Kuznetsova, Brockhoff, & RHD, 2017) in R x64 3.5.2 (R Core Team, 2013). Riverplots were created using the riverplot function (Weiner, 2017) to visualize variance accounted for by predictors, which were calculated using the r2MLM function (Rights & Sterba, 2018). Five models were estimated with each protein predicting to change in total depressive symptoms between each timepoint. To test for bidirectional effects, five additional models were run with total depressive symptoms predicting change in each biomarker between each timepoint.

Depression symptoms and biomarkers were lagged for temporal precedence. Gender, race, and family income were modeled as person-level predictors (Alanna et al., 2011; Deverts, Cohen, Kalra, & Matthews, 2012; Hankin et al., 1998). All repeated measures (biomarkers, depressive symptoms, age at measurement of the outcome, BMI, time of blood draw, hormonal birth control use, and taking a medication that can influence inflammation [e.g. preventative asthma inhaler]) used as predictors were separated into within-person (person-centered) and between-person (the mean across observations per

individual) components (Dominguez-Rodriguez, Abreu-Gonzalez, & Kaski, 2009; Mills, Scott, Wray, Cohen-Woods, & Baune, 2013; Skovlund, Mørch, Kessing, & Lidegaard, 2016). BMI was lagged in all models because it is prospectively associated with both inflammation and depression symptoms (Mac Giollaibhui et al., in press). Medication status, birth control, and time of blood draw were used from the visit of the immune data (e.g., lagged when predicting to depression, contemporaneous when predicting to inflammatory biomarkers). Additionally, the number of months between blood draws was used as an observation-level predictor. Given this study's longitudinal design, months-to-follow-up was not centered or aggregated. Consequently, months since blood draw estimates a combination of within- and between-person effects. Inflammatory biomarker values > 3 SD from the mean were winsorized. This resulted in the winsorization of eighteen CRP, nine IL-6, eight IL-8, four IL-10, and five TNF- α values. All models were run with and without log-transforming ($\text{Log}(100 \times \text{value})$) CRP, IL-6, IL-8, IL-10, and TNF- α values (pre-log-transformation skewness = 1.72, 2.93, 4.71, 7.80, 1.14, respectively; post-log-transformation skewness = -.04, .50, .79, 1.80, -.22, respectively). Plots of the resulting model residuals were checked visually and the model that best satisfied the assumption of normality (i.e., residuals most closely followed the linear model) was interpreted. Both level 1 and level 2 residuals were investigated. Normality of level-1 residuals was prioritized due to this study's focus on time-varying predictors and the fact that the majority of the variance in the models was observation-level variability. In cases of negligible differences in distribution of residuals, the log-transformed model was interpreted to be consistent with convention. To investigate the potential for covariances to differ over time, all models originally were tested with interaction terms between all predictors and months-to-follow-up. To preserve degrees of freedom, only interaction terms with significant associations were included in the final models described below. In addition to the universal covariates described above, this resulted in the addition of interaction terms between a) time and the between-person effect of birth control predicting change in IL-6, IL-8, and IL-10, b) between time and gender predicting change in IL-8, IL-10, and TNF- α , and c) between time and the between-person effect of medication status predicting change in IL-10. There were

no significant interactions between months-to-follow-up and any of our key IVs (biomarkers or symptoms) or in any of the models predicting to change in symptoms.

Multilevel modeling was chosen over traditional regression techniques because the data were clustered within individuals, which would result in greater probability of Type I error and less efficient estimates of coefficients for regression compared to multilevel modeling. Models were estimated using restricted maximum likelihood. Family-wise Holm-Bonferroni (Holm, 1979) corrections were used for all analyses. Exploratory analyses were run using identical models as the primary analyses, except substituting CDI subscales for total depressive symptoms. Although the American Statistical Association does not recommend selective reporting of results on the basis of p -values, in the interest of space, only results significant at $p < .05$ will be described in the exploratory analyses below. Exact p -values will be reported for all primary analyses, regardless of significance.

Results

Preliminary Analyses

Descriptive statistics and bivariate correlations for the main study variables at T1, including untransformed inflammation variables, are presented in Supplemental Table 1. Correlations were calculated with log-transformed biomarker values to improve normality of the distributions, but descriptive statistics are reported using raw variables to increase interpretability. Independent sample t -tests tested whether average levels of total depressive symptoms or inflammatory biomarkers differed by race or sex. Significant sex differences were observed for CRP [males = 1.12 (SD = 1.17); females = 1.77 (SD = 1.79), $t(128.868) = -2.59$, $p = .01$] and IL-6 [males = .32 (SD = .21); females = .44 (SD = .26), $t(138) = -2.95$, $p < .01$], but not TNF- α , IL-8, IL-10, or depressive symptoms ($p = .52$, .23, .16, .77, respectively). Significant racial differences were observed for TNF- α [Caucasians = 1.55 (SD = .33); African Americans = 1.38 (SD = .29), $t(138) = 3.21$, $p < .01$], but not CRP, IL-6, IL-8, IL-10, or depressive symptoms ($p = .27$, .83, .08, .16, .08, respectively). Independent samples t -tests (for

continuous variables) and chi-square tests (for categorical variables) found no significant differences in total depression symptoms, income, race, gender, or any of the biomarkers between the total group of adolescents with at least one blood draw and the analytic sample. The analytic sample was significantly younger at their first blood draw than the total sample of participants with blood draws ($p < .01$, $M_{age} = 16.13$ years and 17.02 years, respectively), likely reflecting participants dropping out of the study as they transitioned into emerging adulthood. The analytic sample also was compared to the entire ACE sample, for which blood draws were an optional component, on demographics and baseline depression symptoms. The analytic and total samples did not differ on total depression symptoms at their first study visit, household income, race, or gender (all p 's $< .05$). Participants in the analytic sample were significantly older than the rest of the sample at their first study visit ($p = .02$, $M_{age} = 12.72$ years and 12.53 years, respectively).

Primary Analyses

Inflammatory biomarkers predicting depressive symptoms

Log TNF- α had a small within-person effect (following the guidelines for interpreting standardized betas as effect sizes; Acock, 2014) on change in total depressive symptoms ($\beta = 0.156$, $SE = 0.060$, $p = .009$; see Table 1 for model results and Figure 1 for variance decomposition), such that increases in TNF- α predicted increases in depressive symptoms. The association between within-person TNF- α and total depressive symptoms was robust to Holm-Bonferroni corrections (adjusted p -value = .045). Raw CRP, raw IL-6, log IL-8, and raw IL-10 did not have significant within-person effects on change in total depressive symptoms ($p = .993$, $p = .657$, $p = .890$, $p = .850$, respectively). Results with the between-person variance components are provided in Supplemental Materials (no significant results).

Depressive symptoms predicting inflammatory biomarkers

Total depressive symptoms did not have a significant within-person effect on change in log CRP, log IL-6, log IL-8, log IL-10, or log TNF- α ($p = .671$, $p = .091$, $p = .341$, $p = .249$, $p = .077$, respectively;

see Table 2). Results with the between-person variance components are provided in Supplemental Materials (no significant results).

Exploratory Analyses

Inflammatory biomarkers predicting depressive symptom subtypes

Both log TNF- α and log IL-10 had small within-person effects on changes in dysphoria ($\beta = 0.168$, $SE = 0.059$, $p = .005$ and $\beta = 0.166$, $SE = 0.063$, $p = .009$, respectively) such that increases in these cytokines predicted increases in dysphoric symptoms (Figure 2). Both results survived family-wise (grouped by inflammatory protein) Holm-Bonferroni corrections (adjusted p -values = .045 and .048, respectively). No other within-person effects of inflammatory biomarkers on symptom subtypes reached significance. Results with the between-person variance components are provided in Supplemental Materials). See Figure 2 for a summary of significant exploratory results.

Depressive symptom subtypes predicting inflammatory biomarkers

Dysphoria had a small within-person effect on changes in log IL6 ($\beta = 0.192$, $SE = 0.059$, $p = .001$), log IL-10 ($\beta = 0.178$, $SE = 0.057$, $p = .002$), and a moderate within-person effect on log TNF- α ($\beta = 0.220$, $SE = 0.058$, $p = .0002$), such that increases in dysphoria predicted increases in these cytokines (Figure 2). These significant findings were robust to familywise (grouped by symptom subtype) Holm-Bonferroni corrections (adjusted p -values = .005, .006, .001, respectively). No other within-person effects of symptom subtypes on inflammatory biomarkers reached or approached significance. Results with the between-person variance components are provided in Supplemental Materials (no significant results).

Discussion

There is substantial evidence of a relationship between inflammatory physiology and depressive symptoms; however, there is still much to be learned about the directionality and degree of causality between these variables. This study extends previous literature on the associations between inflammatory

physiology and depressive symptoms in adolescents by concurrently testing the extent to which average levels of, and changes in, inflammatory biomarkers predict changes in depressive symptoms, and vice-versa. Additionally, it provides some support for bidirectional associations between inflammatory biomarkers and dysphoric symptoms. In these analyses, we found a within-person effect of TNF- α on the development of total depressive symptoms in adolescents. Importantly, the significant within-person association of TNF- α with change in depressive symptoms is suggestive of a potential causal relationship. Further, the presence of a significant within-person association of this cytokine with total depressive symptoms, but lack of a within-person association of total depressive symptoms with any biomarkers, provides support for viewing inflammation as a risk factor rather than just as a downstream consequence of depression. However, results from our exploratory analyses suggest there might be important differences in the strength and direction of these associations by symptom domains.

Exploratory analyses were conducted using depressive symptom subscales, in line with theory that different depressive symptoms have different risk factors and that inflammatory physiology may be more closely related to some depressive phenotypes than others (Dooley et al., 2018; Felger et al., 2018; Fried, Nesse, Zivin, Guille, & Sen, 2014; Krishnadas & Harrison, 2016). The results indicated that biomarkers were differentially predictive of different subscales of symptoms, providing further insight into the development of an inflammatory phenotype of depression and potential sources of heterogeneity among existing studies that use clinical diagnoses or total depressive symptoms without consideration of the types of symptoms endorsed by participants. Specifically, increases in TNF- α and IL-10 predicted increases in dysphoric symptoms and increases in dysphoric symptoms predicted increases in TNF- α , IL-10, and IL-6. Importantly, there were significant, bidirectional within-person effects of TNF- α and IL-10 with dysphoric symptoms, supporting a bidirectional and potentially causal relationship (Falkenström et al., 2017). The results also are in keeping with the conclusions of Dooley et al. (2018), who concluded that changes in inflammation might induce greater affective reactivity. Additionally, this result is congruent with Kuhlman and colleagues (2018), which also focused on within-person effects of

inflammation on depression symptoms, as was the null findings predicting to somatic symptoms.

Although the analysis provided preliminary evidence for specificity, fifty models were run in total for the exploratory analyses, resulting in an increased chance of Type-I error. However, it is worth noting that all significant results were robust to Holm-Bonferroni corrections.

The observation that only the within-person effect of TNF- α predicted increases in total depression symptoms potentially could indicate that it is associated with a less specific set of symptoms than IL-6 or IL-10, which only were associated with dysphoria. Alternatively, differences in the association between an inflammatory protein and behavioral outcomes could be due to differences in the synthesis and release of specific biomarkers. For example, it has been hypothesized that TNF- α might be an important inflammatory protein in the pathogenesis of depression because of its role in maintaining proinflammatory states and the ability to dampen synaptic plasticity (see Brymer, Romay-Tallon, Allen, Caruncho, & Kalynchuk, 2019 for a review on the role of TNF- α in depression). However, until this specific pattern of results is replicated, it is more parsimonious to interpret that this result was driven by TNF- α 's association with dysphoria symptoms, as this was the only depression subscale that TNF- α was associated with. Either way, these results suggest that TNF- α should be strongly considered when selecting inflammatory biomarkers for depression studies, as it is currently studied much less frequently than CRP or IL-6.

Given the fact that the significant effects in our models were all within-person effects, some heterogeneity in the extant literature might be due to reliance on parameters that solely represent between-person effects or conflate between- and within-person effects. Further, considering the results using symptom subscales, another source of variation in extant studies using depression diagnoses or total depressive symptoms might be the distribution of symptoms endorsed in the sample. Indeed, in addition to more significant associations, the effect sizes for significant associations with dysphoria were larger than the significant effect size between TNF- α and total depression symptoms. For example, given these results, significant associations between TNF- α , IL-6, or IL-10 and depression diagnoses/total depression

symptoms would be most likely in samples with high rates of endorsement of dysphoric symptoms. In addition to providing evidence that inflammatory activity could be a precipitating cause of depressive symptoms, and vice-versa, the selective linkages with specific symptom subtypes may inform how best to characterize and diagnose inflammation-related depression in adolescents.

Finally, our evidence that inflammation was predictive of change in depressive symptoms in adolescents also concurs with the recent interest in anti-inflammatory drugs as a therapeutic modality for depression resistant to traditional treatment modalities. Although the majority of our effect sizes were modest, it is important to note that these effect sizes were for single proteins. Thus, broad-acting anti-inflammatory treatments might have a larger effect on symptom reduction. Specifically, our results suggest they may be useful for targeting specific symptoms, such as dysphoria and affective reactivity (Dooley et al., 2018). The likelihood of some reciprocal association also is consistent with the efficacy reported for several cognitive-behavioral interventions (e.g., mindfulness-based stress-reduction) that have been found to reduce both inflammatory activity and negative affect in practitioners (Malarkey, Jarjoura, & Klatt, 2013; Raison et al., 2013; Rosenkranz et al., 2013).

Contrary to the a priori hypotheses, but consistent with several other studies (Copeland et al., 2012; Khandaker et al., 2014; Miller & Cole, 2012), CRP was not predictive of depressive symptoms in any model. This lack of association may partially reflect the young age of the participants, because it is known that CRP levels typically will rise in adulthood. In addition, the rise of CRP in middle-aged adults often is associated with obesity, which is also a risk factor for depression (Preiss, Brennan, & Clarke, 2013). Even in these adolescent participants, log CRP was correlated significantly with BMI at $r = .56$; thus, including BMI as a covariate in all models would have accounted for more variance in the models with CRP compared to models with other biomarkers. Additionally, CRP remained fairly stable over time, resulting in less variation to explain within-person changes in depression symptoms.

Strengths and Limitations

This study had several important strengths. First, it included a large, racially diverse, community sample of adolescents, a population still understudied in behavioral medicine research. Second, most adolescents typically are subject to fewer potential confounds of the association between inflammatory physiology and depression (e.g., medication status, cumulative life stress, cumulative illness) compared to an identical study with adults. Third, the delineation of symptom and biomarker predictor variables into between-person and within-person components in a lagged-model design is a powerful and novel statistical approach to this area of research, which can be applied to other datasets interrogating temporal relationships between physiology and biobehavioral health. It allows for an examination of potential causal effects resulting from changes over time vs. differences between individuals in average levels of a predictor (Falkenström et al., 2017). Finally, the exploratory analyses using symptom subscales were informative for potential explanations for inconsistencies in the extant literature as well as generating additional hypotheses about inflammatory phenotypes of depression.

However, several limitations also should be acknowledged. First, participants were at the upper end of the age-range for which the CDI was validated. Second, this study used self-reported depressive symptoms, rather than clinical interviews, and thus, did not include diagnosed depressive episodes. Although inflammatory activity is believed to be associated with discrete depressive symptoms, not just clinical depression diagnoses, inclusion of analyses testing clinically relevant episodes would have improved the clinical relevance of this study. This concern was mitigated to some degree by the presence of at least mild levels of depressive symptoms in 21% of the sample. Fourth, although within-person predictive associations may suggest potential causal relationships, there remains the possibility that unmeasured, time-varying confounding variables might be driving the effects (e.g., changes in exercise, life stress, and sleep). Fifth, although Moriarty and colleagues (2019) found that at least 13 months was ideal for finding associations between inflammatory biomarkers and change in depression symptoms, it is important to note that this previous study only tested between-person effects. Thus, this time lag might not be ideal for testing the within-person effects that were the focus of this paper, potentially resulting in

an underestimation of effects. Finally, it should be acknowledged that the majority of observed associations were modest. This concern is not surprising, given typical effect sizes of biological variables on psychiatric symptoms and the use of models separating predictors into two different components. However, the translational relevance for clinical practice should be considered with respect to the magnitude of the observed relationships.¹

Conclusion

This study provides support for predictive, and potentially causal, relationships between some aspects of inflammatory physiology and depressive symptoms, specifically dysphoria, in adolescents. The findings have implications both for the etiology of depression and specific inflammatory profiles as well as the classification of an inflammatory phenotype of depression. Furthermore, the prospective associations between inflammatory physiology and depressive symptoms in adolescents suggests that treatments with anti-inflammatory properties (pharmacological or otherwise) might be beneficial in treating symptoms of depression, specifically dysphoria.

¹ There was negative shared variance between biomarkers/depressive symptoms and covariates in several models. Although this is nonintuitive, this is likely due to the coefficients of some of the biomarker/symptom predictors being larger in the described models, which included covariates that might account for variance in the outcome variable unrelated to the primary predictors, compared to the models containing only the biomarker/symptom predictors that were used to calculate proportions of variance accounted for by the r2MLM function (Rights & Sterba, 2018).

References

- Acock, A. C. (2014). *A Gentle Introduction to Stata* (4th ed.). Stata Press.
- Alanna, M., Zhao, L., Ahmed, Y., Stoyanova, N., Hooper, W. C., Gibbons, G., ... Vaccarino, V. (2011). Association between depression and inflammation—differences by race and sex: the META-Health study. *Psychosomatic Medicine*, 73(6), 462–468. <https://doi.org/10.1097/PSY.0b013e318222379c>
- Alloy, L. B., Black, S. K., Young, M. E., Goldstein, K. E., Shapero, B. G., Stange, J. P., ... Abramson, L. Y. (2012). Cognitive vulnerabilities and depression versus other psychopathology symptoms and diagnoses in early adolescence. *Journal of Clinical Child and Adolescent Psychology*, 41(5), 539–560. <https://doi.org/10.1080/15374416.2012.703123>
- Balázs, J., Miklósi, M., Keresztény, Á., Hoven, C. W., Carli, V., Wasserman, C., ... Wasserman, D. (2013). Adolescent subthreshold-depression and anxiety: Psychopathology, functional impairment and increased suicide risk. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 54(6), 670–677. <https://doi.org/10.1111/jcpp.12016>
- Bang, Y. R., Park, J. H., & Kim, S. H. (2015). Cut-off scores of the children's depression inventory for screening and rating severity in Korean adolescents. *Psychiatry Investigation*, 12(1), 23–28.
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Bell, J. A., Kivimäki, M., Bullmore, E. T., Steptoe, A., Bullmore, E., Vértes, P. E., ... Carvalho, L. A. (2017). Repeated exposure to systemic inflammation and risk of new depressive symptoms among older adults. *Translational Psychiatry*, 7(8), e1208. <https://doi.org/10.1038/tp.2017.155>
- Breen, E. C., Reynolds, S. M., Cox, C., Jacobson, L. P., Magpantay, L., Mulder, C. B., ... Norris, P. J. (2011). Multisite comparison of high-sensitivity multiplex cytokine assays. *Clinical and Vaccine Immunology*, 18(8), 1229–1242. <https://doi.org/10.1128/CVI.05032-11>

- Brymer, K. J., Romay-Tallon, R., Allen, J., Caruncho, H. J., & Kalynchuk, L. E. (2019). Exploring the potential antidepressant mechanisms of TNF α antagonists. *Frontiers in Neuroscience*, 13(98), 1–9. <https://doi.org/10.3389/fnins.2019.00098>
- Calder, P. C. (2006). n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. *The American Journal of Clinical Nutrition*, 83(May 2005), 1505–1519. <https://doi.org/10.1093/ajcn/83.5.1505>
- Copeland, W. E., Shanahan, L., Worthman, C., Angold, A., & Costello, E. J. (2012). Cumulative depression episodes predict later C-reactive protein levels: A prospective analysis. *Biological Psychiatry*, 71(1), 15–21. <https://doi.org/10.1016/j.biopsych.2011.09.023>
- Dabitao, D., Margolick, J. B., Lopez, J., & Bream, J. H. (2011). Multiplex measurement of proinflammatory cytokines in human serum: comparison of the Meso Scale Discovery electrochemiluminescence assay and the Cytometric Bead Array. *Journal of Immunological Methods*, 372(1–2), 71–77. <https://doi.org/10.1038/jid.2014.371>
- Dantzer, R., & Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain Research*, 21(2), 153–160. <https://doi.org/10.1016/j.bbi.2006.09.006>
- Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Reviews Neuroscience*, 9(1), 46–57.
- de Ferranti, S. D., Gauvreau, K., Ludwig, D. S., Newburger, J. W., & Rifai, N. (2006). Inflammation and changes in metabolic syndrome abnormalities in US adolescents: Findings from the 1988-1994 and 1999-2000 National health and nutrition examination surveys. *Clinical Chemistry*, 52(7), 1325–1330.
- Deverts, D. J., Cohen, S., Kalra, P., & Matthews, K. A. (2012). The prospective association of socioeconomic status with C-reactive protein levels in the CARDIA study. *Brain Behavior and*

Immunity, 26(7), 1128–1135. <https://doi.org/10.1016/j.bbi.2012.07.017>

Dhabhar, F. S., Burke, H. M., Epel, E. S., Mellon, S. H., Rosser, R., Reus, V. I., & Wolkowitz, O. M. (2009). Low serum IL-10 concentrations and loss of regulatory association between IL-6 and IL-10 in adults with major depression. *Journal of Psychiatric Research*, 43(11), 962–969. <https://doi.org/10.1016/j.jpsychires.2009.05.010>

Dominguez-Rodriguez, A., Abreu-Gonzalez, P., & Kaski, J. C. (2009). Inflammatory systemic biomarkers in setting acute coronary syndromes - effects of the diurnal variation. *Current Drug Targets*, 10(10), 1001–1008.

Dooley, L. N., Kuhlman, K. R., Robles, T. F., Eisenberger, N. I., Craske, M. G., & Bower, J. E. (2018). The role of inflammation in core features of depression: Insights from paradigms using exogenously-induced inflammation. *Neuroscience and Biobehavioral Reviews*, 94(March), 219–237. <https://doi.org/10.1016/j.neubiorev.2018.09.006>

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., & Lanctôt, K. L. (2010). A meta-analysis of cytokines in major depression. *Biological Psychiatry*, 67(5), 446–457. <https://doi.org/10.1016/j.biopsych.2009.09.033>

Duivis, H. E., Kupper, N., Vermunt, J. K., Penninx, B. W., Bosch, N. M., Riese, H., ... de Jonge, P. (2015). Depression trajectories, inflammation, and lifestyle factors in adolescence: The Tracking Adolescents' Individual Lives Survey. *Health Psychology*, 34(11), 1047–1057. <https://doi.org/10.1037/hea0000210>

Eisenberger, N. I., Moieni, M., Inagaki, T. K., Muscatell, K. A., & Irwin, M. R. (2017). In sickness and in health: The co-regulation of inflammation and social behavior. *Neuropsychopharmacology*, 42(1), 242–253. <https://doi.org/10.1038/npp.2016.141>

Falkenström, F., Finkel, S., Sandell, R., Rubel, J. A., & Holmqvist, R. (2017). Dynamic models of

individual change in psychotherapy process research. *Journal of Consulting and Clinical Psychology*, 85(6), 537–549.

Felger, J. C., Haroon, E., & Miller, A. H. (2018). What's CRP got to do with it? Tackling the complexities of the relationship between CRP and depression. *Brain Behavior and Immunity*, 73, 163–164. <https://doi.org/10.1016/j.bbi.2018.08.003>

Fried, E. I., Nesse, R. M., Zivin, K., Guille, C., & Sen, S. (2014). Depression is more than the sum score of its parts: individual DSM symptoms have different risk factors. *Psychological Medicine*, 44, 2067–2076. <https://doi.org/10.1017/S0033291713002900>

Gilman, S. E., Kawachi, I., Fitzmaurice, G. M., & Buka, S. L. (2003). Socio-economic status, family disruption and residential stability in childhood: relation to onset, recurrence and remission of major depression. *Psychological Medicine*, 33, 1341–1355.

Gimeno, D., Kivimäki, M., Brunner, E. J., Elovainio, M., De Vogli, R., Steptoe, A., ... Ferrie, J. E. (2009). Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychological Medicine*, 39(3), 413–423. <https://doi.org/10.1017/S0033291708003723>

Goodwin, R. D., Fergusson, D. M., & Horwood, L. J. (2004). Asthma and depressive and anxiety disorders among young persons in the community. *Psychological Medicine*, 34(8), 1465–1474. <https://doi.org/10.1017/S0033291704002739>

Hankin, B. L., Abramson, L. Y., Moffitt, T. E., Silva, P. A., McGee, R., & Angell, K. E. (1998). Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study. *Journal of Abnormal Psychology*, 107(1), 128–140. <https://doi.org/10.1037/0021-843X.107.1.128>

Hart, B. L. (1988). Biological basis of the behavior of sick animals. *Neuroscience & Biobehavioral*

Reviews, 12(2), 123–137. [https://doi.org/10.1016/S0149-7634\(88\)80004-6](https://doi.org/10.1016/S0149-7634(88)80004-6)

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, 6(2), 65–70.

Howren, M. B., Lamkin, D. M., & Suls, J. (2009). Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosomatic Medicine*, 71(2), 171–186.
<https://doi.org/10.1097/PSY.0b013e3181907c1b>

Huang, C., & Dong, N. (2014). Dimensionality of the Children's Depression Inventory : Meta-analysis of pattern matrices. *Journal of Child and Family Studies*, 23, 1182–1192.
<https://doi.org/10.1007/s10826-013-9779-1>

IBM Corp. (2016). IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

Janeway, C. A. (1989). Approaching the asymptote? Evolution and revolution in immunology. *Journal of Immunology (Baltimore, Md. : 1950)*, 54(9), 1–13. Retrieved from
<http://www.ncbi.nlm.nih.gov/pubmed/24141854>

Khandaker, G. M., Pearson, R. M., Zammit, S., Lewis, G., & Jones, P. B. (2014). Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life a population-based longitudinal study. *JAMA Psychiatry*, 71(10), 1121–1128.
<https://doi.org/10.1001/jamapsychiatry.2014.1332>

Klein, D., Dougherty, L. R., & Olino, T. M. (2005). Toward guidelines for evidence-based assessment of depression in children and adolescents. *Journal of Clinical Child & Adolescent Psychology*, 34(3), 412, 432. <https://doi.org/10.1207/s15374424jccp3403>

Kovacs, M. (1985). The Children's Depression Inventory (CDI). *Psychopharmacology Bulletin*, 21(4), 995–998.

Krishnadas, R., & Harrison, N. A. (2016). Depression phenotype, inflammation, and the brain:

Implications for future research. *Psychosomatic Medicine*, 78(4), 384–388.

<https://doi.org/10.1097/PSY.0000000000000339>

Kuhlman, K. R., Robles, T. F., Dooley, L. N., Boyle, C. C., Haydon, M. D., & Bower, J. E. (2018).

Within-subject associations between inflammation and features of depression : Using the flu vaccine as a mild inflammatory stimulus. *Brain Behavior and Immunity*, 69, 540–547.

<https://doi.org/10.1016/j.bbi.2018.02.001>

Kuznetsova, A., Brockhoff, P., & RHD, C. (2017). lmerTest package: Tests in linear mixed effects models, 82(13), 1–26. <https://doi.org/10.18637/jss.v082.i13>.

Mac Giollabhui, N., Swistun, D., Murray, S., Moriarity, D., Kautz, M., Ellman, L., ... Alloy, L. (n.d.).

Executive dysfunction in depression in adolescence: The role of inflammation and higher body mass. *Psychological Medicine*.

Malarkey, W. B., Jarjoura, D., & Klatt, M. (2013). Workplace based mindfulness practice and inflammation: A randomized trial. *Brain, Behavior, and Immunity*, 27(1), 145–154.

<https://doi.org/10.1016/j.bbi.2012.10.009>

Meyer, T., Stanske, B., Kochen, M. M., Cordes, A., Yuksel, I., Wachter, R., ... Herrmann-lingen, C.

(2011). Serum levels of interleukin-6 and interleukin-10 in relation to depression scores in patients with cardiovascular risk factors. *Behavioral Medicine*, 37(3), 105–112.

<https://doi.org/10.1080/08964289.2011.609192>

Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biological Psychiatry*, 65(9), 732–741.

<https://doi.org/10.1016/j.biopsych.2008.11.029>

Miller, G. E., Cohen, S., & Ritchey, A. K. (2002). Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model. *Health Psychology*, 21(6), 531–541.

<https://doi.org/10.1037//0278-6133.21.6.531>

Miller, G. E., & Cole, S. W. (2012). Clustering of depression and inflammation in adolescents previously exposed to childhood adversity. *Biological Psychiatry*, 72(1), 34–40.

<https://doi.org/10.1016/j.biopsych.2012.02.034>.

Mills, N. T., Scott, J. G., Wray, N. R., Cohen-Woods, S., & Baune, B. T. (2013). Research review: The role of cytokines in depression in adolescents: A systematic review. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 54(8), 816–835. <https://doi.org/10.1111/jcpp.12080>

Moriarty, D. P., Giollabhui, N. Mac, Ellman, L. M., Klugman, J., Coe, C. L., Abramson, L. Y., & Alloy, L. B. (2019). Inflammatory proteins predict change in depressive symptoms in male and female adolescents. *Clinical Psychological Science*, 1–17. <https://doi.org/10.1177/2167702619826586>

Ohayon, M. M., & Schatzberg, A. F. (2003). Using chronic pain to predict depressive morbidity in the general population. *Archives of General Psychiatry*, 60(1), 39–47.

<https://doi.org/10.1001/archpsyc.60.1.39>

Pan, A., Keum, N., Okereke, O. I., Sun, Q., Kivimaki, M., Rubin, R. R., & Hu, F. B. (2012). Bidirectional association between depression and metabolic syndrome: A systematic review and meta-analysis of epidemiological studies. *Diabetes Care*, 35(5), 1171–1180. <https://doi.org/10.2337/dc11-2055>

Preiss, K., Brennan, L., & Clarke, D. (2013). A systematic review of variables associated with the relationship between obesity and depression. *Obesity Reviews*, 14, 906–918.

<https://doi.org/10.1111/obr.12052>

Raison, C. L., Rutherford, R. E., Woolwine, B. J., Shuo, C., Schettler, P., Drake, D. F., ... Miller, A. H. (2013). A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression. *JAMA Psychiatry*, 70(1), 31–41.

<https://doi.org/10.1001/2013.jamapsychiatry.4>

- Rights, J. D., & Sterba, S. K. (2018). Quantifying explained variance in multilevel models: An integrative framework for defining R-squared measures. *Psychology Methods*.
- Rosenkranz, M. A., Davidson, R. J., MacCoon, D. G., Sheridan, J. F., Kalin, N. H., & Lutz, A. (2013). A comparison of mindfulness-based stress reduction and an active control in modulation of neurogenic inflammation. *Brain, Behavior, and Immunity*, 27(1), 174–184.
<https://doi.org/10.1016/j.bbi.2012.10.013>
- Skovlund, C., Mørch, L., Kessing, L., & Lidegaard, O. (2016). Association of hormonal contraception with depression. *JAMA Psychiatry*, 73(11), 1154–1162.
<https://doi.org/10.1001/jamapsychiatry.2016.2387>
- Stewart, J. C., Rand, K. L., Muldoon, M. F., & Kamarck, T. W. (2009). A prospective evaluation of the directionality of the depression-inflammation relationship. *Brain, Behavior, and Immunity*, 23(7), 936–944. <https://doi.org/10.1016/j.bbi.2009.04.011>
- Team, R. C. (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. Retrieved from <http://www.r-project.org>
- van Lang, N. D. J., Ferdinand, R. F., & Verhulst, F. C. (2007). Predictors of future depression in early and late adolescence. *Journal of Affective Disorders*, 97, 137–144.
<https://doi.org/10.1016/j.jad.2006.06.007>
- Watkins, L. R., & Maier, S. F. (1999). Implications of immune-to-brain communication for sickness and pain. *Proceedings of the National Academy of Sciences of the United States of America*, 96(14), 7710–7713. <https://doi.org/10.1073/pnas.96.14.7710>
- Weiner, J. (2017). Sankey or Ribbon Plots.
- Whooley, M. A. (2006). Depression and cardiovascular disease healing the broken-hearted. *JAMA*, 295(24), 2874–2881.

Zalli, A., Jovanova, O., Hoogendijk, W. J. G., Tiemeier, H., & Carvalho, L. A. (2016). Low-grade inflammation predicts persistence of depressive symptoms. *Psychopharmacology*, 233(9), 1669–1678. <https://doi.org/10.1007/s00213-015-3919-9>

Table 1. Effects of Inflammatory Biomarkers on Change in Total Depressive Symptoms (CDI)

	CRP	IL-6	Log IL-8	IL-10	Log TNF- α
Fixed Effects	Coefficient (SE)	Coefficient (SE)	Coefficient (SE)	Coefficient (SE)	Coefficient (SE)
Person-level N	130	130	130	130	130
Intercept	-.962 (4.760) <i>B</i> = .000 <i>p</i> = .840	-1.102 (4.770) <i>B</i> = .000 <i>p</i> = .840	.670 (6.014) <i>B</i> = .000 <i>p</i> = .911	-.831 (4.755) <i>B</i> = .000 <i>p</i> = .861	1.789 (8.499) <i>B</i> = .000 <i>p</i> = .833
Observation-Level N	310	310	310	310	310
Within-person effect of biomarker	-.002 (.211) <i>B</i> = .000 <i>p</i> = .993	.545 (1.229) <i>B</i> = .029 <i>p</i> = .657	-.232 (1.671) <i>B</i> = -.009 <i>p</i> = .890	-.259 (1.363) <i>B</i> = -.132 <i>p</i> = .850	8.571 (3.265) <i>B</i> = .156 <i>p</i> = .0091**
Between-person effect of biomarker	-.109 (.231) <i>B</i> = -.037 <i>p</i> = .637	-.535 (1.313) <i>B</i> = -.030 <i>p</i> = .684	-.712 (1.582) <i>B</i> = -.028 <i>p</i> = .653	-1.202 (1.274) <i>B</i> = -.064 <i>p</i> = .346	-1.465 (2.921) <i>B</i> = -.031 <i>p</i> = .616
Random Effects	Variance Components				
Individual (Person-level) Variance	0	0	0	0	0
Residual (Observation-level) Variance	19.90*	19.89*	19.90*	19.85*	19.42*

Note: * $p < .05$, ** $p < .01$. CRP= C-reactive protein, IL=interleukin, TNF- α = tumor necrosis factor alpha.

Table 2. Effects of Total Depressive Symptoms (CDI) on Change in Inflammatory Biomarkers

	Log CRP	Log IL-6	Log IL-8	Log IL-10	Log TNF- α
Fixed Effects	Coefficient (SE)	Coefficient (SE)	Coefficient (SE)	Coefficient (SE)	Coefficient (SE)
Person-level N	132	132	132	132	132
Intercept	.078 (.690) <i>B</i> = .000 <i>p</i> = .910	-.264 (.356) <i>B</i> = .000 <i>p</i> = .459	.233 (.282) <i>B</i> = .000 <i>p</i> = .409	-.016 (.307) <i>B</i> = .000 <i>p</i> = .959	-.013 (.151) <i>B</i> = .000 <i>p</i> = .930
Observation-Level N	333	333	333	333	333
Within-person effect of symptoms	.006 (.013) <i>B</i> = .027 <i>p</i> = .671	.012 (.007) <i>B</i> = .103 <i>p</i> = .091	-.005 (.005) <i>B</i> = -.059 <i>p</i> = .341	.007 (.006) <i>B</i> = .068 <i>p</i> = .249	.005 (.003) <i>B</i> = .107 <i>p</i> = .077
Between-person effect of symptoms	-.006 (.007) <i>B</i> = -.057 <i>p</i> = .383	.000 (.003) <i>B</i> = -.007 <i>p</i> = .903	.001 (.003) <i>B</i> = .018 <i>p</i> = .776	.000 (.003) <i>B</i> = .003 <i>p</i> = .964	-.001 (.001) <i>B</i> = -.038 <i>p</i> = .544
Random Effects	Variance Components				
Individual (Person-level) Variance	0	0	0	0	0
Residual (Observation-level) Variance	0.33*	0.09*	0.05*	0.06*	0.02*

Note: **p* < .05, ****p* < .001. CRP= C-reactive protein, IL=interleukin, TNF- α = tumor necrosis factor alpha.

Figure 1. TNF- α predicting variance in change in total depressive symptoms

Figure 2. Summary of significant exploratory results

Note: $*p < .05$, $**p < .01$, $***p < .001$, CRP= C-reactive protein, IL = interleukin, TNF- α = tumor necrosis factor alpha

Supplemental Results

Primary Analyses

Between-person variance of inflammatory biomarkers predicting change in depressive symptoms

Between-person effects of raw CRP, raw IL-6, log IL-8, raw IL-10, and log TNF- α ($p = .637$, $p = .684$, $p = .653$, $p = .346$, $p = .616$, respectively) did not have significant associations with change in total depressive symptoms.

Between-person variance of depressive symptoms predicting change in inflammatory biomarkers

Between-person effects of total depression symptoms did not have significant associations with changes in log CRP, log IL-6, log IL-8, log IL-10, or log TNF- α ($p = .383$, $p = .903$, $p = .776$, $p = .964$, $p = .544$, respectively).

Exploratory Analyses

Between-person variance of inflammatory biomarkers predicting change in depressive symptom subtypes

There were no significant between-person effects of any inflammatory biomarkers on change in any symptom subtypes (all $p > .05$).

Between-person variance of depressive symptom subtypes predicting change in inflammatory biomarkers

There were no significant between-person effects of any symptom subtypes on change in inflammatory biomarkers (all $p > .05$).

Supplemental Table 1. Bivariate Correlations of Primary Study Variables

Measure	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. CDI	-								
2. CRP	.02	-							
3. Log IL-6	.15†	.56***	-						
4. Log TNF- α	-.08	.00	.18*	-					
5. Log IL-8	-.20*	-.08	-.07	.08	-				
6. Log IL-10	-.05	.22**	.38***	.43***	.16*	-			
7. Age	.11	.13	.18**	-.05	-.28*	.06	-		
8. BMI	.09	.56***	.42***	-.03	.01	.02	.10	-	
9. Income	-.14	-.04	-.04	.12	.10	.19*	-.09	-.09	-
N	140	140	140	140	140	140	140	140	132
Mean (raw)	7.18	1.17	0.43	1.51	5.57	0.35	16.13	23.24	4.00
SD (raw)	6.21	1.70	0.35	0.48	6.75	0.60	1.41	5.54	2.04

Note: CDI: Children's Depression Inventory; CRP = C-Reactive Protein; IL = Interleukin; TNF- α = Tumor Necrosis Factor – Alpha; BMI = Body Mass Index; *** = $p < .001$; ** = $p < .01$; * = $p < .05$; † = $p < .10$