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Longitudinal Association of Inflammation with Depressive Symptoms: A 7-Year Cross-lagged Twin Difference Study

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Abstract

**Background:** The direction of the association between inflammation and depressive symptoms remains inconsistent. The objective of this study was to evaluate the temporal relationship between inflammation and depressive symptoms, and to assess the role of genetic factors on this association.

**Methods:** In this longitudinal cross-lagged twin difference study, we examined 166 (83 pairs) middle-aged male twins recruited from the Vietnam Era Twin Registry, who were assessed at baseline and after 7 years of follow-up. We assayed plasma levels of two inflammatory biomarkers, interleukin-6 (IL-6) and high sensitivity C-reactive protein (CRP) and measured depressive symptoms using the Beck Depression Inventory-II (BDI). To evaluate the direction of the association, we constructed multivariable mixed-effects regression models and calculated standardized beta-coefficients to compare the strength of the within-pair association for both

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All authors meet the criteria for authorship based on the following four criteria: 1) Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; 2) Drafting the work or revising it critically for important intellectual content; 3) Final approval of the version to be published; and 4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.
pathways. We then conducted a stratified analysis by zygosity and assessed the associations in monozygotic and dizygotic twin pairs separately.

**Results:** The 166 twins were 95% white and had a mean (SD) age of 54 (3) years at baseline. The cross-lagged analysis showed significant and positive associations from visit 1 IL-6 to visit 2 BDI across all models (beta-coefficients ranging from 0.18 to 0.22). However, the opposite pathway (visit 1 BDI to visit 2 IL-6) was not significant after adjusting for confounding factors. In contrast, visit 1 BDI was significantly associated with visit 2 CRP in all models (beta-coefficients ranging from 0.23 to 0.33), while the opposite pathway (visit 1 CRP to visit 2 BDI) showed no significant association. When stratifying by zygosity, significant associations from IL-6 to depression were only seen in monozygotic twins, but associations from depression to CRP were more robust in dizygotic twins, which implies that genetic factors may play a role in this association.

**Conclusions:** The association between inflammation and depression may be bidirectional. Elevated IL-6 levels are more likely to be a risk factor of depression rather than a consequence, while the opposite may be true for elevated CRP. The biological underpinnings of these bidirectional pathways need further evaluation.

**Keywords**
Depression; Inflammation; Cardiovascular disease; Twins; Veterans

**INTRODUCTION**

Depression is common in the United States and worldwide, with a lifetime prevalence between 8% and 17%, and is becoming a primary cause for cardiovascular disease, disability and mortality.1–5 Growing evidence6–11 has linked depressive symptoms to inflammation indexed by elevated levels of inflammatory markers and acute phase proteins, including interleukin-6 (IL-6) and C-reactive protein (CRP).12,13

IL-6 is an interleukin that acts as a pro-inflammatory cytokine and is secreted by T cells and macrophages to stimulate immune response as well as by adipose tissue.14 CRP is an acute-phase protein of hepatic origin that increases following IL-6 secretion, and is a non-specific marker for many diseases related to chronic inflammation. Elevated circulating levels of IL-6 and CRP signal systemic inflammation and have been consistently shown to predict cardiovascular risk.15–17 However, results have been conflicting regarding the causal direction between depression and inflammation. In some studies, after adjusting for demographic factors and other potential confounders, higher levels of IL-6 and CRP independently predicted depressive symptoms longitudinally,9,18–20 while other research suggested that the opposite pathway may also play a role, in that depression may induce or promote inflammation.21–23 Most prior studies were cross-sectional and thus limited in their ability to assess the temporal association between depression and inflammatory markers.24–26 In addition, depression and inflammation may have a common pathophysiology, such as shared genetic influences or shared behavioral or environmental precursors.27,28 Another limitation of prior studies is the lack of adjustment for shared genetic and environmental factors which may confound the association.29
The objective of this study was to investigate the direction of association between inflammation (measured by IL-6 and CRP) and depressive symptoms using a cross-lagged longitudinal twin difference study design. The study of within-pair twin differences provides a natural “counterfactual” design to examine phenotypic associations while controlling for shared genetic and familial factors, because monozygotic (MZ) twins share 100% of their genes while dizygotic (DZ) twins share, on average, 50%. We employed a cross-lagged longitudinal framework to assess the directionality of the association between inflammation and depressive symptoms. The inclusion of both MZ and DZ twins enabled us to assess the role of genetic factors on the associations.

METHODS

Study population

The objective of the Emory Twins Studies (ETS) was to evaluate the role of biological, psychological, and behavioral risk factors in the development of subclinical cardiovascular disease (CVD). This program of research included samples in two companion studies: the Twins Heart Study (THS) and the Stress and Vascular Evaluation in Twins (SAVEIT). The participants in the Emory Twins Studies were recruited from the VET Registry, which is a national sample of 7,369 male MZ and DZ twins who served on active duty during the Vietnam war. For the current study, we included twin pairs discordant for depression or posttraumatic stress disorder (PTSD), as well as control pairs free of depression and PTSD. All the twins were born during 1946–1956, with no self-reported history of CVD based on survey data obtained by the Registry in 1990. A subgroup of twins in the ETS, who were discordant for major depressive disorder (MDD) at any point before, whose examination (visit 1) was carried out between March 2002 and March 2006, and whose members were both still alive, not institutionalized and still part of the Registry, were selected for a follow-up visit (visit 2), as previously described. Of 121 twin pairs who met these criteria, 106 pairs were successfully contacted and 83 pairs (n=166) completed visit 2, on average of 7 years later. The construction of the study population was described previously.

At both visits, twin pairs were examined on the same day using identical assessment protocols. Comprehensive medical history data were collected during a two-day admission under controlled conditions. We obtained participants’ blood samples, as well as anthropometric measurements, behavioral and psychosocial assessments using the same instruments and protocols at both time points. Zygosity information was verified by DNA typing, as previously described. We obtained written informed consent from all participants, and the Institutional Review Board at Emory University approved this study.

Assessment of inflammatory biomarkers

Inflammatory biomarkers, including IL-6 and CRP, were measured in plasma in resting conditions after an overnight fast at both visit 1 and visit 2. Time of blood draw was standardized across the two visits (early morning upon awakening around 6 am). Plasma samples were frozen at −80 °C until analysis. IL-6 was assessed using commercially available enzyme-linked immunosorbent assay (ELISA) kits obtained from R and D Systems (Minneapolis, MN); inter- and intra-assay variability for these assays is <10%. CRP was
measured using the high-sensitivity Beckman Coulter assay on the Synchron LX-20 analyzer (Beckman Coulter; Brea, CA); the inter- and intra-assay precision of this test is <5%. All biochemical assays for each twin pair were processed in the same analytical run at both visits.

**Assessment of depressive symptoms**

At both time points we administered the Beck Depression Inventory-II (BDI-II), a validated and standardized 21-item scale providing a continuous measure of depressive symptoms. Scoring ranges for each item from 0 to 3 with a total maximum cumulative score of 63, with a higher score indicating more depressive symptoms. It has been used widely in community samples and has excellent psychometric properties. At both time points, we also administered the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV), or SCID, which we used to classify participants based on a lifetime or current history of MDD. Given the small number of individuals with current history of MDD (n=6, or 3.6% at visit 1 and n=13, or 7.8% at visit 2), we chose to examine the BDI score over MDD for the primary analysis. The SCID was also used to provide a diagnosis of current PTSD (past month).

**Other measurements**

Medical history and physical examination were obtained by a research nurse or physician assistant at both visits using identical protocols. We collected weight and height data to calculate the BMI, and used standardized questionnaires to obtain data on cigarette smoking status (current versus past or never smoker) and alcohol drinking (number of drinks of alcoholic beverages per week: wine, beer, or cocktail). Physical activity was assessed using the modified Baecke Questionnaire of Habitual Physical Activity used in the Atherosclerosis Risk in Communities (ARIC) study. This is a 16-question instrument documenting levels of physical activities at work, during sports and non-sports activities, and yielding a global physical activity score. Systolic and diastolic blood pressure (SBP and DBP) were measured using a mercury sphygmomanometer on the right arm with the subject in a sitting position after 10 minutes of rest; the average of the two measurements 5 minutes apart was used in the analysis. Venous blood samples were drawn for the measurements of plasma glucose and lipid profile after an overnight fast. Glucose was assessed on the Beckman CX7 chemistry autoanalyzer. Direct low-density and high-density cholesterol levels were measured with homogeneous assays (Equal Diagnostics, Exton, PA). History of hypertension was defined as SBP >140 mmHg or DBP >90 mmHg or self-reported use of antihypertensive medication. A history of coronary artery disease (CAD) that might have occurred after the time of initial screening in 1990 was defined as a diagnosis of myocardial infarction or angina pectoris, or previous coronary revascularization procedures. Diabetes mellitus was defined as having a fasting glucose of more than 126 mg/dL or being treated with insulin or oral hypoglycemic agents. Current use of beta-blockers, antidepressants, statins, and angiotensin-converting enzyme (ACE) inhibitors were also recorded. All the health-related data were obtained at both time points.
**Statistical analysis**

Initial descriptive analyses compared means and percentages of study variables within the same individual twin at visit 1 and visit 2. P-values were calculated using paired *t* tests (for continuous variables) and McNemar statistics (for categorical variables). Inflammatory biomarkers were log transformed due to their non-normal distribution. Correlation between IL-6 and CRP were evaluated using Spearman correlation coefficients at both visits.

We used a cross-lagged twin difference study design to assess bidirectional pathways linking inflammation and depressive symptoms across time, as previously described.\(^{35}\) This approach evaluates both cross-sectional and longitudinal associations between two phenotypes across two time points.\(^{30,42,43}\) Our primary analysis focused on the longitudinal association between IL-6 (or CRP) at visit 1 and BDI at visit 2, and the converse (BDI at visit 1 and IL-6 (or CRP) at visit 2). The comparison of the magnitude of the associations between these two pathways provides information on the temporal sequence, or directionality, between the two variables. This approach focuses on within-pair differences for both variables at two time points. The within-pair differences are inherently controlled for potential confounding by demographics, shared familial and early environmental influences. Moreover, this approach controls for environmental factors during the day of examination, because twins in a pair were assessed at the same time and under the same conditions.

We fitted multivariable mixed-effects linear regression models with a random effect for each twin pair. To avoid overfitting, we constructed a series of models, beginning with a base model that only adjusted for baseline within-pair differences of the dependent variable and relevant between-pair effects without controlling for other covariates. Next, in Model 2, we added baseline factors that were deemed potential confounders (education, smoking, alcohol use, physical activity, history of CAD, and beta-blocker use). In Model 3 we further adjusted for cardiovascular risk factors that could be in the pathway between depression and inflammation (BMI, hypertension, and diabetes), and finally, in Model 4 we added antidepressant use due to its complex role in the association between depression and inflammation.\(^{44-46}\) Prior studies have found that antidepressant treatment may inhibit inflammatory response by reducing the level of inflammatory cytokines.\(^{47}\) It has also been shown that depressed patients with a higher levels of inflammatory markers are less likely to respond to antidepressants.\(^{44-46}\) Hence, it becomes critical to understand the role of antidepressants in the association between depression and inflammation. To allow comparisons across directional paths, the model beta-coefficients and 95% confidence intervals (CI) were standardized, and can be interpreted as the number of standard deviations (SDs) the dependent variable will change, per SD difference in the predictor variable. For example, for our longitudinal associations between visit 1 BDI and visit 2 IL-6, the beta-coefficients describe the number of SDs the two twins will differ in IL-6 at visit 2, per one within-pair SD difference in BDI at visit 1.

To test if there was a genetic influence underlying the association between the two phenotypes, we evaluated effect modification between zygosity and depression (or inflammation) by adding an interaction term to the model. We then stratified the association by zygosity and evaluated the causal pathways in MZ and DZ twins separately. Because MZ
twins share 100% of genes, while DZ twins on average only share 50%, if a larger difference in BDI (or inflammation) at visit 2 is found in relation to inflammation (or BDI) at visit 1 within DZ pairs than MZ pairs, this suggests that the genetic background may play a role in this association. Conversely, if the paired effect is seen in MZ twins, this rules out that genetic factors play a role in the association. Given the known comorbidity between depression and PTSD, we also examined if the results persisted when adjusting for current PTSD.

Missing data were rare (0–3%) for all variables so we used all available data without imputation. We assessed linearity of all continuous variables using locally weighted scatterplot smoothing (LOWESS) of martingale residuals from the regression models. Multicollinearity was evaluated by variance inflation factors. A two-sided p-value of <0.05 was used for statistical significance and 95% CI were calculated for model parameters. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and Stata 14.0 (StataCorp, College Stata, TX).

RESULTS

Twins’ characteristics

Of the 166 twins in the study, 95% were white, with a mean age ± SD of 54 ± 3 years at baseline (Table 1). The mean follow-up time ± SD was 7 ± 2 years. Of the twins, 47 pairs (n=94, 57%) were MZ twins and 36 pairs (n=72, 43%) were DZ twins.

Compared to the twins at visit 1, the same twins at visit 2 had higher BMI, were more often current smokers, less physically active, and more often reported a history of CAD or diabetes. The prevalence of current major depressive episode was also significantly higher at visit 2 compared to visit 1 (7.8% vs. 3.6% respectively), but the prevalence of current PTSD was similar at the two time points. Twins at visit 2 also reported higher medication use than at visit 1. Overall, 29% reported antidepressant use at visit 2, vs. 22% at visit 1. The median BDI score was also higher at visit 2 than at visit 1. There was no significant change in the levels of inflammatory biomarkers (IL-6 and CRP) from visit 1 to visit 2. The Spearman correlation coefficients between IL-6 and CRP were 0.47 at visit 1 (p<0.05) and 0.51 at visit 2 (p<0.05).

Association between IL-6 and BDI

There was no significant cross-sectional association between IL-6 and BDI at visit 1, but there was a positive cross-sectional association at visit 2 (Table 2).

Cross-lagged longitudinal analyses showed consistently significant associations between visit 1 IL-6 and visit 2 BDI across all models (Table 2). After adjusting for potential confounders in Model and cardiovascular risk factors in Model 3, the associations remained significant and minimally changed, with beta-coefficients changing from 0.21 in the base model to 0.20 in Model 3. Adjustment for antidepressant use in Model 4 did not substantially change the association, with a beta-coefficient of 0.22. The results indicated that with one SD increase in log(IL-6) in a twin compared to his co-twin at visit 1, he is
expected to have, on average, 0.22 SD (corresponding to 22%) higher BDI score than his cotwin at visit 2.

In contrast, the converse longitudinal association (from visit 1 BDI to visit 2 IL-6) was only significant in the base model, and became attenuated in Model 2 that adjusted for confounding factors (Table 2). Adjusting for antidepressant use in Model 4 further diminished the beta-coefficient towards zero. Figure 2 illustrates the cross-lagged associations using Model 4 results.

**Association between CRP and BDI**

The cross-sectional association between BDI score and CRP (Table 3) showed similar results as for IL-6, with a significant association only found at visit 2 (beta-coefficients ranging from 0.18 to 0.21), but not at visit 1 (beta-coefficients: -0.11 to 0.01). Contrary to our findings for IL-6, however, there was a significant longitudinal association between visit 1 BDI and visit 2 CRP (beta-coefficients from 0.23 to 0.33), while the longitudinal association in the opposite pathway (from visit 1 CRP to visit 2 BDI) was weaker in all models (beta-coefficients: 0.06 to 0.09).

**Zygosity-Specific Results**

Figure 2 shows the results of the stratified analysis by zygosity adjusted for cardiovascular risk factors as in Model 3 above. For the path going from visit 1 IL-6 to visit 2 BDI, the longitudinal association was significant in MZ twins but not in DZ twins, with beta-coefficient of 0.35 (95% CI, 0.16, 0.53) and -0.06 (95% CI, -0.26, 0.14) in MZ and DZ twins, respectively, p=0.035 for the interaction. In the opposite pathway, the longitudinal association was not significant in either MZ or DZ twins. However, the association between CRP and BDI was observed in both DZ and MZ twins in the direction from visit 1 BDI to visit 2 CRP, and was somewhat more robust among DZ twins, although the interaction was not significant, at p=0.37 (Figure 2).

Adding PTSD in any models did not materially change any of the above results. Results also remained similar when considering current major depression in place of the BDI score in Model 4 (Supplement Figure 1).

**DISCUSSION**

In this longitudinal evaluation of pathways linking inflammation and depressive symptoms in Vietnam-era veteran twin pairs, higher plasma levels of IL-6 at baseline predicted increased depressive symptoms at follow-up, and conversely, higher levels of depressive symptoms at baseline predicted increased CRP at follow-up. These results suggest that the link between inflammation and depression is bidirectional and that IL-6 and CRP may signal different causal pathways in this relationship.

The association between elevated IL-6 levels at baseline and increasing depressive symptoms at follow-up was not explained by behavioral factors, cardiovascular risk factors, or use of medications. The opposite pathway, from BDI levels at visit 1 to IL-6 at visit 2, was largely explained by lifestyle factors related to depression (smoking, alcohol use,
physical inactivity) and medications. The significant association from visit 1 BDI to visit 2 CRP was similarly not explained by lifestyle factors, cardiovascular risk factors, or antidepressants use. Thus, inflammation, reflected by elevated IL-6, is more likely to be a cause of depression, rather than a consequence, and depression itself may also cause chronic inflammation as measured by CRP.

Our results expand previous predominantly cross-sectional studies of a positive association between inflammation and depression, and support the hypothesis that such association is bidirectional. Our data agree with prior literature showing that elevated inflammatory markers such as IL-6 have a positive and significant prospective association with subsequent development or worsening of depressive symptoms. For example, Khandaker and colleagues showed that higher IL-6 levels in childhood were associated with subsequent risk of depression after 9 years of follow-up, even after adjusting for age, sex, BMI, ethnicity, social class, past psychological and behavioral problems, and maternal postpartum depression.

We found a stronger cross-sectional association between depressive symptoms and inflammation at visit 2 compared to visit 1. This unexpected finding may reflect the worsening of comorbidities and risk factors we observed during follow-up, such as higher BMI, smoking, diabetes and CAD. Although we adjusted for most of these risk factors, residual confounding may still contribute to the difference in the strength of the cross-sectional associations at the two time points.

There are a few proposed mechanisms underlying the link between depressive symptoms and inflammation. Animal studies showed that systemic inflammatory cytokines, such as IL-6, can communicate with the brain, and lead to increased sleep, reduced locomotor activity and social interactions, and increased anxiety. A recent study suggests that depression may be linked to chronic stress through direct signaling of the brain by IL-6. Specifically, chronic social stress alters the integrity of the blood-brain barrier (BBB) at the nucleus accumbens, promoting IL-6 passage across the BBB. Furthermore, in human studies that used interferon-α as a source of cytokine exposure, inflammatory activation reliably provoked depressive symptoms. These findings may reflect an integration of inflammatory pathways in a mind-body system evolved to cope with environmental danger. Research also suggests that higher circulating levels of cytokines may lead to decreased availability of serotonin and other neurotransmitters, to activation of the hypothalamic-pituitary-adrenal axis, and to increased oxidative stress in the brain, which all could be pathways contributing to negative affect and the development of depressive symptoms.

We did not find a significant association for the pathway linking depressive symptoms at baseline to elevated levels of IL-6 at follow-up. Contrary to our findings, Stewart and colleagues found that baseline BDI was a predictor of IL-6 six years later, while the association was not significant in the opposite direction; they also found a bidirectional but non-significant longitudinal association between BDI and CRP. Differences in population characteristics and in the statistical analysis may account for some of the inconsistent results. Failure to control for genetic predisposition and familial factors may also explain part of the differences. Consistent with our results, a recent meta-analysis reported that...
depressed patients who are treatment resistant have higher baseline inflammation than responders, suggesting that elevated levels of inflammation contribute to depression treatment resistance.60

In contrast to the IL-6 results, we did not find a significant association linking visit 1 CRP levels to visit 2 depression. However, higher levels of depressive symptoms at visit 1 were related to increased levels of CRP at visit 2, which is consistent with other community-based studies.21,23,61 The discrepant results compared to IL-6 may be due to different functions that each marker has in the inflammation process. Elevated IL-6 levels signal acute inflammatory effects with a possible direct action on the brain, whereas CRP levels may require days or weeks to go up after an inflammatory stimulus, and in fact a sustained elevation of IL-6 over time is necessary in order for circulating CRP levels to increase.62 Thus, IL-6 may be a better marker of the effect of acute inflammation on the brain, while CRP is more a marker of chronic, long-term inflammatory response. Another explanation is that CRP, in contrast to IL-6, does not directly affect neurobiology, and therefore cannot influence mood given that it does not cross the blood-brain barrier. In contrast, CRP could be more directly linked to sustained behavioral and physiological consequences of depression, such as changes in body weight, diet, physical activity and sleep.

In addition to causal pathways, it is possible that depression and inflammation are linked, at least in part, by shared pathophysiological processes. This is suggested by previous research that has related the same genetic variants to both phenotypes. For example, genetic variations involving the solute carrier family 6 member 4 gene, or SLC6A4, were associated with both depression and elevated IL-6 levels,27 and polymorphisms of inflammatory cytokine genes were associated with depression.28,63 However, based on our results, such “genetic confounding” may not play a role in the longitudinal association between IL-6 and depression, since the association was noted within MZ twins who are genetically identical. On the other hand, shared genetic factors could be implicated in the longitudinal association between depression and elevated CRP, since this association was more robust within DZ twins (who only share on average 50% of their genes) than MZ twins.

A better understanding of the role of inflammation in the pathogenesis and clinical course of depression may lead to improved prevention and treatment of depression. Our findings not only suggest that inflammatory biomarkers may aide in identifying people at high risk for depression and its consequences, but also suggest that the use of anti-inflammatory agents may help in the prevention and treatment of depression. If depression is a consequence of inflammation, then reducing inflammation would theoretically alleviate depression. Consistent with this view, a recent systematic review of 14 randomized clinical trials showed that anti-inflammatory treatment, in particular celecoxib, was effective in decreasing depressive symptoms.64 Future studies may help devise more targeted interventions, which may reduce depression and at the same time potentially reduce the risk for cardiovascular morbidity and mortality.

The strengths of this study include its longitudinal design with subjects recruited from a national registry of veteran twins. This is also one of the few studies that evaluated the causal pathways linking depressive symptoms and inflammation longitudinally. The twin
study design allowed us to control for genetic factors and early familial confounding factors. However, this study also has some limitations. First, our sample only included middle-aged, male, predominantly white twins who served in the military, thus the generalizability to other populations is unknown. Because the sample was only male, we were unable to assess sex differences in our results. Previous studies have reported that depression may predict elevated inflammation in men but not in women. On the other hand, inflammation may predict worsening depression in women but not in men, as women appear more vulnerable to inflammation-induced mood changes. Although a homogeneous population reduces generalizability, it does improve validity. Causal inference is aided by studies that carefully control for confounding factors rather than those seeking representative samples. Causal inference was the most important goal of this study. Second, the relatively small sample size (n=166) may have increased the type II error in some analyses, and the small number of twins who had a current major depressive episode limited our ability to fully examine clinical depression. Third, we only had two time points for our longitudinal analysis. However, most previous studies were cross-sectional and thus our design is clearly a step forward in order to assess the temporal association between depression and inflammation. Future studies with larger and more diverse study populations and more time points are needed to confirm our findings. However, despite these limitations, the within-pair twin study design improved the internal validity of our results.

CONCLUSIONS

The association between inflammation and depressive symptoms is bidirectional. Inflammation as measured by plasma levels of IL-6 is positively associated with future depressive symptoms, but depressive symptoms also predict future inflammation measured by CRP. These associations are not influenced by genetic or environmental confounding factors. Our findings add to the growing knowledge of underlying causal pathways linking inflammation and depression, and may aide future efforts directed at identifying and managing individuals at risk for depression and its comorbidities. Finally, our results may inform future research directions on mechanisms underlying the link between depression and cardiovascular disease incidence and mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCE


## Highlights

- The direction of association between depression and inflammation remains unclear
- We used a longitudinal twin difference study design to address this question
- We found that inflammation measured by interleukin-6 predicted future depression
- However, depression also predicted inflammation indexed by C-reactive protein
- Our study suggests a bidirectional association between depression and inflammation
Figure 1. Illustration of the cross-lagged association between IL-6 (or CRP) and BDI score using Model 4 results (adjusted for smoking, beta-blocker use, education, alcohol use, physical activity, prevalent CAD, BMI, hypertension, diabetes, and antidepressant use).

Abbreviations: IL-6: interleukin-6; CRP: C-reactive protein; BDI: Beck Depression Inventory; CAD: coronary artery disease; BMI: body mass index. Numbers indicate standardized beta-coefficients from mixed-effects regression models. * Indicates significant association at p < 0.05.
Figure 2.
Standardized cross-sectional and longitudinal association between IL-6 (or CRP) and BDI stratified by zygosity adjusted for Model 4 variables (smoking, beta-blocker use, education, alcohol use, physical activity, prevalent CAD, BMI, hypertension, diabetes, and antidepressant use). N=94 monozygotic twins and N=72 dizygotic twins. Abbreviations: IL-6: interleukin-6; CRP: C-reactive protein; BDI: Beck Depression Inventory; MZ: monozygotic; DZ: dizygotic; CAD: coronary artery disease; BMI: body mass index. Numbers indicate standardized beta-coefficients from mixed-effects regression models;
vertical lines indicate 95% confidence intervals. * Indicates significant association at p <0.05.
Table 1.
Characteristics of twin participants at baseline and at 7 years of follow-up (n=166)

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<th>Characteristics</th>
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<td>39.3 (8.5)</td>
</tr>
<tr>
<td>Prevalent CAD, n (%)</td>
<td>13 (7.8)</td>
<td>30 (18.1)</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL, mean (SD)</td>
<td>100.5 (17.3)</td>
<td>106.9 (26.4)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>20 (12.0)</td>
<td>29 (17.6)</td>
</tr>
<tr>
<td>IL-6, pg/mL, mean (SD)</td>
<td>2.5 (5.5)</td>
<td>2.0 (1.4)</td>
</tr>
<tr>
<td>CRP, mg/L, mean (SD)</td>
<td>3.7 (17.2)</td>
<td>2.7 (4.4)</td>
</tr>
<tr>
<td>BDI score, median (IQR)</td>
<td>4 (1–9)</td>
<td>5 (2–10)</td>
</tr>
<tr>
<td>Current major depressive episode, n (%)</td>
<td>6 (3.6)</td>
<td>13 (7.8)</td>
</tr>
<tr>
<td>Current PTSD, n (%)</td>
<td>13 (7.8)</td>
<td>15 (9.0)</td>
</tr>
<tr>
<td><strong>Medication use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blockers, n (%)</td>
<td>13 (7.8)</td>
<td>36 (21.8)</td>
</tr>
<tr>
<td>Antidepressants, n (%)</td>
<td>36 (21.7)</td>
<td>48 (28.9)</td>
</tr>
<tr>
<td>Statin, n (%)</td>
<td>44 (26.5)</td>
<td>75 (45.5)</td>
</tr>
<tr>
<td>ACE inhibitor, n (%)</td>
<td>26 (15.7)</td>
<td>42 (25.3)</td>
</tr>
</tbody>
</table>

Abbreviations: SD: standard deviation; n: number; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; CAD: coronary artery disease; IL-6: interleukin-6; CRP: C-reactive protein; BDI: Beck’s Depression Inventory; IQR: interquartile range; PTSD: posttraumatic stress disorder; ACE: angiotensin-converting enzyme.

* indicates statistical significance at p <0.05. P-values were calculated using paired t-test (continuous variables) and McNemar statistics (categorical variables).

a Physical activity level was assessed by the Baeecke questionnaire score.

b Interleukin-6 data were log-transformed due to non-normality; data presented as geometric mean.
Table 2.
Standardized cross-sectional and longitudinal association between IL-6 and BDI score

<table>
<thead>
<tr>
<th></th>
<th>Cross-sectional</th>
<th></th>
<th>Longitudinal</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>7-year</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-6 ←→ BDI</td>
<td>0.23 *</td>
<td>0.21 *</td>
<td>0.15 *</td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.05</td>
<td>0.17 *</td>
<td>0.18 *</td>
<td>0.09</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.06</td>
<td>0.19 *</td>
<td>0.20 *</td>
<td>0.11</td>
</tr>
<tr>
<td>Model 4</td>
<td>0.03</td>
<td>0.18 *</td>
<td>0.22 *</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Abbreviations: IL-6: interleukin-6; BDI: Beck depression inventory.

Results are shown as standardized beta-coefficients from mixed-effects regression models. Model 1: base model; model 2: model 1 variables + confounding factors (smoking, beta-blocker use, education, alcohol use, physical activity, prevalent CAD); model 3: model 2 variables + cardiovascular risk factors (BMI, hypertension, diabetes); model 4: model 3 variables + antidepressant use.

* Indicates significant association at p <0.05.
<table>
<thead>
<tr>
<th>Model</th>
<th>Baseline</th>
<th>7-year follow-up</th>
<th>Longitudinal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRP ←→ BDI</td>
<td>CRP ←→ BDI</td>
<td>CRP → BDI</td>
</tr>
<tr>
<td>Model 1</td>
<td>−0.11</td>
<td>0.18</td>
<td>0.09</td>
</tr>
<tr>
<td>Model 2</td>
<td>−0.09</td>
<td>0.21</td>
<td>0.06</td>
</tr>
<tr>
<td>Model 3</td>
<td>−0.09</td>
<td>0.19</td>
<td>0.06</td>
</tr>
<tr>
<td>Model 4</td>
<td>0.01</td>
<td>0.21</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Abbreviations: CRP: C-reactive protein; BDI: Beck depression inventory; CAD: coronary artery disease; BMI: body mass index.

Results are shown as standardized beta-coefficients from mixed-effects regression models. Model 1: base model; model 2: model 1 variables + confounding factors (smoking, beta-blocker use, education, alcohol use, physical activity, prevalent CAD); model 3: model 2 variables + cardiovascular risk factors (BMI, hypertension, diabetes); model 4: model 3 variables + antidepressant use.

* indicates significant association of p <0.05.