

# Chronic Interpersonal Stress Predicts Activation of Pro- and Anti-Inflammatory Signaling Pathways 6 Months Later

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**Objective:** To understand the mechanisms underlying chronic interpersonal difficulties and their detrimental influence on mental and physical health. **Methods:** A total of 103 healthy young women (mean age = 17 years) were administered a structured interview to assess the degree of chronic interpersonal stress in their lives. At the same time, blood was drawn to measure systemic inflammation, the expression of signaling molecules that regulate immune activation, and leukocyte production of the cytokine interleukin-6 after *ex vivo* stimulation with lipopolysaccharide. All of the immunologic assessments were repeated 6 months later. **Results:** To the extent subjects were high in chronic interpersonal stress at baseline, their leukocytes displayed greater increases in messenger ribonucleic acid (mRNA) for the proinflammatory transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B) over the next 6 months. They also showed larger increases in mRNA for inhibitor of  $\kappa$ B, a molecule that sequesters NF- $\kappa$ B in the cytoplasm and minimizes its proinflammatory activities. Chronic interpersonal stress at baseline was unrelated to changes in biomarkers of systemic inflammation but was associated with increasingly pronounced interleukin-6 responses to lipopolysaccharide. These associations were independent of demographics, lifestyle variables, and depressive symptoms. **Conclusions:** These findings suggest that chronic interpersonal difficulties accentuate expression of pro- and anti-inflammatory signaling molecules. Although this process does not result in systemic inflammation under quiescent conditions, it does accentuate leukocytes' inflammatory response to microbial challenge. These dynamics may underlie the excess morbidity associated with social stress, particularly in inflammation-sensitive diseases like depression and atherosclerosis. **Key words:** stress, social support, social conflict, inflammation, glucocorticoid receptor, cytokines.

**CRP** = C-reactive protein; **GR** = glucocorticoid receptor; **I $\kappa$ B** = inhibitor of  $\kappa$ B; **IL** = interleukin; **mRNA** = messenger ribonucleic acid; **NF- $\kappa$ B** = nuclear factor- $\kappa$ B.

## INTRODUCTION

Considerable research indicates that socially integrated persons enjoy better mental and physical health than their more isolated peers (1,2). However, in a series of recent studies, it has become evident that social ties can also have detrimental influences on health, especially when they are marked by conflict, mistrust, and instability (3–5). Among patients recovering from a mood disorder, for example, family tensions double the odds of a relapse occurring, and similar patterns are seen in patients with eating disorders and schizophrenia (6). Interpersonal difficulties are also associated with heightened susceptibility to respiratory infections, delayed healing of wounds, accelerated emergence of the metabolic syndrome, and increased morbidity and mortality from cardiovascular disease (7–11). These effects have been well substantiated in a series of tightly controlled studies with rodents and nonhuman primates (12–14).

Despite this robust pattern of findings, little is known about the responsible underlying mechanism(s). One candidate hy-

pothesis is that chronically abrasive relationships foster low-grade systemic inflammation, which then contributes to the evolution and/or expression of psychiatric, infectious, metabolic, and coronary diseases (15–17). This view received initial support in a study of married couples who had conflictual interactions in the laboratory; those who expressed greater hostility showed higher levels of the inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor- $\alpha$  the next morning (8).

In this article, we examine a broader spectrum of interpersonal relationships, including romantic relationships, friendships, and familial relationships, and consider whether their quality relates to two major biomarkers of systemic inflammation, C-reactive protein (CRP) and interleukin (IL)-6. To identify the molecular signaling pathways involved in these dynamics, we also assess the expression of messenger ribonucleic acid (mRNA) for the chief proinflammatory transcription factor, nuclear factor- $\kappa$ B (NF- $\kappa$ B), and for the  $\alpha$  and  $\beta$  isoforms of the glucocorticoid receptor (GR). When it is ligated by cortisol, GR- $\alpha$  has potent anti-inflammatory properties, mediated by its inhibition of NF- $\kappa$ B signaling (18). This inhibition occurs through direct protein-protein interactions, as well as GR-mediated induction of inhibitor of  $\kappa$ -B (I $\kappa$ B), a molecule that sequesters NF- $\kappa$ B in the cytosol and thereby prevents it from switching on proinflammatory genes. The functions of GR- $\beta$  are not fully defined, but it is thought to inhibit the activity of GR- $\alpha$  and may, thus, facilitate proinflammatory signaling (19). Finally, to model the dynamics of these signaling pathways under conditions of immune challenge, we quantified expression of IL-6 by leukocytes that had been stimulated with bacterial product *ex vivo*.

These analyses were carried out within the context of a short-term prospective study, in which interpersonal difficulties were assessed at baseline and inflammatory processes were measured at that time and again 6 months later. This design has a number of strengths compared with the cross-sectional analyses that are more common in psychoneuroim-

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munology. First, it allows changes over time in the outcomes of interest to be modeled, which is presumably more relevant to disease pathogenesis than one-time assessments. Second, it minimizes the likelihood that erroneous conclusions about directionality will be made, which is an important consideration here because inflammatory cytokines can have profound influences on social behavior (20). Finally, a design like this is able to capture stress-related changes that take time to evolve, such as those that result from gradual “wear and tear” on bodily systems. Based on these considerations, we hypothesized a prospective association between interpersonal difficulties and inflammatory processes. Specifically, to the extent that subjects were high in chronic interpersonal stress at study entry, we expected them to display greater activation of pro-inflammatory signaling pathways and higher levels of inflammatory biomarkers 6 months later.

## METHODS

### Subjects

These data were collected between October 2004 and December 2007 as part of a larger project on depression and atherosclerosis among young women at high risk for mood disorders. Subjects were recruited from the Vancouver, British Columbia community through advertisements in local media. Eligibility criteria included being a) female and 15 to 19 years old; b) fluent in the English language; c) free of acute and chronic medical conditions and standing medication regimens other than oral contraceptives; d) without a lifetime history of psychiatric disorders; and e) at high risk for developing an initial episode of depression. Subjects' medical histories were ascertained through detailed interviews and laboratory testing. Psychiatric backgrounds were evaluated with the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) Non-Patient Edition. High-risk was defined as having a first-degree relative with a history of affective disorder, and/or scoring in the top quartile of the population distribution on one of two indices of cognitive vulnerability to depression, the Dysfunctional Attitudes Scale or the Adolescent Cognitive Style Questionnaire (21).

This article focuses on 103 subjects who had been assessed at study entry and 6 months later. The sample had a mean  $\pm$  standard deviation (SD) age of  $17.19 \pm 1.37$  years at study entry. Forty-five percent of subjects self-identified as East Asian, 45% as white, and the other 10% as East Indian, Aboriginal, or other. Participants came from homes in which parents averaged  $14.98 \pm 3.63$  years of education. The larger project was reviewed and approved by the University of British Columbia's Research Ethics Board. Written consent was obtained from all subjects. For those who were <18 years old, a parent or guardian also provided consent.

### Chronic Interpersonal Stress

To assess the extent of chronic interpersonal stress in subjects' lives, we administered the UCLA Life Stress Interview-Adolescent Version (22) at study entry. This semistructured instrument probes stressors in multiple domains of life. In each domain, the interviewer asks a series of open-ended questions and uses the data to rate the degree of chronic stress over the last 6 months. For this paper, we averaged ratings across the interview's four social domains: romantic partner, closest friendship, other friendships, and family relationships. Ratings in these domains were modestly interrelated with correlations ranging from  $r = .10$ ,  $p = .33$  (romantic partner and closest friendship) to  $r = .56$ ,  $p = .001$  (closest friendship and other friendships). The average interdomain correlation was  $r = .25$ . Although the interdomain associations were modest, we elected to collapse across domains for two reasons. Conceptually, we did not have any a priori reason to believe that the domains would associate differentially with the project's outcomes, so collapsing them into a broader index reflecting “abrasive social relations” seemed most appropriate. This strategy also made sense from a statistical

perspective because treating the domains separately would have quadrupled the number of analyses performed, and in doing so produced unacceptably high odds of Type 1 error. Scores on the final index could range from 1 to 5, with lower values reflecting warm, intimate, and supportive relationships, and higher values suggesting conflict, mistrust, and instability. The Life Stress Interview has been used widely in psychiatric research and there is robust evidence to support its reliability and validity in diverse populations like ours (22,23). In our project, interviewers showed excellent reliability on ratings, with agreement ranging from 89% (closest friendship) to 96% (romantic partner).

### GR, NF- $\kappa$ B, and I $\kappa$ B

Expression of pro- and anti-inflammatory signaling molecules was quantified through real-time reverse transcription polymerase chain reaction (RT-PCR) at study entry and 6 months later. Total RNA was extracted from leukocytes using PAXgene Blood RNA kits (PreAnalytix, Hombrechtikon, Switzerland). RT-PCR reactions were carried out (Prism 7000 Sequence Detection System, Applied Biosystems, Foster City, California), using one-step assays based on 5' nuclease activity of FAM-labeled TaqMan probes (Applied Biosystems). For NF- $\kappa$ B and I $\kappa$ B, commercially available assays were used (#HS00765730\_m1 and #HS00153283\_m1, Applied Biosystems). For the GR isoforms, we developed a new TaqMan assay in collaboration with Applied Biosystems. The primer sequences were 5'-AGTGGTTGAAAATCTCCTTAAGTATTGCT-3' (forward) and 5'-GGTATCTGATTGGTGATGATTTCAGCTA-3' (reverse) for GR- $\alpha$  and 5'-AGAAGATTATGTGCACTTCGTTGTCA-3' (forward) and 5'-GGCACAGCTTCTTTCCCATTTAAT-3' (reverse) for GR- $\beta$ . All assays used a standard thermal cycling protocol recommended by the manufacturer. As an internal control, 18S mRNA (for GR isoforms) or  $\beta$ -actin mRNA (for NF- $\kappa$ B and I $\kappa$ B) were quantified in parallel with target genes. The data were normalized using the  $\Delta C_T$  method ( $\Delta C_T = C_T \text{ target} - C_T \text{ control}$ ). Results are expressed as relative quantities of each target, calculated by subtracting each patient's  $\Delta C_T$  from the highest  $\Delta C_T$  in the distribution. Thus, higher relative quantities indicate greater expression of target genes.

### Systemic Inflammation and Cytokine Production

Systemic inflammation was assessed, using serum levels of CRP and IL-6, at study entry and 6 months later. CRP was measured using a high-sensitivity chemiluminescence technique (Immulite 2000, Diagnostic Products Corporation, Los Angeles, California). This assay has an intra-assay variability of 2.2% and a minimum detection threshold of 0.20 mg/L. IL-6 was measured, using commercially available high-sensitivity enzyme-linked immunosorbent assays (ELISAs) (R&D Systems, Minneapolis, Minnesota) with a minimum detection threshold of 0.039 pg/ml. Inter- and intra-assay variability were <10%. To model the dynamics of inflammatory signaling pathways under immune challenge, we quantified leukocyte production of IL-6 after stimulation with lipopolysaccharide (LPS) at study entry and 6 months later. Whole blood sample was drawn into Lithium-Heparin Vacutainers and diluted 10:1 with saline, and then co-incubated with LPS at a concentration of 50 ng/ml (Sigma, Saint Louis, Missouri) for 6 hours at 37°C with 5% CO<sub>2</sub>. The supernatants were then harvested and frozen at -80°C until assayed for IL-6 by ELISA (DuoSet ELISA Development Systems; R&D Systems). These kits have a minimum detectable threshold of 0.7 pg/ml and inter- and intra-assay variability of <10%.

### Potential Confounders

To determine whether behavioral and biomedical characteristics might be acting as confounders, we collected information regarding age, ethnicity, oral contraceptive use, socioeconomic status, smoking history, central adiposity, and strenuous physical activity. Each of these factors has been linked to interpersonal difficulties and/or immune functions in past work (1,7,8,23). Socioeconomic status was assessed with the adolescent version of the MacArthur Scale of Subjective Social Status (24), and central adiposity was indexed as the ratio of waist to hip circumference. Strenuous physical activity was measured as minutes each week engaged in “regular activity akin to brisk walking, jogging, bicycling, etc, long enough to work up a sweat” (25). Because depressive symptoms can arise from chronic interpersonal difficul-

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ties (26), and themselves bring about systemic inflammation (27), we also administered the Beck Depression Inventory (28) to subjects at the 6-month visit.

## Statistical Analyses

To evaluate the study's major hypotheses, we estimated a series of partial correlations between chronic interpersonal stress at baseline and inflammatory parameters 6 months later. Each analysis controlled for the potential confounding influences of age, ethnicity, oral contraceptives, socioeconomic status, central adiposity, and strenuous activity as well as values of the outcome variable at baseline. (In other words, each of these covariates was held constant when the correlations were estimated.) Thus, significant associations indicate that chronic interpersonal difficulties at study entry presage changes in biological outcomes over the next 6 months, and do so in a fashion that is independent of the demographic and biobehavioral covariates included in the model. Smoking was not included as a covariate because only three patients reported daily use of cigarettes. (The findings were similar regardless of whether these subjects were included or excluded from analyses, so we kept them in.) All results are based on two-tailed tests of significance.

## RESULTS

### Preliminary Analyses

Table 1 describes the sample's characteristics and provides descriptive statistics for major predictors, covariates, and outcomes. The study had a diverse sample of teen-age females who mirrored the broader Vancouver population in terms of racial/ethnic background. Because of the project's strict inclusion criteria, all were in good health, free of psychiatric disorders, and without standing medication regimens other than oral contraceptives. On the whole, the sample was rated as having modest levels of chronic interpersonal stress (2.36 on a 1–5 scale), but there was a good deal of variance around this average. Analyses of psychometric characteristics revealed that all of the study's predictors and outcomes were distributed normally. The only exceptions to this rule were CRP and serum IL-6, both of which had positive kurtosis, with more observations at the left tail of the distribution than

expected. To ensure that this did not affect the results of statistical analyses, we repeated all of them involving CRP and IL-6 with nonparametric techniques.

Analyses of the project's various inflammatory parameters indicated that several of them were intercorrelated. Specifically, there were significant associations between GR- $\alpha$  and GR- $\beta$  mRNA, NF- $\kappa$ B and I $\kappa$ B mRNA, CRP and IL-6, and GR- $\alpha$  mRNA and IL-6 production at both study entry and at follow-up (all  $r \geq .22$ , all  $p \leq .05$ ; mean  $r = .60$ ). None of the other pairs of outcomes displayed consistently significant associations. Analyses of stability over the 6-month follow-up period revealed considerable variability across parameters. Whereas CRP, IL-6, and LPS-stimulated IL-6 were moderately stable over time (all  $r \geq .46$ , all  $p \leq .001$ ; mean  $r = .51$ ), none of the intracellular signaling molecules was (all  $r < .08$ ,  $p > .43$ ). The one exception to this pattern was for NF- $\kappa$ B mRNA, which to our surprise was inversely correlated over time,  $r = -.26$ ,  $p = .01$ .

Before conducting primary statistical analyses, we examined relationships between predictors, outcomes, and covariates. Chronic interpersonal stress was higher among subjects with more central adiposity ( $r = .23$ ,  $p = .02$ ), and marginally higher among those low in socioeconomic status ( $r = -.18$ ,  $p = .06$ ), but did not vary by age, ethnicity, strenuous activity, or oral contraceptive use (all  $p > .10$ ). None of the covariates was related to the project's major outcome variables—changes over time in the various inflammatory parameters, all  $p > .09$ . We nonetheless elected to include all covariates in the models presented below. This approach safeguards against the possibility of spurious relationships emerging between chronic stress and biological outcomes as a result of the variance they share with potential confounders.

### Chronic Stress and Inflammatory Dynamics

At study entry, there was a marginally significant inverse association between chronic interpersonal stress and NF- $\kappa$ B mRNA,  $r = -.19$ ,  $p = .06$ . However, none of the other cross-sectional associations between chronic stress and inflammatory parameters reached statistical significance (all  $r \leq .17$ , all  $p \geq .09$ ). (This was also true in nonparametric analyses of CRP and IL-6,  $p > .34$ .)

By contrast, chronic interpersonal difficulties at study entry were related to changes over time in a number of outcomes. Table 2 describes the results of these analyses. To the extent that they had chronic interpersonal stress at the time of study entry, subjects displayed larger increases in LPS-stimulated production of IL-6 over the 6-month follow-up (Figure 1). Over the same time frame, subjects with interpersonal difficulties showed larger increases in mRNA for the proinflammatory molecules GR- $\beta$  and NF- $\kappa$ B, and in mRNA for I $\kappa$ B. The latter molecule sequesters NF- $\kappa$ B in the cytoplasm and minimizes its proinflammatory activities. Chronic interpersonal stress at study entry was unrelated to changes in GR- $\alpha$ , serum CRP, and serum IL-6 over the follow-up period, all  $p > .23$ . (Identical results emerged in nonparametric analyses of CRP and IL-6,  $p > .27$ .)

TABLE 1. Characteristics of the Sample ( $n = 103$ )

Characteristic	Mean $\pm$ SD or $n$ (%)
Age	17.19 $\pm$ 1.37
White	47 (45.7%)
East Asian	46 (44.7%)
Baseline chronic interpersonal stress (1–5)	2.36 $\pm$ 0.49
Baseline C-reactive protein (mg/L)	0.55 $\pm$ 0.69
Baseline interleukin-6 in serum (pg/ml)	0.61 $\pm$ 0.54
Baseline interleukin-6 production (pg/ml)	42,985 $\pm$ 15,546
Baseline glucocorticoid receptor- $\alpha$ mRNA (RQ)	3.58 $\pm$ 1.82
Baseline glucocorticoid receptor- $\beta$ mRNA (RQ)	3.51 $\pm$ 1.71
Baseline nuclear factor- $\kappa$ B mRNA (RQ)	5.04 $\pm$ 2.14
Baseline inhibitor of $\kappa$ B mRNA (RQ)	5.86 $\pm$ 2.50
Daily cigarette smoker	3 (2.1%)
Oral contraceptive user	18 (17.3%)
Central adiposity (waist-hip ratio)	0.75 $\pm$ 0.05
Strenuous exercise per week (minutes)	123.05 $\pm$ 151.68
Beck Depression Inventory (0–63)	7.21 $\pm$ 6.32
Subjective social status (1–10)	6.73 $\pm$ 1.17

SD = standard deviation; mRNA = messenger ribonucleic acid.

TABLE 2. Associations Between Chronic Interpersonal Stress at Study Entry and Inflammatory Parameters 6 Months Later

	Serum CRP	Serum IL-6	Stimulated IL-6	GR- $\alpha$	GR- $\beta$	NF- $\kappa$ B	I $\kappa$ B
Model A	-0.04	-0.11	0.19**	0.09	0.21*	0.26*	0.20*
Model B	-0.08	-0.12	0.22*	0.13	0.20*	0.22*	0.22*
Model C	-0.07	-0.10	0.22*	0.13	0.21*	0.22*	0.21*

\*  $p < .05$ ; \*\*  $p < .06$ .

Model A displays Pearson's correlations between chronic interpersonal stress at study entry and each of the outcome variables 6 months later. In Model B, the covariates age, race, oral contraceptives, socioeconomic status, central adiposity, and strenuous physical activity have been partialled out, as has the value of the outcome variable at the time of study entry. (In other words, all of the covariates have been "held constant" when estimating the correlation.) Model C is identical to Model B except that it also includes depressive symptoms at 6 months as a covariate.  $n = 103$ . Analyses have 88 to 101  $df$ , depending on analysis and outcome. CRP = C-reactive protein; IL = interleukin; GR = glucocorticoid receptor; NF- $\kappa$ B = nuclear factor- $\kappa$ B; I $\kappa$ B = inhibitor of  $\kappa$ B.

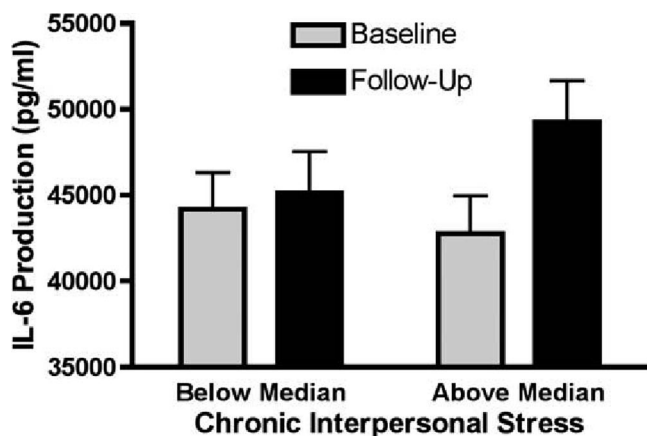


Figure 1. LPS-stimulated production of IL-6 increases over time in subjects with chronic interpersonal stress. The sample was stratified at the median of chronic interpersonal stress (2.4 on a 1–5 scale), and the groups' IL-6 values were compared using a repeated-measures ANOVA. Covariates included age, ethnicity, oral contraceptives, socioeconomic status, central adiposity, and physical activity. The analysis yielded a significant Stress  $\times$  Time interaction,  $F(1,95) = 4.11$ ,  $p = .05$ , indicating that the low and high stress groups changed differentially over the 6-month follow-up. Data are plotted as mean  $\pm$  standard error. LPS = lipopolysaccharide; IL = interleukin; ANOVA = analysis of variance.

All of the significant prospective associations were independent of age, ethnicity, socioeconomic status, oral contraceptives, central adiposity, and strenuous activity (Table 2, middle row). These associations also were independent of depressive symptoms at the 6-month assessment (Table 2, bottom row), suggesting that observed changes in inflammatory signaling were directly related to interpersonal difficulties and not mediated by any consequent increases in dysphoria. (Incidentally, there was not a consistent pattern of cross-sectional or prospective associations between depressive symptoms and inflammatory parameters, all  $r < .14$ , all  $p > .17$ . The one exception to this was for I $\kappa$ B mRNA, which was positively associated with depression in both cross-sectional and prospective analyses,  $r > .20$ ,  $p < .05$ .)

## DISCUSSION

Mounting evidence suggests that chronic interpersonal stressors have a detrimental influence on mental and physical health, and one emerging hypothesis regarding the mechanism of these effects involves the ability of stress to alter physiologic inflammatory processes. The present results support that

hypothesis in identifying significantly greater 6-month increases in expression of gene products involved in the transduction of inflammatory signals (NF- $\kappa$ B, GR- $\beta$ , and I $\kappa$ B mRNA) in leukocytes from young people who experienced chronic interpersonal stress. These alterations in inflammatory gene expression seem to have significant consequences for leukocyte functional responses to stimulation. In response to a model bacterial stimulus (LPS), leukocytes from those showing higher levels of interpersonal difficulty showed greater increases over time in the production of the proinflammatory cytokine IL-6 than did cells from those experiencing low levels of interpersonal difficulty. These conflict-related changes in expression of inflammatory signaling pathway genes seem to predominately affect the leukocyte's potential to respond to a pathogen challenge, rather than its basal production of proinflammatory cytokines. That is, subjects experiencing high levels of interpersonal difficulty at baseline did not show elevations in serum biomarkers of basal inflammatory activity (i.e., circulating IL-6 or CRP). Differences only emerged when inflammatory signal transduction pathways were actively engaged by a model stimulus (i.e., in LPS-stimulated production of IL-6 ex vivo).

These results imply that, at least in young healthy people with comparatively low levels of chronic inflammation, social conflict creates a potential for hyperinflammatory responses that requires an exogenous immunological stimulus for realization. Thus, any health consequences of these dynamics, would likely involve a person  $\times$  situation interaction in which interpersonal difficulties (person) acts to amplify the effects of a pathogenic insult (situation) to affect inflammation-related disease pathogenesis. Such dynamics could have relevance in a number of mental and physical illnesses that are known to be exacerbated by interpersonal difficulties. For example, depression is particularly sensitive to inflammation, and so are a number of infectious, metabolic, and coronary diseases (15–17,29).

The mechanisms responsible for stress-related changes in inflammatory signaling remain to be elucidated. Because of the project's strict inclusion criteria and use of statistical controls, we can be reasonably confident that lifestyle variables, psychiatric conditions, medical illnesses, and demographic factors are not responsible for the observed relationship between interpersonal difficulties and altered inflammatory signaling. One plausible hypothesis is that sympathetic nervous

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system activation underlies this phenomenon. Acute bouts of social conflict provoke the release of norepinephrine (30), which can potentially increase NF- $\kappa$ B expression or enhance its deoxyribonucleic acid (DNA)-binding activity (31). However, the available evidence suggests that these dynamics unfold over fairly short periods of time and, as such, they would be expected to manifest in a “real-time” association between interpersonal difficulties and inflammatory parameters. By contrast, our data suggest that there is a time lag of up to 6 months between exposure to social stress and changes in leukocyte functions.

To some degree, cortisol may help to explain these time-lagged patterns. Individuals without a regular schedule of warm social contacts tend to have unstable rhythms of cortisol output (32,33). With repeated exposure to high doses of cortisol over time, such persons may eventually become resistant to the hormone’s influence. In the immune system, the development of such resistance would enable inflammation to flourish without its usual hormonal constraints (23,34). Chronic social stress has been shown to foster resistance to the anti-inflammatory properties of glucocorticoids in rodent models (13,35). Similar dynamics have been found in humans: recent genome-wide microarray experiments demonstrated that people who are chronically lonely or facing significant interpersonal stress, e.g., caring for a spouse with cancer, show heightened NF- $\kappa$ B activity and simultaneous impairment of cortisol-mediated signaling (34,36). Collectively, these results suggest that social difficulties may provoke cortisol abnormalities, which over time foster resistance to glucocorticoids and expression of inflammatory mediators. The time required for this chain-of-events to unfold may help to explain why our data yielded evidence of prospective associations between interpersonal difficulties and inflammatory processes but no cross-sectional relationships.

This study had several limitations worth noting. First, because the sample was chosen to be at high risk for depression, they are not representative of the general population. Although this constrains the generalizability of the findings, it does not seriously complicate interpretation of them. Future research will need to be done, however, to substantiate the effects in the broader population.

Second, the study quantified mRNA for inflammatory signaling molecules but did not measure their associated proteins or directly assess the functional activity of each individual protein (although the integrated activity of the pathway as a whole was assayed, using the LPS stimulation model). Assessment of individual protein alterations will need to be done in future research.

Third, the study only had two points of inflammatory assessment. As a result, we are unable to specify how long the “incubation period” is between exposure to interpersonal difficulties and subsequent alterations in inflammatory processes. Without more points of assessment, we are also unable to specify if and when these alterations resolve, or what influence earlier social conflict had on biological processes captured at study entry. Multiwave studies will be needed to address these questions.

Fourth, the sample was composed of young women who, on average, had fairly good social relationships and limited amounts of systemic inflammation. The modest stress levels may have restricted the magnitude of associations we were able to observe, and the low CRP and IL-6 values may explain why these outcomes were unrelated to interpersonal difficulties in our sample but have been linked to stress in other projects (34,36).

Finally, we did not measure any clinical outcomes in this project, so it remains unclear whether these dynamics have disease implications. It will be important for the next wave of studies in this area to do so, and determine whether inflammatory processes are the mechanism through which chronic interpersonal stress “gets under the skin” to undermine mental and physical health. In the meantime, these findings extend into humans a large corpus of animal research (37,38), suggesting that an organism’s physiology is intimately regulated by the social context in which s/he resides.

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