

# A Preliminary Study of Daily Interpersonal Stress and C-Reactive Protein Levels Among Adolescents From Latin American and European Backgrounds

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**Objective:** To examine the association between the experience of daily interpersonal stress and levels of C-reactive protein (CRP), an inflammatory marker that is a key indicator of cardiovascular risk, during the teenage years. **Methods:** A total of 69 adolescents ( $M_{\text{age}} = 17.78$  years) completed daily diary checklists each night for 14 days in which they reported their experience of negative interpersonal interactions in the domains of family, peers, and school (e.g., conflict with family and friends, peer harassment, punishment by parents and teachers). Blood samples were obtained an average of 8.63 months later and assayed for circulating levels of CRP, using enzyme-linked immunosorbent assay. Measures of body mass index (BMI), socioeconomic status (SES), substance use, stressful life events, rejection sensitivity, and psychological distress were obtained. **Results:** A greater frequency of daily interpersonal stress was associated with higher levels of CRP, even after controlling for BMI, SES, substance use, life events, rejection sensitivity, psychological distress, and frequency of daily interpersonal stress 2 years earlier. **Conclusions:** Experiencing a high frequency of interpersonal stressors that are typical of adolescent life is associated with higher levels of inflammation even among a normative, healthy sample of adolescents. Additional work should focus on other daily experiences during the adolescent period and their implications for elevated risk for later cardiovascular disease. **Key words:** C-reactive protein, inflammation, daily interpersonal stress, adolescence.

**BMI** = body mass index; **CRP** = C-reactive protein; **CVD** = cardiovascular disease.

## INTRODUCTION

Although not high in frequency, negative interpersonal interactions such as conflict with family and friends, teasing or harassment by peers, and being reprimanded by parents or teachers are a feature of the adolescent experience (1,2). Interpersonal stressors such as these are examples of low-frequency events that nevertheless have a high psychological impact. Conflict with parents occurs at a rate of only once or twice per week during adolescence, but it is significantly associated with elevated levels of psychological distress (1). Similarly, peer harassment occurs infrequently, but it is a significant predictor of depression and anxiety (3). Interpersonal stressors such as these are among the most frequent and powerful predictors of psychological distress among individuals (4).

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Less is known, however, about the impact of these normative interpersonal stressors for the physical health of adolescents. Increased evidence has shown that general stress is associated with lower levels of physical health, as measured by both self-reports of symptomatology and objective measures, such as cholesterol, high blood pressure, and obesity (5). Much of the previous research has focused on stress in a general sense, using measures and inventories that include stressors and major life events in a variety of domains, such as work, relationships, and finances (6,7). Less work has examined the impact of actual interpersonal interactions that are typical of everyday life, and very little has examined this potential link among younger samples. Nevertheless, there are recent findings to suggest that such interpersonal stressors may be associated with physical health. For example, adolescents who experience more frequent harassment from peers also report more physical complaints, such as headaches and fatigue (8).

Given the increased interest in identifying the early risk factors for later health problems, such as cardiovascular disease (CVD) (9), it is important to examine the years before adulthood and to examine the impact of conditions and experiences that are particularly relevant for children and adolescents. The focus of this study was to examine the linkage between daily interpersonal stress and levels of C-reactive protein (CRP) among a sample of adolescents from Latin American and European backgrounds. The focus was on this biomarker because of the accumulation of findings that elevated levels of CRP, an indicator of chronic inflammation, are associated with a greater risk for the later development of CVD (10,11). There also is an emerging body of evidence that stress can lead to greater inflammation because the stimulation of the hypothalamic-pituitary-adrenal (HPA) axis by stress can upregulate inflammatory processes, as indicated by higher levels of CRP and proinflammatory cytokines (6,12).

Adolescents' experience with interpersonal stress was measured using the daily diary checklist method. The aim was to focus on the normative, everyday experience of interpersonal stress this method is superior to traditional questionnaire tech-

niques because it “captures life as it is lived” and is less susceptible to recall biases (13). As described below, participants completed a checklist each night for a period of 2 weeks. They reported their daily experiences of negative interpersonal interactions (e.g., conflict, harassment) in the primary domains of adolescent life: family, peers, and school. These daily reports provide a more direct measure of actual experience than more typical questionnaire inventories, which usually ask respondents to generalize their reports across time and experiences.

Another goal of the study was to distinguish the impact of these daily interpersonal stressors from other potential factors. Stressful life events have been linked to higher levels of cardiovascular risk (14) and adolescents’ reports of life events was measured to determine whether daily interpersonal stressors predicted CRP even after accounting for this factor. Given the links between interpersonal stress, personality, and psychological well-being, adolescents’ sensitivity to interpersonal rejection and their reports of anxious and depressive feelings were assessed to determine whether any link between interpersonal stress and CRP was independent of these factors.

## METHODS

### Sample and Design

The sample of 69 adolescents in the current study is a subsample of a group of 383 adolescents from three high schools in the Los Angeles area who took part in a larger study of the daily experience of adolescents when they were in the 12th grade ( $M_{\text{age}} = 17.78$  years in the 12th grade). There were no significant differences between the subsample of 69 and those who did not take part in the current study on any of the key 12th grade measures that were used in the current study and described below,  $t$  values(352–378) = 0.20–1.50,  $p$  values = .13–.85.

The sample consisted of 42 participants from Latin American backgrounds, most (95%) of whom reported Mexican as their ethnic background, and 27 participants from a mix of European backgrounds (e.g., Irish, Jewish, German). All adolescents spoke and read English fluently, although this was not a requirement for participation in the study. The sample was 53.6% female.

During the spring of the 12th grade in 2006, participants filled out questionnaires during school hours and then completed a daily diary checklist each night before going to bed for 14 consecutive days. The diary checklists were only 3 pages long and took about 5 to 10 minutes to complete. To monitor completion of the diary checklists, participants were also provided with 14 manila envelopes and an electronic time stamper (Dymo Corporation, Stamford, Connecticut). The time stamper is a small, handheld device that imprints the current date and time and is programmed with a security code so that the correct date and time cannot be altered. Participants were instructed to place their completed diary checklist into a sealed envelope each night, and to stamp the seal of the envelope with the time stamper. At the end of the 2-week period, the adolescents returned the completed materials to the school and received \$30 for participating in the study. In addition, the adolescents were told that they would receive two movie passes if inspection of the data indicated that they had completed the diaries correctly and on time. The time stamper method of monitoring the completion of the diaries and the cash and movie pass incentives resulted in a high rate of compliance, with 98.6% of the diaries being completed.

Of the total sample of 69, 55 participants completed identical diary checklists, using the same protocol 2 years earlier during the spring of the 10th grade. These reports were used to conduct additional analyses controlling for stable individual differences in the experience of daily interpersonal stress and to examine the association of change in daily interpersonal stress with CRP.

In the fall and winter after the 12th grade, in 2006/2007, the participants were recontacted and recruited to participate in an additional round of data collection. Participants came to a laboratory at the University of California, Los Angeles (UCLA) where they completed questionnaires, had height and weight measurements taken using a stadiometer, and provided blood samples for the evaluation of CRP. Appointments took place an average of 8.67 months (range = 4.25–13.25 months) after the participants completed their questionnaires and diary checklists during the 12th grade. Blood samples were drawn at various times during the day, with the majority (85.8%) being obtained between the hours of 12 PM and 4:45 PM. The modal time was 1:30 PM. All procedures were approved by the UCLA Institutional Review Board, and all participants were 18 years of age at the time of data collection.

### Social and Behavioral Measures

Daily interpersonal stress during the 12th grade was measured by adolescents’ reports of whether any of 10 events occurred to them each day (yes/no): punished or disciplined by parents, something bad happened to someone in the family, parents had an argument, argued with mother, argued with father, argued with other family member, argued with a friend, argued with or punished by teacher, harassed or picked on by a student at school, and harassed or picked on by other person out of school. The events were selected because they are known to be psychological stressors for adolescents across the primary domains of family, peers, and school (1,3). Although two of the items (something bad happened to someone in the family, parents had an argument) do not directly involve the adolescent, they are family stressors known to affect psychological well being (15,16). A summary variable was created that indicated the percentage of days on which any 1 of these 10 events occurred to the adolescent. This approach was chosen rather than analyzing each experience separately because each is a low-frequency event with very little variance. Collapsing across all events provided us with sufficient range and variability to conduct analyses. The  $\alpha$  coefficient (0.83) indicated that the measure possessed good internal consistency.

Stressful life events were measured using an inventory appropriate for teenagers in which adolescents indicated if any of 23 events occurred in the past 12 months (e.g., a parent lost a job, there was a death of a family member, you or someone close to you became seriously ill, your grades went down a lot, you broke up with a boyfriend or girlfriend (17)). The scale has been shown to be significantly predictive of adolescent depression (18) and has substantial overlap with life events and chronic stress measures used among adults that have been shown to be predictive of CRP (14). A sum of the total number of events that occurred was created to measure participants’ overall major life events in the past year.

Daily psychological distress during the 12th grade was assessed with items on the daily checklist that were obtained from the Profile of Mood States (19). Adolescents used a 5-point scale (1 = “Not at all” to 5 = “Extremely”) to indicate the extent to which they felt anxious and depressive feelings (items: “sad,” “hopeless,” “discouraged,” “on edge,” “unable to concentrate,” “uneasy,” “nervous”). Scores on the seven items were averaged for each day, and then a mean level of daily distress was obtained by taking the average of scores across the 14 days. The  $\alpha$  coefficient (0.80) indicated that the measure possessed good internal consistency.

Rejection sensitivity was measured using the Rejection Sensitivity Questionnaire, which has been shown to be predictive of individuals’ perceiving intentional rejection in response to ambiguous behavior and in the insensitive behavior of relationship partners (17). The measure consists of 18 hypothetical situations in which participants rate their expectations of acceptance or rejection (e.g., “You ask your parents for extra money to cover living expenses”). For each situation, participants first indicate the degree of concern or anxiety they have about the outcome of the situation, using a 6-point scale (1 = very unconcerned to 6 = very concerned; e.g., “How concerned or anxious would you be over whether or not your parents would help you out?”). Participants then use a 6-point scale (1 = very unlikely to 6 = very likely) to rate how likely the other person will be to respond in an accepting fashion (e.g., “I would expect that my parents would not mind helping me out”). Scores were computed by taking the mean of the products of the concern and likelihood ratings for each situation ( $\alpha = 0.75$ ).

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Finally, measures of smoking behavior and caffeine and alcohol use were obtained when participants completed the additional questionnaire before their blood samples were taken. Adolescents indicated how many days (0–7) in the past week they had any caffeinated drink, such as coffee or cola, smoked cigarettes, or had a drink containing alcohol (beer, wine, a mixed drink, any kind of alcoholic beverage).

### Measurement of Body Mass Index (BMI)

BMI was measured by height and weight observations obtained using a stadiometer at the time of the blood collection. BMI was calculated by taking body weight in pounds divided by the square of height in inches, and multiplied by 703 to convert to metric units (20).

### CRP Measurement

Blood samples were drawn from participants through routine venipuncture after they completed the questionnaire and after height and weight measurements were taken. CRP was measured using high-sensitivity immunoassay (BN-II System, Dade-Behring, Newark, Delaware). Samples were automatically diluted 1:20 with N Diluent. This technique has a limit of detection of 0.175 mg/L and intra- and interassay coefficients of variation of <4%.

### Data Analysis

Data were analyzed using SPSS for Windows, Version 15 (SPSS Inc., Chicago, Illinois). Individual levels of CRP were first examined for values of >10 mg/L, and no adolescents had values of CRP that met this criterion suggested by the American Heart Association and the Centers for Disease Control and Prevention as being indicative of an acute inflammatory response (e.g., an infection) that would warrant exclusion from analyses such as these (21).

Hierarchical regressions were conducted to examine the bivariate association between daily interpersonal stress and CRP and to test whether an initial association between the two could be attributable to socioeconomic status (SES), BMI, substance use, life events, rejection sensitivity, and psychological distress. Finally, an exploratory analysis also addressed whether the change in interpersonal stress from the 10th to 12th grade was associated with CRP as a way to control for the possibility that any observed association was due to stable, long-term individual differences.

## RESULTS

Overall, the mean and standard deviation values of the study variables portray a generally healthy and normative sample (Table 1). Average levels of CRP were low: 46 adolescents had CRP levels in the low-risk range (CRP = <1), 13 adolescents fell into the intermediate-risk category (CRP = 1–3), and only 7 adolescents were in the high-risk category (CRP = >3). There were no significant differences in levels of CRP according to adolescents' gender and ethnic background,  $t$  values(67) = 0.65–1.48,  $p$  values = .15–.52. BMI

was within normal range and participants reported low levels of substance use.

Interpersonal stress was not uncommon, occurring on approximately one third of days, but it also was not extremely frequent. In terms of the occurrence of specific events across the 2-week period, the most common event was argued with friend (60.9% of adolescents reported it occurring at least once), followed by argued with mom (58%), argued with dad (37.7%), being punished or disciplined at home (34.8%), argued with another family member (33.3%), something bad happened to someone else in the family (26.1%), parents had argument (20.3%), punished at school (18.8%), harassed at school (8.7%), and harassed outside of school (7.2%). The rates of family conflict and peer harassment are similar to those obtained in other studies of adolescents (1,3), but there have been no previous studies that estimated the daily frequency of the other events. In terms of the average number of events reported per day, 13% of the sample reported no events occurring each day. An additional 72.5% reported an average of >0 but <1 event per day, 13.1% reported 1 to 2 events per day, and 1.4% reported an average of >2 events per day.

Adolescents also reported few stressful life events and fairly low levels of daily emotional distress and rejection sensitivity.

Bivariate correlations among the study variables are presented in Table 2 and show significant associations of interpersonal stress with CRP, life events, rejection sensitivity, and distress. In addition, individuals with a higher BMI also had higher levels of CRP.

Results of the hierarchical regression are presented in Table 3. As shown in Model 1, adolescents who reported more frequent interpersonal stress had significantly higher levels of CRP. This association existed even after controlling for the adolescents' SES, BMI, and substance use (Model 2). In addition, the link between interpersonal stress and CRP was significant above and beyond life events, rejection sensitivity, and psychological distress (Models 3 and 4).

Because the blood samples were obtained at variable periods after the adolescents completed the reports of daily interpersonal stress, an additional analysis was conducted to determine whether the strength of the association between stress and CRP varied according to the number of months between the two measurements. The interaction between daily interpersonal stress and the length of the interval between the measurements in predicting CRP, however, was not significant ( $\beta = 0.08$ ,  $B = 0.02$ ,  $SE = 0.11$ ,  $p = .89$ ).

Finally, using the 55 out of 69 participants for whom an identical measure of daily interpersonal stress was available at the 10th grade, a regression was conducted in which 10th grade level of daily interpersonal stress was controlled at the same time as using 12th grade daily interpersonal stress to predict CRP. This allowed us to control for stable, individual differences and to examine the impact of change in daily interpersonal stress on CRP. Results indicated that the 12th grade measure was a significant predictor of CRP ( $\beta = .34$ ,  $B = 2.52$ ,  $SE = 1.20$ ,  $p < .05$ ), whereas the 10th grade

TABLE 1. Descriptives

Variable	Mean	SD	Range
Interpersonal stress	0.33	0.26	0–0.93
CRP	1.26	1.78	0.20–9.70
BMI	25.31	7.38	13.06–52.31
Substance use	1.84	1.29	0–5
Life events	4.00	2.52	0–12
Distress	1.51	0.41	1.0–3.05
Rejection sensitivity	8.91	3.28	2.13–18.50

$n = 69$ .

SD = standard deviation; CRP = C-reactive protein; BMI = body mass index.

TABLE 2. Correlations

Variable	<i>r</i>							
	1	2	3	4	5	6	7	8
Interpersonal stress	1	.26*	.09	.07	.26*	.24*	.32**	-.10
CRP		1	.34**	.01	.11	.07	.09	.10
BMI			1	-.06	.10	.19	.23	-.18
Substance use				1	.30*	.17	-.12	.22
Life events					1	.19	.08	.00
Rejection sensitivity						1	.38**	-.05
Distress							1	.05
SES								1

\*  $p < .05$ ; \*\*  $p < .01$ .

CRP = C-reactive protein; BMI = body mass index; SES = socioeconomic status.

TABLE 3. Hierarchical Regression Predicting CRP From Interpersonal Stress, SES, BMI, Substance Use, Major Life Events, Rejection Sensitivity, and Distress

	Model							
	1		2		3		4	
	$\beta$	B (SE)	$\beta$	B (SE)	$\beta$	B (SE)	$\beta$	B (SE)
Intercept		0.67 (0.34)		0.68 (0.33)*		0.69 (0.34)*		0.61 (0.36)
Interpersonal stress	0.26	1.84 (0.83)*	0.25	1.77 (0.80)*	0.25	1.75 (0.83)*	0.28	1.99 (0.89)*
SES			0.20	0.43 (0.26)	0.20	0.43 (0.26)	0.22	0.47 (0.27)
BMI			0.35	0.08 (0.03)**	0.35	0.08 (0.03)**	0.37	0.09 (0.03)**
Substance use			-.02	-.06 (0.31)	-.03	-.07 (0.34)	-.04	-.11 (0.35)
Life events					0.01	0.01 (0.09)	0.01	0.10 (0.09)
Rejection sensitivity							-.01	0.00 (0.07)
Distress							-.10	-.43 (0.58)
R <sup>2</sup> /adjusted R <sup>2</sup>		0.07/0.06		0.21/0.16		0.21/0.14		0.22/0.12
F		4.88*		4.10**		3.23*		2.35*

\*  $p < .05$ ; \*\*  $p < .01$ .

CRP = C-reactive protein; SES = socioeconomic status; BMI = body mass index.

measure was not ( $\beta = -0.01$ ,  $B = -0.06$ ,  $SE = 1.25$ ,  $p = .95$ ). These results were obtained despite the fact that the 10th grade and 12th grade measures of interpersonal stress were highly correlated with one another ( $r = .59$ ,  $p < .001$ ).

## DISCUSSION

Daily interpersonal stress experienced during the high school years was found to be associated with increased levels of inflammation, as measured by CRP, even among a normative, healthy population of teenagers. This association could not be attributed to differences in SES, BMI, or substance use. In addition, analyses suggest that the association was not due to stressful life events, the personality characteristic of rejection sensitivity, and average daily psychological distress. These results are consistent with the emerging body of evidence pointing to the link between stress and increased inflammation, placing individuals at risk for the later development of CVD (6,10).

The findings also contribute uniquely to this body of research in several ways. First, the impact of interpersonal stress was identified, which previously has been shown to be one of the most significant classes of stressors for psychological well-being (4). Results suggest that the same may be true for

inflammation, and future work would profit from distinguishing between different types of stressors to determine which are most important for inflammation.

Second, the intensive measurement of interpersonal stressors at the daily level shows the value of such a technique, and points to the importance of daily negative interactions for physical health, above and beyond the occurrence of stressful life events. Nevertheless, it would be useful to compare the daily measure of interpersonal stress with additional measures that tap global appraisals of stress, such as that used in a recent study of adults (6).

Third, results suggest that the association of interpersonal stress with inflammation is unexplained by individuals' psychological appraisal of distress or a tendency to be particularly sensitive to social rejection.

Finally, although most research on stress and inflammation has focused on adulthood, these results show that such links can occur as early as the teenage years among a healthy sample, suggesting that alterations in the biological substrates that initiate CVD begin before adulthood.

The nonexperimental nature of the data does not allow for definitively arguing for the causal effect of daily interpersonal

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stress on CRP. The longitudinal analyses controlling for earlier levels of daily interpersonal stress do allow for ruling out the possibility that chronic individual differences in stress account for the association. Nevertheless, more frequent measurements of both stress and CRP across time would better allow us to examine the directionality of the association across time. Recent studies that have manipulated stressors experimentally have found a link between stress and inflammation (22), providing more support for the possibility that daily interpersonal stress contributes to elevated levels of CRP in the sample.

An important limitation of the study is the timing of the measurement of CRP, which occurred months after the measurement of daily interpersonal stress and at different time points for different individuals. Follow-up analyses showed that the strength of the association did not vary significantly according to the timing of the CRP measurement, suggesting that this variability did not make a difference in the results obtained in this study. In addition, the results suggest that the potential impact of interpersonal stress on inflammation can go beyond the immediate time after the experience and last several months. We do not know, however, whether the participants in our study continued to experience similar interpersonal stress in the interval between the measurement of such stress and CRP, and the sizable association between the 10<sup>th</sup> grade and 12th grade reports of interpersonal stress suggests a fair degree of continuity in these experiences. Elevated levels of CRP are thought to be part of an inflammatory cascade that is upregulated by HPA axis responses to stressors, such as those reported in the measure of daily interpersonal stress (12). CRP is a relatively downstream indicator of this cascade, being produced by the liver in response to increased levels of proinflammatory cytokines. As such, one would not expect to observe elevated levels of CRP immediately after the occurrence of daily interpersonal stress. Nevertheless, future work should include a more contemporaneous measurement of CRP along with multiple measurements of both interpersonal stress and CRP over a period of time in order to more definitely determine the long-term association of experiences of daily interpersonal stress with inflammation.

The relatively small sample size was a limitation that should be rectified in future studies in order to provide the statistical power necessary to explore the role of additional potential explanatory factors as well as the interaction between interpersonal stress and individual characteristics, such as gender and ethnicity. Finally, because the occurrence of acute illness was not assessed in this study, the potential role of this factor in increasing levels of CRP could not be examined.

In conclusion, findings indicate the importance of focusing on actual daily stressful experiences when examining the implications of psychological and social factors for the development of risk for CVD during the adolescent years. Although the frequency of some of these experiences may be low, they could have a significant impact on long-term physical health during adulthood.

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