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Hostility, Anger and Depression Predict Increases in C3 over a 10-Year Period

Stephen H. Boyle[1], **William G. Jackson**[2], and **Edward C. Suarez**[1]

1 *Department of Psychiatry and Behavioral Sciences, Duke University Medical Center*

2 *Air Force Research Laboratory, Brooks-City Base, Texas*

Abstract

We examined the relation of hostility, anger and depression to 10-year changes in the third (C3) and fourth (C4) complement in 313, apparently healthy male participants enrolled in the Air Force Health Study (AFHS), a 20-year study designed to evaluate the health consequences of dioxin exposure. Hostility, depression and anger were assessed using subscales from the Minnesota Multiphasic Personality Inventory (MMPI), which was administered in 1985. Given the high intercorrelations among these psychological scales, we used a principal component analysis to generate a composite score representing the linear combination of the hostility, anger and depression scales. The dependent variables, C3 and C4 levels, were determined from samples collected in 1992, 1997 and 2002. Regression analyses controlling for age, race, alcohol use, body mass index and cigarette use as well as onset of disease and use of lipid lowering and blood pressure medications during follow-up revealed a significant time X composite score interaction for C3 complement ($p < .0003$), but not C4. Post-hoc analyses revealed that high composite scores were associated with larger 10-year increases in C3. These observations suggest that men who are hostile and are prone to experience frequent and intense feelings of anger and depression show activation of the complement system, and specifically increases in C3, that may contribute to the development of coronary heart disease.

Keywords

Complement; hostility; anger; depression; men

1. Introduction

Epidemiological evidence continues to support the psychosomatic hypothesis that hostility, anger and depression are associated with an increased risk of atherosclerotic cardiovascular disease (ACVD) (e.g., Ahmad, 2000;Ferketich, et al., 2000), Type 2 diabetes (T2D) (Arroyo, et al., 2004) and essential hypertension (Everson, et al., 1998). Although the mechanisms accounting for those associations are not well delineated, one emerging hypothesis posits that

Correspondence should be addressed to: Stephen H. Boyle, PhD or Edward C. Suarez, PhD, Duke University Medical Center, PO Box 3328, Durham, NC USA 27710 Email: boyle020@mc.duke.edu or suare001@mc.duke.edu

[1]As a corollary to the stated hypothesis, we also speculated that each of the individual components, that is, anger, hostility and depression, would also predict changes in C3 and C4 over time.

[2]Secondary analyses examined the relation of each individual scale to C3 and C4 levels. Analyses revealed significant hostility X time ($F(2, 596) = 6.36$, $p < .002$), depression X time ($F(2, 596) = 4.44$, $p < .02$) and anger X time interactions ($F(2, 596) = 3.35$, $p < .04$) for C3. Hostility, anger and depression were positively and significantly associated with 10-year increases in C3. These interactions did not predict C4 changes.

psychological attributes contributes to adverse health via inflammation (Black, 2003). Support for this hypothesis comes from a number of cross-sectional studies that have reported significant associations between biomarkers of inflammation, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α and C-reactive protein (CRP), and anger (Suarez, 2004), hostility (Graham, et al., 2006;Suarez, 2003a) and depression (e.g., Miller, et al., 2002;Suarez, et al., 2003), as well as to a composite score representing the linear combination of those variables (Suarez, 2003b;2004). While compelling, the cross-sectional nature of those prior observations limits any conclusions regarding prospective associations. If inflammation is an important pathophysiological mechanism whereby psychological attributes contribute to the development and progression of chronic diseases, it is critical to demonstrate that individuals characterized by high levels of hostility, anger and depression exhibit increasing or elevated levels of inflammatory biomarkers over time.

One aspect of the immune system that has been associated with ACVD and T2D is the complement system. While there are a number of components to the immune complement system (Ritchie, et al., 2004), studies investigating its role in the development of chronic diseases have focused on the major protein C3, and to a lesser extent, C4. The emphasis placed on C3 is due, in part, to its production by activated macrophages and its role as a cytokine (Zimmer, et al., 1982), and its control of lipid and glucose metabolism (Baldo, et al., 1993), pathways leading to cardiovascular disease and diabetes. C4, on the other hand, has been linked with obesity and glucose metabolism (Engstrom, et al., 2005). Cross-sectional studies have shown that C3 is associated not only with risk factors of CHD (Onat, et al., 2005) and diabetes (Engstrom, et al., 2005), but also with the presence and severity of CAD (Figueredo, et al., 1993;Ylitalo, et al., 1997) and ischemic stroke (Di Napoli, et al., 2001). In one prospective study, C3, but not C4, was associated with incident T2D (Engstrom, et al., 2005). C3 has also been associated with incident myocardial infarction (Muscari, et al., 1995) and incident atrial fibrillation (Dernellis & Panaretou, 2006).

Only a few studies have examined the relation of psychological attributes and/or psychological stress to activation of the complement system. Elevations in C3 and C4 have been noted in depressed patients in some (Berk, et al., 1997;Kronfol & House, 1989;Song, et al., 1994), but not all (Spivak, et al., 1989) studies. Other studies have shown that the stress of academic examination evokes increases in C3c, but only among those students who perceived the examination as stressful, whereas C4 showed a significant reduction in students with low-stress perception (Maes, et al., 1997). Although few in number, the findings of the previous studies suggest that psychological attributes, and emotional stress are associated with activation of the complement system.

To date, no study has examined the relation of the complement system to anger and hostility or has examined these potential associations over time. The aim of the present study, therefore, was to examine the relation of psychological risk factors to changes in serum levels of C3 and C4 over a 10-year period. In light of our previous cross-sectional observations (Suarez, 2004), we hypothesized was that the linear combination of hostility, anger and depression would be similarly associated with elevations in C3 and C4 levels and with greater changes in these proteins over time (Footnote 1).

2. Methods

2.1 Participants

The study sample consisted of men who participated in the Air Force Health Study (AFHS). A detailed description of the study design and the participant selection procedure has been published previously (Wolfe, et al., 1990). Briefly, the AFHS was designed to evaluate the health of the veterans of Operation Ranch Hand, the unit responsible for the aerial spraying of

Agent Orange and other dioxin-contaminated herbicides in Vietnam from 1962 to 1971. Initially, all living Ranch Hands and a matched comparison group of Air Force veterans who served in Southeast Asia, but were not occupationally exposed to herbicides in Vietnam, were invited to participate in the 20-year study. Physical examinations were performed in 1982, 1985, 1987, 1992, 1997, and 2002. The examination content emphasized detection of medical endpoints suspected of being associated with exposure to dioxin, so extensive data on health status and health behaviors were collected. In 1985, the examination included a one-time administration of the Minnesota Multiphasic Personality Inventory (MMPI). The MMPI is a well-established self-report questionnaire that has been used to assess various dimensions of personality and has been used in many studies examining the relation of psychological factors to risk of various chronic medical illnesses and stress-induced pathophysiological mechanisms. Assessment of C3 and C4 levels was included only in the 1992, 1997 and 2002 exams.

Of the participants in the AFHS, 2065 subjects completed the 1985 and 1992 examinations. From this total, participants were excluded if, in the 1992 assessment, they reported any of the following: use of anti-inflammatory medications including statins (N = 232); history of liver disorders (N = 603); myocardial infarction (N = 100); diabetes (N = 265); hypertension (N=101); cancer (N = 284); HIV (N = 4); drug (N = 5) or alcohol dependence (N = 127); and psychosis (N = 54). Four hundred and ninety-five people had more than one of these conditions. An additional 41 participants were excluded because they were taking other medications suggestive of other chronic conditions (e.g., thyroid medication) or acute conditions that might have immunological consequences (e.g., penicillin). Lastly, participants that were missing data on one or more of the covariates (N = 10) were also excluded. These relatively stringent exclusion criteria were implemented in order to evaluate the relation of psychological attributes to complement levels with less confounding from these conditions.

Of the remaining 598 people in the sample, 545 attended the 1997 examination and 519 attended the 2002 examination. Those participants that reported using anti-inflammatory medications at the 1997 (N = 80) or 2002 (N = 178) examinations were also excluded. Our study sample was composed of 313 (133 Ranch Hands) who met inclusion criteria and attended the 1985, 1992, 1997 and 2002 examinations.

In comparison to the study sample (n = 313), (see Table 1 for means) those subjects who were excluded were older (mean = 54.53 yr, $p < .0001$); less well educated; consumed more drinks of alcohol per day (mean = .76, $p < .04$); smoked more cigarettes per day (mean = 5.98, $p < .002$) and had higher body mass index (mean = 28.59 kg/m^2, $p < .0001$); depression (mean = 7.07, $p < .0001$); hostility (mean = 13.34, $p < .0003$); anger (mean = 3.72, $p < .0005$); C3 complement levels (mean = 120.07 mg/dl, $p < .0001$) and C4 complement levels (mean = 29.54 mg/dl, $p < .0001$). Ranch Hand status, racial composition and level of physical activity did not significantly differ between the study sample and those subjects who were excluded ($p's > .05$). Given this pattern of findings, individuals who were excluded would likely have the effect of attenuating the magnitude of the associations examined in these analyses. All of the participants were male (19 African Americans) with a mean age in 1992 of 50.16 yrs. (SD = 6.32). See Table 1 for a further description of study participants.

2.2 Measurement of Psychological Attributes

All participants were administered the 566-item MMPI at the 1985 examination. Assessment of hostility, anger and depression was performed using MMPI-derived subscales. For the 14 subjects with missing items, we multiplied the mean of the completed items by the number of items making up that scale. Simulation studies have demonstrated that this approach to handling missing data yields relatively unbiased estimates and is a reasonable alternative to more complex approaches such as multiple imputation (Schafer & Graham, 2002).

Hostility—Hostility was assessed using 39-items from the Cook-Medley Hostility Scale (CMHS) (Cook & Medley, 1954) of the MMPI. These items, identified by Barefoot et al. (Barefoot, et al., 1989), reflect the cognitive (i.e., cynicism and hostile attributions), affective (i.e., hostile affect) and behavioral (i.e., aggressive responding) dimensions of hostility. Representative items included "I think most people would lie to get ahead" (i.e., cynicism); "I tend to be on my guard with people who are somewhat more friendly than I had expected" (i.e., hostile attribution); "Some of my family have habits that bother and annoy me very much" (i.e., hostile affect); and "I have at times had to be rough with people with people who were rude and annoying" (i.e., aggressive responding). The test-retest correlation for this scale was .74 across a 10-year period (Barefoot, 1997). In our study, the α-coefficient for this scale was 0.85.

Depression—The 40-item Obvious Depression scale (OBD) from the MMPI was used to assess depression. The OBD is a straightforward measure of depressive symptoms experienced outside the psychiatric context (e.g., I am happy most of the time) and has been shown to be more appropriate for nonclinical samples than the D scale, another MMPI based measure of depression (Wiener, 1948). The OBD has been shown to correlate 0.72 with the Center for Epidemiologic Study-Depression (CESD) (Radloff, 1977) and 0.78 with the Zung Self-Rating Depression Scale (Zung, 1986) in a community sample (Barefoot, et al., 2001). In one previous study, the OBD scale predicted MI and mortality in a population sample (Barefoot & Schroll, 1996). The 10-year test-retest correlation for the OBD was reported to be .67 in a previous study (Barefoot & Schroll, 1996). The α coefficient for this scale was 0.70.

Trait anger—Trait anger was assessed with an 11-item scale from the MMPI. The selection of these particular items was based on a previous factor analysis of MMPI-2 items that resulted in a 16-item trait anger scale (Siegman, et al., 2000). That study found a significant relation between trait anger and incident CHD in a sample of 1300 men followed for an average of eight years (Siegman, et al., 2000). The trait anger scale consisted of the 11 items (ex. I am not easily angered) from the 16 MMPI-2 items that also appear in the original MMPI. The total score on these 11-items correlated 0.58 with the SCL-90 hostility subscale in a sample of 774 army veterans. There is no test-retest data available for this scale. However, the ability of the longer scale to predict incident heart disease across a period of many years suggests that it measures a relatively stable trait (Siegman, et al., 2000). The α coefficient for this scale was 0.68.

Blood chemistry—Blood specimens were obtained following an overnight fast. Participants were requested to adhere to a 250-gram carbohydrate diet and avoid alcohol consumption for three days prior to their arrival to prepare for 2-hour postprandial glucose testing. These samples yielded measures of C3 and C4. Complement components were measured using a nephelometric assay and the Beckman 360 Protein System (Beckman Coulter, Fullerton, CA). Although no system changes in the hardware and software occurred during the follow-up period, protein assays for 1997 and 2002 were standardized (Bonhall, 1995). The 1992 values for C3 and C4 complement were converted to these units by multiplying by 1.03 and 1.31 respectively (Bonhall, 1995). The between-assay coefficient of variation based on 1% of the sample at three different concentrations of C3 ranged from 4.6% to 9.3% at the 1997 exam and 6.5% to 6.9% at the 2002 exam. The corresponding coefficients of variation for C4 ranged from 3.4% to 4.9% at the 1997 exam and 6.9% to 7.3% at the 2002 exam. The coefficients of variation were not available from the 1992 exam, but were likely acceptable given the same procedures were used in all three examinations.

2.2 Assessment of chronic disease

Hypertensive status—Hypertensive status was measured as a dichotomous variable due to the dual criteria used to determine status. Subjects were classified as hypertensive if they either

reported taking blood pressure medications or had systolic blood pressure >160 mm/Hg or diastolic blood pressure > 90 mm/Hg.

Diabetes status—Presence of diabetes was measured as a dichotomous variable. Evidence of diabetes was defined either by physician diagnosis or by a 2-hour postprandial glucose ≥ 200 mg/dl.

Cardiovascular Disease (CVD)—History of CVD was evaluated in two ways. First, participants provided a detailed medical history that included questions about previous heart trouble, stroke or transient ischemic attacks. Medical records were used to verify all reported conditions and to determine the time of occurrence of major cardiac conditions. Second, an electrocardiogram (ECG) evaluated the possible existence of a previous heart condition. ECG's were obtained after a four hour fast and abstinence from tobacco. History of CVD was coded positive if there was evidence of a previous MI, stroke or transient ischemic attack at any examination.

Liver disease—Participants' self-report, physician examination and laboratory examination were used to determine the presence or absence of liver disorders. Self-reported liver disease was verified by medical record review for the following categories: liver abscess; cirrhosis; hepatomegaly; hepatitis; jaundice; necrosis of the liver; and other liver disorders. The other liver disorders category included nonspecific elevation of levels of alanine transaminase (ALT > 55 U/L), aspartate transminase (AST > 42 U/L), lactic acid dehydrogenase (LDH > 172 U/L), other nonspecific abnormal serum enzyme levels (e.g., alkaline phosphatase > 107 U/L) and nonspecific abnormal results of liver function tests.

Cancer—Current and past diagnosis of cancer were obtained from medical records. Malignancies were coded following rules and conventions of the International Classification of Diseases, 9th Edition, Clinical Modification (ICD-9-CM). Common cancer diagnoses included basal cell carcinoma, malignant skin neoplasms, prostate cancer and squamous cell carcinoma.

Medication Use—Data concerning use of cardiac medications, statins, other lipid lowering medications, blood pressure medication, aspirin, and anticoagulation medications were also collected at each physical examination. Based on participant self-report current use of medication was coded as 0 (no) or 1 (yes) for each of the above medication categories.

2.3 Measurement of health behaviors

Body Mass Index—Body mass index (BMI) was calculated as weight (kg) divided by height (m^2).

Self Reported Health Behaviors—Participants responded to a series of questions regarding smoking, alcohol use and participation in various forms of exercise. Smoking was measured as the number of cigarettes per day during the two-week period prior to the physical examination. Alcohol use was measured as the average number of drinks per day over the previous month. Finally, based on their responses to the exercise questions, participants were classified as sedentary, moderately active, or active.

2.4 Statistical analyses

The relations of the psychological attributes to C3 and C4 complement levels were examined using a repeated measures approach as described in the general linear model procedure from SAS (SAS Institute, 1999). In the analyses, time (1992, 1997 and 2002) was the within-subject factor and the factor-analytically derived component score, reflecting the linear combination

of hostility, anger, and depression, was the between-subjects factor. Covariates included age, race, BMI, alcohol use, exercise history, cigarette use, liver disease, cardiovascular disease, diabetes, and cancer, and the use of lipid lowering drugs, blood pressure medication or other cardiac drugs over the 10-year follow-up. The time X psychological component score interaction term was used to examine whether the psychological attributes influenced levels of complement proteins over time. In the case that this interaction was not significant, we refitted the model with only main effects for time and psychological component score.

3. Results

Preliminary Analysis

As in previous studies, hostility, anger, and depression were significantly and positively correlated with each other (r-ranged: .37–.51). In order to reduce the number of analyses, we conducted a principal component analysis that yielded a single factor (Eigenvalue = 1.86) that accounted for 62% of the variance. As expected, the three variables loaded positively on this factor (factor loadings > .70). A factor score, termed psychological risk factor (PRF), was generated using this one-factor solution. Higher factor scores indicated greater levels of hostility, proneness to anger, and elevated levels of depressive symptoms. In previous studies, a composite score constructed from measures of these same three constructs proved to be as strong or stronger predictor of inflammatory biomarkers than the individual scales (Suarez, 2003b;2004). The PRF score was used in all reported analyses.

Consistent with previous observations (Engstrom, et al., 2005), C3 and C4 complement levels were significantly and positively associated with each other (r's > .50; $p < .00001$) at each of the three examinations. C3 complement measured in 1992 was positively associated with white blood cell count ($r = .13$, $p < .03$) and BMI ($r = .28$, $p < .0001$), but negatively associated with alcohol consumption ($r = -0.10$, $p < .08$). C3 was not significantly associated with age, exercise history or cigarette use. C4 measured in 1992 was only positively associated with BMI ($r = .16$, $p < .006$).

3.1 The effects of time on C3 and C4 levels

C3 level showed significant increases over the 10-year period ($F(2,624) = 9.61$, $p < .0001$) (See Table 2). Comparisons using a 1-df contrast revealed that C3 levels significantly increased from 1992 to 1997 ($F(1,312) = 6.43$, $p < .02$) and from 1997 to 2002 ($F(1,312) = 4.28$, $p < .04$). C4 level showed significant decreases over the 10-year period ($F(2,624) = 295.03$, $p < .0001$). Contrasts revealed that C4 levels significantly decreased from 1992 to 1997 ($F(1,312) = 122.72$, $p < .0001$) and from 1997 to 2002 ($F(1,312) = 215.33$, $p < .0001$).

3.2 Psychological attributes and Changes in C3 and C4 levels

The time X PRF component score interaction was a significant predictor of C3 complement levels ($F(2, 596) = 7.38$, $p < .0003$) (Footnote 2). Post-hoc decomposition of this interaction revealed that PRF was significantly and positively associated with increases in C3 from 1992 to 2002 ($F(1, 298) = 13.16$, $p < .0004$) and from 1997 to 2002 ($F(1, 298) = 5.07$, $p < .03$). Although increases in C3 from 1992 to 1997 were similarly associated with PRF, this association did not reach significance ($F(1, 298) = 2.51$, $p = .10$). To illustrate this association, we plotted adjusted means for participants in the highest (n = 79) and lowest (n = 80) quartiles of PRF. As shown in Figure 1, individuals characterized by the highest levels of hostility, anger and depression showed greater C3 increases over the 10-year follow-up relative to those in the lowest quartile of the distribution of PRF score.

Neither the main effect for PRF $F(1,298) = .03$, *ns*) nor the time X PRF component score interaction $F(1,596) = 1.70$, *ns*) was a significant predictor of C4 levels.

3.2 Psychological attributes and change in C3 and C4 levels: The effects of dioxin exposure

As noted, the primary aim of the AFHS was to examine health consequences of dioxin exposure. In order to ascertain whether dioxin exposure had an effect on the above relations we refitted the models and included exposure group. Exposure group (n = 113) was not associated with either C3, F(1,297) = .00, *ns*) or C4, F(1,297) = .42, *ns*) levels. Furthermore, the exposure group X component score interaction was not a significant predictor of C3, F (1,296) = .00, *ns*) or C4, F(1,296) = .04, *ns*) complement levels. Finally, the exposure group X component score X time interaction was not a significant predictor of either C3, (F(1, 592) = .25, *ns*) or C4 complement levels, (F(1, 592) = 1.92, *ns*).

4. Discussion

Our longitudinal study of apparently healthy men showed that the shared variance among MMPI-derived measures of hostility, depression and anger significantly predicted increases in levels of C3 over a 10-year period and that these associations were independent of factors known to influence complement activation. Thus, men with low levels of these attributes showed relatively no change in C3 with the 10-year mean level remaining around 111 mg/dl. On the other hand, men with the highest levels of hostility, depressive symptoms and anger exhibited an average increase in C3 of 7.1% over the 10-year period with mean C3 levels rising to 122 mg/dl by the 10-year assessment. While no study has examined the clinical relevance of changes in C3 to disease, one prospective study showed that the highest prevalence of diabetes over an average follow-up period of 6.5 years was among men with C3 levels above 112 mg/dl (Engstrom, et al., 2005). In contrast, the same clustering of psychological attributes failed to predict changes or levels in C4 over time. These data add to the growing body of evidence that psychological attributes, in this case the combination of hostility, anger and depression, are associated with long term inflammatory processes that contribute to CHD, T2D and EH.

One possible mechanism that may account for the current observations is the association of these psychological factors to both stress-induced adrenergic hyperactivation and beta-adrenergic receptor down-regulation. Although sympathetic activation has been associated with reduced stimulated production of proinflammatory cytokines (Watkins, et al., 1999), this is not always the case. For example, evidence suggests that beta-adrenergic receptor density mediates the effects of catecholamines on cytokine production (Wahle, et al., 2005) with norepinephrine inducing cytokine production in mononuclear cells exhibiting lower beta-adrenergic receptor density. Those findings led us to hypothesize that stress-induced adrenergic activity, in the presence of down-regulated beta-receptors, leads to C3 production via activation of transcription factors and proinflammatory cytokines. Consistent with this hypothesis, excessive stress-induced adrenergic responses, and specifically stress-induced increases in norepinephrine, are associated with activation of nuclear factor (NF)-κ b (Bierhaus, et al., 2003), and increases in gene expression for IL-6 and TNF-α (Barnes & Karin, 1997). IL-6 and TNF-α have been associated with upregulated mRNA C3 (Colten & Strunk, 1993). Moreover, IL-6 also stimulates production of CRP, which has been shown to activate the classical complement pathway and stimulate production of C3 (Jarav, et al., 1999). Interestingly, IL-6 (and IL-1) does not appear to affect the biosynthesis of C4 (Falus, et al., 1990;Kulics, et al., 1990) that could explain the nonsignificant relation of psychological attributes to C4.

The above explanation is applicable to the current observations. For example, anger, hostility, and to a lesser extent depression, have been associated with stress-induced increases in norepinephrine (e.g., Light, et al., 1998;Suarez, et al., 1998b). We have also shown that hostile men and women exhibit down-regulation of $beta_2$-adrenoreceptors on circulating peripheral mononuclear cells (Suarez, et al., 1997) and reduced beta-adrenergic responsiveness to agonist infusion (Hughes, et al., 2003;Suarez, et al., 1998a). Evidence from our laboratory has shown

that hostility, anger and depression, alone or in combination, are associated with circulating levels of IL-6 (Suarez, 2003a), expression of tumor necrosis factor (TNF)-α on peripheral monocytes (Suarez, et al., 2002) and CRP (Suarez, 2004), inflammatory proteins that promote complement activation and C3 production. Thus, anger arousal in hostile depressed men may initiate a cascade of events, starting with excessive stress-induced adrenergic responses and beta-adrenergic receptor downregulation, that leads to activation of the NF-κβ complex and production of inflammatory cytokines that initiates the acute phase response (APR) and subsequent production of C3. Over time, chronic hostility, depression and anger may give rise to frequent episodes of emotional stress that initiate this cascade of biological events leading to greater and sustained long-term increases in C3.

Another possible pathway involves the action of insulin (Muscari, et al., 2000). Insulin and insulinemia are closely related to C3 (Weyer, et al., 2000). In addition, insulin resistance has also been linked with C3 (van Oostrom, et al., in press). Previous studies have suggested that hostility, anger and depression are associated with elevated fasting insulin and insulin resistance (Huerta, et al., 1995;Surwit, et al., 2002). We recently showed that arousal of negative emotions in men who exhibited greater insulin resistance was associated with increases in stimulated production of IL-6 and TNF-α (Suarez, et al., 2006), cytokines that have been linked with upregulation of C3 mRNA (Colten & Strunk, 1993). Among hostile men with frequent bouts of anger and depression, increases in C3 may reflect either the direct effects of insulin and insulin resistance on C3 or the moderating effect of insulin resistance on stress-induced increases in cytokines contributing to C3 production.

An important caveat of the current data is the possibility that the longitudinal relation of psychological attributes to C3 reflects subclinical disease. As noted, our exclusion criteria were implemented in order to decrease the likelihood for the confounding effects of clinical disease and medical treatments with anti-inflammatory effects. Nevertheless, given the mean age of the participants, it is likely that some participants developed subclinical atherosclerosis during the 10-year follow-up period. It is possible that the observed increases in C3 reflect upregulated expression of C3 in atherosclerotic lesions (Yasojima, et al., 2001). To minimize the influence of clinical disease on the observed associations, we controlled for onset of manifest CHD disease, as well as hypertension and diabetes, during the 10-year follow-up period. Because no measure of subclinical atherosclerosis was collected those statistical adjustments may have underestimated the role of atherosclerosis in the association of PRF to changes in C3.

The current study has limitations due to the lack of minorities and women. What effects gender and race have on these associations remain to be explored. In our previous studies, however, we failed to observe race- or gender-related differences in the relation of psychological attributes to CRP (Suarez, 2004). Nevertheless, it is recognized that C3 concentrations are determined by a number of factors including female sex hormones (Ritchie, et al., 2004). Thus, it is important that future studies examine whether the relation of complement to psychosocial attributes observed in the current study generalizes to women and minorities.

Although the composite score was associated with 10-year increases in C3, it should not be overlooked that psychological variables, measured in 1985 only, were not significantly associated with initial assessment of C3 concentrations in 1992. This null finding may be due to the exclusion of men with clinical chronic disease, including those believed to have an inflammatory basis (i.e., EH, T2D and CVD), up to and including the 1992 examination. Exclusion of those individuals may have restricted the range in C3 levels and resulted in the lack of an association between PRF and initial C3 levels. Consistent with this explanation, individuals who were excluded had significantly higher initial levels of C3 ($p < .0001$) and scored significantly higher on the component factor ($p < .0001$). When we include these

individuals in a re-analyses, results revealed the expected relation between the psychological component score and initial levels of C3 ($p < .002$).

Another possible explanation for the lack of an association between the psychological variables and initial levels of C3 assessed in 1992 is selective survival. By 1992, many of the participants were at an age where chronic diseases were fairly prevalent. As noted, when selecting the study sample, we removed those individuals with evidence of chronic diseases. The resulting sample may have been a relatively hardy subgroup that was less vulnerable to the effects of hostility, anger and depression. Thus, it may have taken longer for those psychological variables to exert a negative impact on health in this sample. This is consistent with the observation that effect sizes in studies of some psychological variables (e.g., hostility) and health have typically been larger in younger populations (Barefoot, et al., 1995). It should be noted that the proposed explanations are not mutually exclusive. Both restriction of range and selective survival may play a role in the null relations observed between psychological variables and levels of C3 measured in 1992.

5. Conclusion

The results of the current study fill a critical gap in the existing literature with respect to the longitudinal association between inflammatory biomarkers and psychological factors. In so doing, we showed positive associations between psychological attributes and 10-year changes in C3 among initially healthy middle-aged males. These data add to the growing body of evidence supporting the hypothesis that inflammation is an important mediator in the relation of psychosocial factors to chronic medical conditions such as CHD, Type 2 diabetes and essential hypertension.

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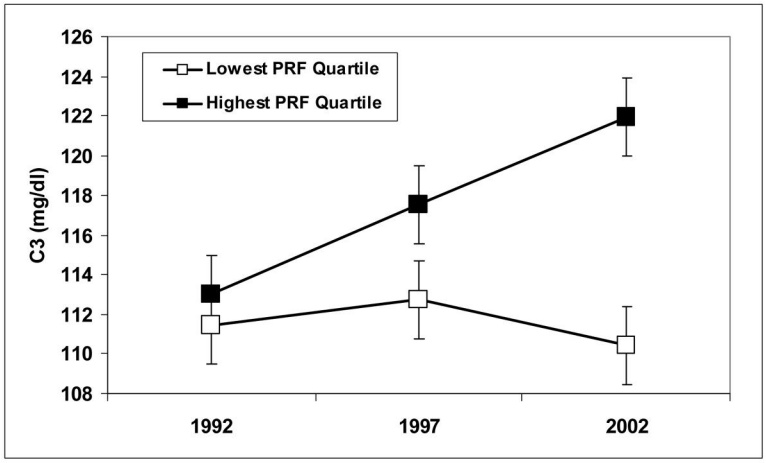

Figure 1.
Adjusted mean (SE) C3 levels for lowest and highest Psychological Risk Factor Quartiles at each examination.

Table 1

Characteristics of Study Participants (n = 313)

Variable	Percentile	Variable	Mean (SD)
Demographic		Demographic	
Ethnicity (% Caucasian)	93.9%	Age	50.2 (6.3)
Education – > High School	56.9%	Health Behaviors	
– High School	43.1%	Body mass index (BMI) (kg/m^2)	27.2 (3.2)
Married	87.5%	Alcohol (0–10 drinks/ day)	.59 (1.1)
Health Behaviors		Psychological Attributes	
Current Smoking (0–60 cigarettes/day)	22%	Anger	3.2 (2.2)
Exercise		Hostility	11.8 (6.4)
Sedentary	55.9%	Depression	5.7 (3.4)
Moderately Active	18.5%		
Active	25.6%		
Onset of Chronic Diseases after 1992			
CVD	3.5%		
Diabetes	5.8%		
Hypertension	23.0%		
Liver Disorder	22.7%		
Cancer	12.1%		
Medication Use after 1992			
Blood Pressure	1.9%		
Cardiovascular	11.8%		
Non-statin lipid lowering	1.6%		

Table 2

Means (SD) of C3 and C4 Levels at each Examination

Complement (mg/dL)	Exam Years 1992	1997	2002
C3	112.9 (16.7)	114.7 (17.4)	116.5 (20.7)
C4	27.8 (6.5)	25.5 (4.8)	22.5 (5.6)