CHRONIC STRESS, INFLAMMATION, AND PROGRESSION OF CAROTID ARTERY ATHEROSCLEROSIS: A MEDIATION MODEL

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Psychosocial stress might account for some of the variance in cardiovascular disease (CVD) risk that is not explained by traditional risk factors. Most studies of stress and CVD have focused on (a) stress in a single life domain, and (b) clinical CVD outcomes. Few studies have examined physiologic mechanisms that might explain the association between stress and CVD. The primary aim of the present study was to examine whether: (1) chronic stress predicts changes in carotid artery intima-media thickness (IMT) and plaque; and (2) the association between chronic stress and changes in these surrogate CVD endpoints is mediated by inflammatory processes. A secondary aim was to investigate whether individual differences in cardiovascular reactivity (CVR) moderates the association between stress and changes in IMT and plaque. The sample (n=276; M age=60.5), was a subset of the Pittsburgh Healthy Heart Project, a longitudinal investigation of the effects of psychosocial and biological risk factors on surrogate CVD endpoints among healthy older adults. Chronic stress was assessed at baseline with the Chronic Stress Scale (CSS; Norris & Uhl, 1993), a self-report survey that measures stress in 7 life domains during the preceding 6 months. Chronic stress was computed in terms of (a) scores on the 7 CSS subscales and (b) average score across all 7 subscales. Ultrasound IMT measures were taken at baseline and 3 years later. Mean IMT was derived by taking the bilateral average of far wall common, internal and bulb measures. IMT change was computed as the arithmetic difference between follow-up and baseline values. Plaque change was computed as the number of visible lesions at follow-up less the number of lesions at baseline. Blood draws for inflammatory markers and CVR testing were conducted at separate baseline visits. Results failed to support the mediation model. Only the CSS physical stress subscale was an independent predictor of IMT change (b=.02, t=2.13, p=.03). CSS scores were unrelated to plaque, or to inflammatory marker levels. Results did not differ according to CVR. Findings question the importance of chronic stress, as measured by global self-report, as a predictor of change in IMT and plaque.

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1.0 INTRODUCTION

1.1 OVERVIEW

Cardiovascular disease (CVD) is a long-term, progressive condition of multifactorial etiology. Much of the variance in prevalent CVD can be attributed to the presence of various biomedical risk factors, such as hypertension, hypercholesterolemia, and diabetes mellitus, in addition to behavioral factors such as smoking and lack of physical activity. The importance of modifying these traditional risk factors in the treatment and prevention of CVD is undisputed. However, there is evidence to suggest that traditional risk factors alone do not account for all CVD risk (Futterman & Lemberg, 1998; Hackam & Anand, 2003). Given that approximately 650,000 United States citizens per year are expected to experience a new acute coronary event, with 47% of those attacks being fatal (American Heart Association, 2003), even modest estimates of variance in CVD that cannot be explained by traditional risk factors represent a substantial population of persons whose early signs of disease are likely to be overlooked. From a public health standpoint, then, identification of novel CVD risk factors may improve the ability to detect preclinical CVD, and thus potentially lower clinical CVD burden at the level of the population.

It has been postulated that psychosocial factors, in particular the experience of psychosocial stress, might account for some of the unexplained variance in CVD morbidity (Krantz & McCeney, 2002; Rozanksi, Blumenthal, Davidson, Saab, & Kubzansky, 2005). Results from the INTERHEART study, for example, have shown that CVD risk associated with psychosocial factors, including perceived stress, is comparable to that associated with other more traditional risk factors such as hypertension, abdominal obesity, and smoking (Yusuf et al., 2004). It should be noted that the INTERHART study, like the majority of investigations that have reported prospective associations between stress and CVD risk, have focused on the prediction of clinical disease endpoints, such as myocardial infarction (MI) and cardiovascular

death. Taken at face value, then, one might conclude from the findings of extant research that exposure to chronic stress increases individuals' susceptibility to experiencing an acute event. In other words, psychosocial stress might influence CVD risk by facilitating the transition form asymptomatic subclinical pathology to symptomatic clinical disease.

That chronic stress appears to play a role in the transition to clinically manifest disease does not exclude the possibility that stress influences CVD risk at earlier stages in the disease process as well. For example, exposure to chronic stress might be associated with (1) the initiation of biologic processes that are fundamental to initial atherosclerotic lesion development, or (2) the progression of fatty streaks to more advanced plaques. The following discussion will focus on the possible role of psychosocial stress in these early disease processes, particularly those aspects of early CVD pathology that can be represented by changes in intima-media thickness and development of atherosclerotic plaque.

Chronic stress exposure may have direct effects on disease progression by exhausting biological resources or by activating a continually aroused state. Alternatively, exposure to chronic stress also may have indirect effects on health via failed coping efforts or deteriorated health behaviors (Lepore, 1995). The role of behavioral factors, such as poor diet and participation in high-risk activities (e.g., smoking), is considerable. However, associations between psychosocial stress and CVD risk frequently persist independent of the effects of such behavioral influences (Manuck, Marsland, Kaplan, & Williams, 1995). Thus, it is of interest to identify plausible physiologic mechanisms that might provide a direct pathway by which psychosocial stress could influence CVD pathogenesis. One proposed mechanism involves activities of immune and inflammatory processes.

The following pages provide a rationale for investigating inflammation as a physiologic mechanism by which the experience of psychosocial stress might be translated into cardiovascular pathology. The discussion begins with an overview of existing human and comparative research that has observed an association between exposure to chronic psychosocial stress and development of CVD-relevant outcomes. The discussion then will proceed to review existing literature supporting the proposition that chronic stress may contribute to CVD pathogenesis via a mechanism involving underlying inflammatory activity. Finally, the paper will conclude with the proposal of an empirical investigation that has been designed to examine the following research questions among a sample of healthy, older adults: (1) Is chronic stress

associated with pathophysiologic changes relevant to early CVD progression; and (2) Is that association mediated by ongoing low-grade inflammatory activity? Prior to beginning this discussion, the pages to follow will examine the question of how best to define and measure the psychosocial construct, "stress."

1.2 CHRONIC STRESS AND CVD

A fundamental challenge of stress and health research is the conceptualization and measurement of the independent variable. Since Selye's introduction of the term in the early part of the previous century, stress has been defined variously. Central to most definitions is the notion of disrupted homeostasis as a function of poor fit between the individual and his or her environment (Menaghan, 1983). Said differently, stress arises when demands imposed by the environment cannot be accommodated by the coping efforts of the individual (Lazarus & Folkman, 1984). "Coping," for the present purposes, refers to deliberate behaviors that are enacted by the individual to reduce the threat associated with a given environmental stimulus (stressor). Associated with coping behaviors are automatic biological responses that compensate for stress-related perturbations of the internal physiologic milieu. An important caveat to the above definition of coping is that it is predicated upon the assumption that the individual consciously recognizes a given environmental stimulus as stressful. Thus, stress might be construed as the outcome of 3 co-occurring processes: (1) stressor exposure; (2) stressor appraisal; and (3) response to the stressor (Lazarus & Folkman, 1984). Stress so defined may influence physical health outcomes via either of the following pathways: insufficient responding and inappropriate responding. In the former case, an inadequate response to toxic environmental stimuli increases the individual's vulnerability to damaging effects associated with the stressor. For example, an impaired immune response to invasion by an infectious pathogen increases susceptibility to bodily infection. Alternatively, an inappropriate response to environmental stimuli that over-compensates for associated effects also may contribute to the generation of a diseased state. For instance, a protracted inflammatory response to infection or injury may result in damage to surrounding tissue. Likewise, exaggerated heart rate response to fearful stimuli may contribute

to the creation of pathogenic conditions at the artery wall, thus predisposing susceptible endothelium to early atherosclerotic processes (Markovitz & Matthews, 1991).

Given the different routes by which stress has been hypothesized to influence organic functioning and ultimately health outcomes, it is unsurprising that agreement on a standardized operationalization of the stress construct has proven difficult for researchers of stress and disease. One point of contention concerns the importance of the appraisal process: Is it necessary for an individual to perceive an environmental stimulus as stressful in order for physiologic responses (with the potential for pathophysiologic sequelae) to take place? Another area of disagreement focuses on whether the effects of chronic stressors may be summated across life domains, or whether specific chronic stressors should be considered separately, thus allowing for examination of interactive effects. The following section will discuss the strengths and weaknesses of several operationalizations of stress that have been examined as predictors of CVD-relevant outcomes among comparative animal and human populations.

1.2.1 Comparative research

One prominent paradigm that has been used to approximate stress among nonhuman animal species is social reorganization. Briefly, this stressor involves rotation of experimental animals through unstable social groupings that may or may not include exposure to a dominant or aggressive conspecific. Given the communal nature of several animal species that are used in the laboratory (e.g., rodents, nonhuman primates) it is assumed that social reorganization constitutes an aversive environmental challenge that likely would elicit a physiologic stress response. This assumption is supported by findings of elevated beta-adrenergic activity among laboratory animals that have been exposed to unstable social conditions (Henry, Meehan, & Stephens, 1967).

The relevance of social reorganization to coronary disease has been demonstrated by a series of studies that were conducted among cynomolgus macaques (J. R. Kaplan, Adams, Clarkson, & Koritnik, 1984; J. R. Kaplan, Manuck, Clarkson, Lusso, & Taub, 1982; J. R. Kaplan et al., 1983). Among male macaques that had been fed a moderately atherogenic diet (i.e., comparable to typical Western diet), assignment to an unstable social condition was unrelated to atherogenesis (Kaplan et al, 1982). However, a significant stress-by-social status interaction

revealed that dominant macaques that were assigned to the unstable condition developed the most advanced atherosclerosis when measured in terms of intima-media thickness and lumen diameter (Kaplan et al, 1982). These findings were independent of the effects of serum cholesterol. However, to rule out the potential influence of the hyperlipidemic diet on atherogenesis, the authors replicated their study among a sample of male macaques that had been fed a "prudent" diet. Results of this study indicated that macaques that were assigned to the unstable condition developed more extensive coronary atherosclerosis than macaques that were assigned to the stable condition (Kaplan et al, 1983)[1]. In the third study, Kaplan and colleagues attempted to extend their findings among male macaques to females of the species. Among female macaques that had been fed an atherogenic diet, there was a main effect for social status such that dominant females developed fewer raised coronary lesions than did their subordinate counterparts regardless of social condition (Kaplan et al, 1984).

A major strength of the above nonhuman primate research is the choice of the cynomolgus macaque as a comparative model. Macaques develop intimal, lipid-laden lesions that are similar in anatomic distribution to, and share pathologic characteristics with atherosclerotic lesions observed in humans. Moreover, the social organization of macaques promotes the exhibition of aggressive and competitive behaviors that are reminiscent of anger and hostility, traits that have been associated with CVD in human research (Kaplan et al, 1985).

Two important inferences might be drawn from the above comparative research. First of all, the findings among macaques provide some indication of when during the disease process psychosocial stress might influence CVD. That social reorganization stress anticipates coronary atherogenesis among nonhuman primates suggests that chronic psychosocial stressors have the capacity to influence CVD at early stages of disease progression. A second conclusion that might be derived from this series of studies concerns the nature of chronic stressors that might influence CVD-relevant outcomes. Though procedurally novel, social reorganization in the laboratory likely approximates a type of psychosocial stressor that nonhuman primates typically encounter as a part of their daily existence. Thus, the above findings suggest that a stressor need be neither exceptional nor traumatic to contribute to CVD pathogenesis. Extrapolating these associations to human populations, a reasonable hypothesis is that ongoing exposure to ordinary

[1] The interaction with social status was not tested as the behaviors of this group of monkeys could not reliably be identified as dominant or subordinate.

stressors, or the "persistent hardships experienced by those engaged in mainstream activities within major institutions" (Pearlin & Schooler, 1978), may be sufficient to place individuals at risk for future CVD.

1.2.2 Human research

Three sources of ordinary, daily life stress frequently have been examined as correlates of health outcomes in humans: work, family, and socioeconomic status (SES). Given the centrality of work and family roles to individuals' daily lives (Pearlin, 1983), stress encountered in either of these 2 domains may be of particular relevance to the development of CVD. Because of the pervasive influence of SES across several life domains, stress associated with socioeconomic disadvantage similarly may contribute importantly to CVD risk. In contrast to the experimental work among nonhuman primates, the correlational nature of research on chronic stress and CVD among humans precludes the drawing of causal inferences. However, findings from a number of prospective studies suggest that chronic stress derived from occupational, family and socioeconomic sources is a reliable antecedent of clinical cardiovascular pathology.

1.2.2.1 Occupational stress

A prominent model of occupational stress that frequently has been investigated as a risk factor for CVD is the "job strain" construct proposed by Karasek (R.A. Karasek, 1979). This model describes the condition of job strain as resulting from an imbalance of high demand and low decision latitude (control) in the workplace (R.A. Karasek, 1979). In general, 2 methods have been used to assess job strain: self-reports and inference based on job title. Self-reports, though designed to assess objective stressors associated with the work environment (e.g., Does your job require you to work hard?), also capture indirectly the stressor appraisal process. Positive endorsement of a given workplace stressor may be influenced both by objective observation of the associated environmental stimulus as well as by individuals' perceptions of that stimulus as stressful, particularly if respondents are asked to rate the duration, intensity, or frequency of stressor exposure. By comparison, inference-based measures of occupational stress circumvent the influence of individual stressor appraisal by assigning scores on various job characteristics to individuals based on their job titles. Because assigned scores have been derived from pooled

survey data, it reasonably can be assumed that the majority of persons who are employed in a given occupation would perceive a similar level of job strain. A major limitation of inference-based methods, however, concerns intra-occupational variability in job strain (Schwartz, 2000). For example, the manager of a small, independent grocery store may be exposed to different levels of occupational stress relative to a regional manager of a Fortune 500 company, even though both individuals carry the same job title.

Both self-report and inference-based methods have been incorporated into research on job strain and CVD. A recent review of the literature on workplace environment and CVD risk indicated that self-report measures appear to be more reliable predictors of hard CVD endpoints than inference-based measures (Belkic et al., 2000). Among 10 prospective studies reviewed by Belkic, 6 reported positive associations between low workplace control, or the combination of low control with high demand (job strain) and risk for clinical CVD. All 6 positive studies employed self-report measures of job strain. By comparison, 3 of the 4 studies that reported negligible associations between job strain or its components and risk for CVD employed inference-based methods (Belkic et al., 2000).

It is important to note that Belkic's review was limited to studies that included only male participants. A comparatively smaller literature has examined the effects of job strain on risk for CVD among women. Results from these studies largely have been mixed. An earlier review of job strain and CVD research included 3 studies that examined both men and women (Schnall et al., 1994). Two of these investigations reported no sex difference in the risk of clinical CVD endpoints associated with high job strain, whereas the third study reported a stronger association among women[2] (Schnall et al., 1994). More recently, it has been shown that occupational stress may not be as important a predictor of CVD outcomes among women relative to men. For example, findings from the Whitehall II cohort indicated that self-reports of neither job strain nor workplace control were important predictors of CVD among women (Kuper & Marmot, 2003). Similarly, Lee and colleagues reported no effect of job conditions on CVD risk among female participants in the Nurses' Health Study (Lee, Colditz, Berkman, & Kawachi, 2002).

In addition to the research that has documented a prospective association between occupational stress and risk for clinical CVD outcomes, there is some evidence to suggest that

[2] It should be noted that the finding of a stronger effect of job strain among women relative to men was reported in an unpublished doctoral dissertation.

stress in the workplace may be related to progression of subclinical CVD as well. In one of 2 known studies to examine occupational stress as a predictor of change in markers of subclinical CVD, Lynch and colleagues found that men who reported a combination of high workplace demands and low income showed greater progression of carotid atherosclerosis relative to men who reported low demands and high income (J. Lynch, Krause, Kaplan, Salonen, & Salonen, 1997). Additionally, Everson and colleagues found that the association between work stress and carotid artery atherosclerosis progression in this sample was especially marked among men who showed high cardiovascular reactivity in anticipation of an exercise task (Everson et al., 1997).

Summary. Though not entirely consistent, findings from research on job strain and CVD suggest that certain features of the work environment may predispose individuals to risk for clinical CVD outcomes. Specific conclusions based on this literature include the following: (1) Self-report measures appear to be more reliable correlates of CVD outcomes than inference-based measures; (2) men and women may differ on the extent to which occupational stress, particularly the low-control dimension of job strain, predicts CVD; and (3) occupational stress may influence CVD at early stages in the disease process.

1.2.2.2 Family stress

Within the family domain, chronic psychosocial stress may arise from a number of sources. One potential source of chronic stress is the marital relationship. Because of the enduring nature of this social tie and the centrality of the spouse role to many adults' lives, chronic stress within the context of marriage may be of particular relevance to CVD risk. A second important source of chronic stress within the family domain is that which arises as a function of taking on informal caregiving responsibilities for a chronically ill spouse or relative. Results from 2 recent meta-analyses suggest that caregiver status may be related to higher levels of self-reported stress and depression, and a slightly greater risk of physical morbidity (Pinquart & Sorensen, 2003; Vitaliano, Zhang, & Scanlan, 2003). Thus, caregiving may be an additional source of family stress that could influence CVD pathogenesis.

Marital stress. The extant literature on marriage and health is replete with research on marital status and risk for CVD morbidity and mortality, with the married having a protective advantage over the unmarried (Kiecolt-Glaser & Newton, 2001). By comparison, only 3 known studies have investigated a prospective relation between marital stress and CVD outcomes

(Coyne et al., 2001; Gallo et al., 2003; Orth-Gomer et al., 2000). In the first 2 studies, high marital stress at admission to hospital predicted a greater risk of recurrent CVD events or mortality among persons with pre-existing disease (Coyne et al., 2001; Orth-Gomer et al., 2000). Orth-Gomer and colleagues followed a sample of 187 married or cohabiting women for 5 years following hospitalization for an acute myocardial infarction. Marital stress was measured with the Stockholm Marital Stress Scale (SMSS), a structured interview that was developed by the authors. After controlling for age, baseline CVD severity, use of estrogen, education, and traditional biomedical risk factors, women who reported marital stress at baseline were at greater risk of experiencing a recurrent event (recurrent MI, CVD mortality, or revascularization procedure) relative to women who reported low or no marital stress at baseline (Orth-Gomer et al., 2000).

Coyne and colleagues investigated whether baseline marital stress predicted 4-year survival among 189 patients (21% female, 17% non-white) who had been hospitalized for congestive heart failure. Marital stress was assessed with a composite measure that was comprised of responses to individual patient and spouse interviews and an observer-coded rating of positive behaviors during a videotaped marital problem discussion. Analyses that adjusted for gender and baseline disease severity revealed that more marital stress predicted shorter survival time. Although men and women differed on a number of baseline characteristics, with women showing worse functional impairment, a shorter time since diagnosis, and reporting less marital stress, gender was not a significant predictor of survival. Gender did, however, moderate the effect of marital stress on survival such that the association was stronger among women relative to men (Coyne et al., 2001).

In the third study, Gallo and colleagues examined changes in carotid artery atherosclerosis among healthy, middle-aged women who could be classified into one of the following 3 groups: satisfied with marriage; dissatisfied with marriage; or unmarried. Marital satisfaction was assessed with a 7-item self-report measure that was administered at baseline and 3 years later. Results indicated that women who were satisfied with their marriages at baseline showed the least carotid atherosclerosis progression during the 3 years between the 11- and 14-year follow-up assessments, whereas women who were not satisfied with their marriages showed the greatest progression (Gallo et al., 2003).

Caregiver stress. Like the literature on marital stress and CVD, extant research on caregiver stress and CVD-relevant outcomes is sparse. Despite the research to suggest that caregivers have worse health compared to noncaregivers (Vitaliano et al., 2003), only one published prospective study has examined whether caregivers have a higher relative risk for developing CVD, specifically. Lee and colleagues examined whether being a caregiver for a disabled or ill spouse, parent, or other relative is associated with an elevated risk of incident nonfatal or fatal CVD (MI) among participants in the Nurses' Health Study (Lee, Colditz, Berkman, & Kawachi, 2003). Results indicated that women who reported more than 9 hours per week caring for an ill spouse showed an elevated risk of incident CVD relative to non-caregivers; perceived strain associated with caregiving did not moderate these results. Interestingly, CVD risk among women who provided care for ill parents or other relatives did not differ from that of women with no caregiving responsibilities (Lee et al., 2003).

The findings reported by Lee and colleagues suggest that being the primary informal caregiver for an ill or disabled spouse may be associated with elevated risk of incident CVD among previously healthy middle-aged women. That the risk associated with caregiver status was independent of perceived caregiver strain stands in contrast to findings from an earlier study that examined caregiver stress as a predictor of all-cause mortality (Schulz & Beach, 1999). Schulz and Beach found that only caregivers who reported experiencing strain associated with providing care were at higher risk of mortality relative to non-caregivers; caregivers who reported no strain did not differ from non-caregivers in mortality risk. A number of factors might have contributed to the discrepant findings between these 2 studies. Schulz and Beach studied male and female caregivers, whereas the sample employed by Lee and colleagues was comprised entirely of women. Men are less likely than women to act as sole or primary caregivers, and more likely than women to receive respite from other informal caregivers or from formal care providing services (R. Stone, Cafferata, & Sangl, 1987). Thus, the inherent stressfulness of being a caregiver may be reduced for men relative to women. In this way, caregiver status may represent a different type of stressor for men than it does for women. That the sample employed by Schulz and Beach was not large enough to examine associations by cause of death also might explain differences between the 2 studies: patterns of relations between caregiver stress and mortality may differ between all-cause and CVD death. This explanation is unlikely, however, given that CVD is a leading cause of premature mortality

(American Heart Association, 2003). Finally, whereas Lee and colleagues controlled for CVD risk factors in their analyses, Schulz and Beach did not make these adjustments.

Summary. Findings from the above studies suggest that stressors encountered within the family domain may be associated with an elevated risk for cardiovascular morbidity and mortality. Perceptions of marital stress appear to predict worse outcomes among persons with pre-existing CVD, and may influence early atherogenesis among women without history of clinical cardiovascular pathology. Stress associated with being an informal family caregiver also may influence risk for future CVD. However, it is unclear whether CVD risk associated with caregiving depends upon level of perceived caregiver burden. The pattern of findings to date suggests that stress in the family domain may be a more important contributor to risk for CVD among women relative to men. However, given the paucity of studies that have conducted gender comparisons, it has yet to be determined whether this apparent trend is indicative of a true sex difference.

1.2.2.3 Low socioeconomic status (SES)

In addition to stressors that are encountered within the work and family domains, low socioeconomic status (SES) might be conceived of as another type of pervasive psychosocial stressor with the capacity to influence risk for developing CVD. There is a recognized gradient of CVD risk associated with SES such that the probability of CVD morbidity and mortality increases continuously with decreasing status (G. A. Kaplan & Keil, 1993; Pickering, 1999). In addition to the elevated risk for clinical CVD associated with low SES, there also is evidence to suggest that socioeconomic disadvantage may predict progression of subclinical atherosclerosis (J. Lynch, Kaplan, Salonen, & Salonen, 1997). Lynch and colleagues examined whether (a) level of education and (b) income each were associated with 4-year progression of carotid artery atherosclerosis among male participants in the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD). Results from analyses that were adjusted for age and baseline atherosclerosis indicated a significant inverse association between both SES indices and carotid atherosclerosis progression (J. Lynch, Kaplan et al., 1997).

A number of factors may influence the CVD risk associated with low SES both at the level of the individual (access to health care, poor health behaviors) and at the level of the community (air and water quality, crowding) (Evans & Kantrowitz, 2002). However, these

"physical" factors account for only some of the observed gradient of CVD risk associated with socioeconomic disadvantage. Thus, it is possible that psychological factors, such as perceived psychosocial stressors associated with low SES, also may play an important role in determining CVD risk (Pickering, 1999).

1.2.3 Limitations of previous research on chronic stress and CVD

1.2.3.1 Outcome measures

As previously discussed, much of the existing prospective research on chronic stress and CVD in human populations has focused on clinical endpoints, such as incident or recurrent MI, or cardiovascular mortality. Notable exceptions are the 3 studies that employed progression of carotid artery atherosclerosis as the outcome measure (Gallo et al., 2003; J. Lynch, Kaplan et al., 1997; J. Lynch, Krause et al., 1997). Findings from studies that employ "hard" CVD endpoints are compelling in that they provide suggestive evidence that the experience of psychosocial stress might influence individuals' risk of future clinical CVD. However, these studies provide no information regarding the stage of disease development at which exposure to psychosocial stress is likely to influence cardiovascular pathogenesis. When interest is in examining the role of psychosocial stress in earlier stages of the disease process, it may be of benefit to examine surrogate endpoints that reflect developing subclinical pathogenesis rather than manifest clinical disease.

1.2.3.2 Characteristics of sample populations

A second notable limitation of the above reviewed research on psychosocial stress and CVD concerns characteristics of the studied populations. For example, much of the occupational stress research has been conducted on male samples, thus limiting the extent to which associated findings might be generalized to women. By comparison, 2 of 3 marital quality studies (Gallo et al., 2003; Orth-Gomer et al., 2000) employed entirely female samples. Given the moderating effect of gender reported by Coyne and colleagues (Coyne et al., 2001), it seems it would benefit future investigations to include both male and female participants. Interpretation of the marital quality literature also is complicated by the fact that Orth-Gomer and Coyne (Coyne et al., 2001; Orth-Gomer et al., 2000) each employed samples with pre-existing disease. Although both

studies controlled for CVD severity, the possibility that some feature of the disease experience was influencing participants' reports of marital quality cannot be excluded. Thus, the proposed direction of causality is called into question.

1.2.3.3 Lack of emphasis on explanatory mechanisms

Another limitation of the extant research on psychosocial stress and CVD is the lack of emphasis on identifying plausible physiologic mechanisms by which psychosocial stress might be translated into observable cardiovascular pathology. One notable exception is the Gallo study (Gallo et al., 2003) wherein findings showed that pulse pressure and serum triglyceride levels were partial mediators of the association between marital satisfaction and atherosclerotic progression. Despite the growing speculation that inflammatory processes may mediate the association between chronic stress and CVD (Black & Garbutt, 2002), however, no known studies have examined this specific explanatory mechanism.

1.2.4 Summary

Psychosocial stress frequently has been observed to correlate with risk for clinical CVD outcomes. Chronic stress associated with work, family, and socioeconomic domains may be especially relevant to CVD risk. That chronic or repeated exposure to psychosocial stress might be related causally to the initiation and progression of CVD has been suggested by findings from comparative research. It is difficult to draw causal inferences based on the human literature, however, due to the employ of correlational designs. Additional limitations of the above-reviewed human stress research with regard to understanding the role of stress in early cardiovascular pathogenesis include the following: (a) a predominant focus on clinical CVD end points; (b) lack of generalizability due to characteristics of the employed sample populations; and (c) the lack of emphases on physiological mechanisms.

1.3 PROPOSED MECHANISMS

The present study was designed to address some of the acknowledged limitations of the extant literature on chronic stress and CVD in human samples. Specifically, the present study examines the role of inflammatory processes in stress-related subclinical CVD pathogenesis among persons without known history of CVD. Before presenting the employed methodology, the following pages will examine relevant literatures that make the case for investigating inflammation as a mediator in the stress-CVD association. The discussion will begin with a brief overview of the evidence implicating activation of the sympathetic nervous system (SNS) in the process of early cardiovascular pathogenesis. Attention is given to this literature because SNS activity is one proximal physiologic mechanism by which psychosocial stress may be linked to more distal inflammatory processes.

1.3.1 Sympathetic nervous system activation and CVD

Hemodynamic and neuroendocrine changes associated with chronic SNS activation have been shown to initiate pathophysiologic processes relevant to CVD risk (Manuck, Kaplan, Muldoon, Adams, & Clarkson, 1991; Manuck et al., 1995). The most compelling evidence for a mediating role of SNS activation in the development of CVD has been derived from research conducted among nonhuman primates (Kaplan et al., 1987; 1989). As described above, Kaplan and colleagues found that dominant male cynomolgus macaques that were exposed to an unstable social environment showed more severe coronary atherosclerosis than macaques not exposed to this chronic stressor. Involvement of SNS activation as a mediator of the relation between stress and vascular pathology was suggested by the additional finding that atherogenesis in macaques that were placed in unstable social settings was eliminated by administration of a beta-adrenoreceptor antagonist (J. R. Kaplan & Manuck, 1989; J. R. Kaplan, Manuck, Adams, Weingand, & Clarkson, 1987). These findings are corroborated by results from 3 prominent non-primate models that examined the association between chronic exposure to laboratory stressors and development of hypertension, an important risk factor for CVD (Henry et al., 1967; Julius, Brant, Krause, & Buda, 1989; Lawler, Cox, Sanders, & Mitchell, 1988). In all 3 of these

studies, SNS activation associated with repeated exposure to a psychological stressor predicted development of sustained hypertension.

1.3.2 Inflammation as a mediator between SNS activation and CVD

The above comparative studies support an association between stressor-induced elevations in SNS activity and CVD-relevant outcomes. Various mechanisms have been proposed to explain how SNS activation might be translated into cardiovascular pathology. These include direct effects of catecholamines on vascular smooth muscle synthesis (deBlois et al., 1996), and indirect effects via changes in hemoconcentration (Allen & Patterson, 1995), hemostasis (von Kanel, Mills, Fainman, & Dimsdale, 2001), platelet activation (Markovitz & Matthews, 1991), and blood pressure (Treiber et al., 2003). Another proposed mechanism involves activation of immune and inflammatory processes (Black & Garbutt, 2002). Given that atherosclerosis increasingly has come to be understood as a chronic inflammatory condition (Ross, 1993), it is important to examine whether psychosocial stress plays a role in the initiation of inflammatory activities that ultimately contribute to atherogenesis. As the major focus of the present study is to examine inflammation as a mediator in the path linking stress and CVD, the evidence for this mechanism will be discussed in detail.

1.3.2.1 CVD as a chronic inflammatory condition

Ross proposed a "response-to-injury" theory of atherogenesis that involves ongoing interaction between "activated" endothelium and circulating immune, inflammatory, and haemostatic factors (Ross, 1993). Proatherogenic changes characteristic of the early stages of this process include (1) increased permeability of the endothelium; (2) upregulation of leukocyte adhesion molecules; and (3) migration of leukocytes into the artery wall (Ross, 1999). Monocytes and monocyte-derived macrophages are present in the endothelium at all stages of atherogenesis, as well as helper and cytotoxic T-lymphocytes. Once activated, these cells release cytokines, proteolytic enzymes, and growth factors. In addition to facilitating the recruitment of additional inflammatory cells, these soluble mediators may contribute directly to further endothelial damage (Ross, 1999). Thus, it has been proposed that low-grade elevations in circulating levels of these mediators that result from systemic infection or inflammation may contribute to further

endothelial damage and/or exacerbation of the ongoing local inflammatory response at the artery wall (J. Danesh et al., 1999).

Converging evidence from histopathologic, epidemiologic, and experimental research has suggested that 2 specific markers of inflammation, interleukin-6 (IL-6) and C-reactive protein (CRP) may factor importantly in the development of CVD. IL-6 is a pleiotropic cytokine with a broad range of humoral and cellular immune effects associated with inflammation, host defense, and tissue injury. IL-6, like IL-1 and tumor necrosis factor-alpha (TNF-α), has been identified as a major pro-inflammatory cytokine, and is produced by endothelial, smooth muscle, and adipose cells, as well as by macrophages (Rabin, 1999). IL-6 also plays an essential role in the initiation of the acute phase response (APR), a systemic reaction to bodily infection or injury, that is comprised of a number of physiological and behavioral adjustments including fever, leukocytosis, hypersomnia, hyperalgesia, anorexia, and depression (Baumann & Gauldie, 1994). CRP is a positive acute phase protein that is synthesized and released by the liver during the APR. Though only trace amounts of CRP are detectable in the circulations of healthy adults (< 1.0 mg/L), CRP concentrations may increase up to 10,000-fold during an acute infection (Hirschfield & Pepys, 2003). Hepatic release of CRP is thought to depend primarily on stimulation by circulating IL-6, and the sole determinant of CRP concentration in the circulation is its own rate of synthesis. Accordingly, mild but persistent elevations in CRP concentration have been used as an indirect marker of IL-6 activity, and thus of underlying pathogenic inflammatory processes (Whicher, Rifai, & Biasucci, 2001).

Histopathologic identification of IL-6 and CRP in atherosclerotic lesions. Several studies that have examined human and animal atheroma specimens for the presence of IL-6 and CRP have demonstrated that both of these markers are detectible in greater quantities in atherosclerotic compared to healthy vessels. A full review of this literature is beyond the scope of the present paper. However, findings from a few selected studies that were conducted with human autopsy or surgical specimens are discussed in detail here.

Seino and colleagues (Seino et al., 1994) compared expression of IL-6 gene transcripts in atherosclerotic and non-atherosclerotic artery sections that were obtained from patients undergoing surgical revascularization. Results showed that IL-6 mRNA expression was significantly greater in atherosclerotic relative to healthy arteries, specifically in the intimal layer of atherosclerotic lesions (Seino et al., 1994). Rus and colleagues (Rus, Vlaicu, & Niculescu,

1996) detected IL-6-specific deposits in the intima of both diseased and healthy aortic, iliac, and femoral artery samples. However, the number of cells expressing IL-6 mRNA was higher in areas of increased intimal thickening and in fibrous plaques relative to normal intima and media. Moreover, compared to healthy intima, atherosclerotic intima was characterized by larger deposits of IL-6 in the extra-cellular connective tissue matrix (Rus et al., 1996).

Studies that have examined levels of CRP in human coronary artery specimens provide further evidence for an association between inflammatory markers and atherosclerosis. Zhang and colleagues (Zhang, Cliff, Schoefl, & Higgins, 1999) used indirect immunofluorescence to examine coronary arteries that were resected at autopsy. Results revealed that grades of CRP immunoreactivity correlated positively with intimal thickness and negatively with lumen diameter (Zhang et al., 1999). Torzewski and colleagues (Torzewski et al., 2000) compared CRP levels in sequential sections of diseased coronary artery specimens. CRP was detectible in artery sections with evidence of atherosclerotic lesion development, but not in lesion-free sections. Moreover, CRP was apparent in lesions that were morphologically less-advanced, whereas macrophages were detectible only in comparatively more advanced lesions. In other words, presence of CRP preceded that of macrophage accumulation (Torzewski et al., 2000). Findings from this latter study suggest that CRP may be involved in the migration of macrophages to the endothelium, a fundamental step in early atherogenesis.

Epidemiologic studies of inflammatory markers and CVD risk. An impressive collection of epidemiologic studies have provided evidence for both cross-sectional and prospective associations between (a) IL-6 and (b) CRP and clinical cardiovascular endpoints. The majority of prospective studies have been conducted among healthy community populations without known history of pre-existing CVD. Findings from these studies are summarized below.

To date, 8 prospective studies have examined whether circulating IL-6 levels predict long-term CVD outcomes among previously healthy adults (Cesari et al., 2003; Harris, Ferrucci, Tracy et al., 1999; Jenny et al., 2002; Luc et al., 2003; Pai et al., 2004; Pradhan et al., 2002; Ridker, Hennekens, Buring, & Rifai, 2000; Ridker, Rifai, Stampfer, & Hennekens, 2000). All of these studies reported an increasing crude risk of incident fatal or nonfatal CVD with increasing levels of IL-6. With the exception of 2 studies (Pai et al., 2004; Ridker, Hennekens et al., 2000), CVD risk associated with IL-6 remained significant even after controlling for the effects of demographic variables and conventional biological and behavioral risk factors. It should be

noted that Ridker and colleagues examined a model that incorporated multiple inflammatory markers in addition to IL-6 (CRP, serum amyloid A, soluble intercellular adhesion molecule-1, homocysteine). Inclusion of these factors, specifically, accounted for the attenuation to nonsignificance of the risk associated with IL-6 (Ridker, Hennekens et al., 2000).

CRP has gained considerable attention as a potential non-traditional risk factor for CVD as well. Danesh and colleagues recently conducted a meta-analysis of 22 prospective studies that examined the association between circulating CRP and risk of incident CVD (J. Danesh et al., 2004). Samples largely were drawn from the general population of healthy, middle-aged and older adults. Data analyses from individual studies included controls for demographic variables and established CVD risk factors. Across all 22 studies, a comparison of CVD cases in the top versus the bottom third of CRP concentrations resulted in a combined odds ratio of 1.58 (CI = 1.48, 1.68). This calculated risk was somewhat smaller than that which was reported in an earlier meta-analysis that included only the 11 studies that were published before 2000 (OR = 1.9) (J. Danesh, Whincup, & Walker, 2000). The authors attributed the trend toward more extreme findings in the earlier studies to positive publication bias. Nine additional prospective studies of CRP and incident CVD were published between January 2003 and December 2004 (Ballantyne et al., 2004; Cesari et al., 2003; Koenig, Lowel, Baumert, & Meisinger, 2004; Luc et al., 2003; Pai et al., 2004; Ridker & Cook, 2004; Rutter, Meigs, Sullivan, D'Agostino, & Wilson, 2004; St-Pierre et al., 2003; van der Meer et al., 2003). Consistent with the findings reviewed by Danesh (2004), results from all 9 studies indicated a higher relative risk of CVD at higher CRP concentrations.

Experimental support for a causal role for IL-6 and CRP in atherogenesis. The epidemiologic research described above suggests that the appearance of low-grade inflammatory activity, as indicated by modest elevations in basal IL-6 and CRP, antedates clinically manifest cardiovascular pathology. However, the correlational nature of epidemiologic studies precludes the ability to draw causal inferences from these data. Compelling evidence in support of a direct causal role for IL-6 and CRP in CVD pathogenesis may be derived from experimental research findings.

Comparative research in vivo and in vitro has indicated that IL-6 may have proatherogenic effects on aortic endothelium. For example, addition of murine recombinant IL-6 in vitro to isolated rat aortic vascular smooth muscle cells (VSMCs) has been associated with

accelerated cell growth (Ikeda et al., 1991). More recently, daily treatment of atherosclerosis-prone mice with recombinant IL-6 in vivo has been associated with increased lesion size relative to non-treated mice (Huber, Sakkinen, Conze, Hardin, & Tracy, 1999). Finally, in vitro exposure of mouse aortic VSMCs to recombinant murine IL-6 has been associated with increased expression of angiotensin-II type 1 receptors with subsequent enhanced production of reactive oxygen species (ROS) (Wassmann et al., 2004). Intra- and extracellular production of ROS has been found to trigger and exacerbate endothelial dysfunction, which has important implications for atherogenesis (Griendling, Sorescu, Lassengue, & Ushio-Fukai, 2000).

Findings from experimental research have suggested that CRP, as well, may be more than a passive marker of inflammatory atherogenesis. For example, atherosclerosis-prone apolipoprotein-E knockout mice that were transgenic for human CRP developed larger aortic lesions relative to mice that did not express human CRP, regardless of whether treated with turpentine to induce inflammation (Antoni et al., 2004). Moreover, lesions from transgenic mice were found to express higher amounts of vascular cellular adhesion molecule 1 (VCAM-1) mRNA and higher amounts of angiotensin-II type 1 receptor mRNA (Antoni et al., 2004). Exposure of human saphenous vein VSMCs in culture to recombinant CRP has been associated with increased VSMC DNA synthesis, enhanced angiotensin-II type 1 receptor expression, and amplification of basal intracellular production of ROS (C.-H. Wang et al., 2003). Finally, incubation of rat aortic VSMCs with human recombinant CRP in vitro has been associated with induction of monocyte chemoattractant peptide and nitrogen oxide synthase gene expression (Hattori, Matsumura, & Kasai, 2003).

Results from 2 recently published clinical trials contribute further support for a causal association between CRP and CVD (Nissen et al., 2005; Ridker et al., 2005). The first study, which was conducted among persons who had been hospitalized for acute coronary syndromes, examined (a) the effects of statin therapy on CRP levels; and (b) whether resultant reductions in CRP are associated with a decreased risk of experiencing a recurrent event. As expected, patients who were assigned to statin therapy were less likely to experience recurrent events relative to patients who were not taking statins. Also as expected, treatment with statins predicted lower levels of CRP. Reductions in CRP following statin therapy predicted a lower rate of recurrent events, regardless of associated LDL cholesterol levels (Ridker et al., 2005). The second study examined the association between reduced CRP subsequent to intensive statin

treatment and progression of coronary artery atherosclerosis among patients with angiographically documented coronary disease (Nissen et al., 2005). Intravascular ultrasonographic examination over 18 months revealed that reductions in CRP following statin treatment were correlated negatively with progression of coronary atherosclerosis during this period. Once again, the association between reduced CRP and less progression was independent of the effects of concurrent reductions in LDL cholesterol (Nissen et al., 2005).

Summary. Histopathology research has shown that the inflammatory markers, IL-6 and CRP, are present in atherosclerotic lesions, thus implicating them in the process of atherogenesis. The clinical relevance of these histopathologic findings is suggested by data from epidemiologic research which demonstrate a reliable association between these 2 inflammatory markers and risk of incident CVD. Findings for IL-6 appear slightly stronger than analogous results for CRP. However, given the comparatively smaller size of the IL-6 literature, it cannot be concluded with certainty that IL-6 is a better predictor of CVD outcomes. Findings from in vitro and in vivo experimental research suggest that IL-6 and CRP may be more than passive bystanders in the initiation and progression of atherosclerosis. Outcomes from 2 recent clinical trials provide additional evidence to support a causal role for CRP that is independent of the effects associated with other known risk factors.

1.3.2.2 Autonomic activity and inflammation

The following conclusions might be drawn based on the research discussed thus far: (1) The association between stress and cardiovascular pathogenesis among laboratory animals appears to be mediated by activation of the SNS; and (2) inflammatory processes, in particular those that are mediated by IL-6 and CRP, play an important role in atherogenesis. Whether inflammation is a plausible mechanism whereby activity of the SNS might be translated into cardiovascular pathology depends on whether SNS activation is associated with pro-inflammatory effects[3]. Such effects might be realized via a direct pathway wherein SNS mediators activate beta-adrenergic receptors that are expressed on the surface of circulating immune cells. Alternatively,

[3] It should be noted that the role of the HPA axis in inflammation is much better understood than the role of the SNS. However, as beta-adrenergic activation has been linked to CVD risk, the primary outcome of the present study, the following discussion will focus on the role of SNS activity on inflammatory processes.

the SNS may influence systemic inflammation indirectly by affecting other immune processes that ultimately result in inflammatory effects.

Direct pathway. The physiologic response to stress is associated with activation of the SNS and the hypothalamic adreno-cortical (HPA) axis. Early conceptualizations of stress and immunity maintained that stressor-induced upregulation of the activity of either of these 2 stress-response systems resulted in global suppression of immunity. More recent evidence has suggested that the immunologic response to stress is associated with attenuation of some components of immunity and enhancement of others. Paradigmatic of this change in perspective is the model of the TH1/TH2 shift. Briefly, T-helper lymphocytes may be characterized by functions associated with promotion of (a) cellular immunity (TH1); or (b) humoral immunity (TH2). It is thought that stress, via activation of the SNS, shifts the balance of TH1 and TH2 immune activity in favor of the latter. Given that TH1, but not TH2, lymphocytes express beta-adrenergic receptors on their cell surfaces, it further has been proposed that the shift to a predominantly TH2-mediated immunity is a consequence of selective suppression of TH1 activity by the SNS (Rabin, 1999).

As IL-6 is released from lymphocytes that carry out TH2 functions, it might be expected that SNS activation is associated with relatively elevated IL-6 production. Evidence from experimental animal research is consistent with this hypothesis. Szabó and colleagues found that IL-6 release in response to stimulation with lipopolysaccharide (LPS) was exacerbated in mice that were pretreated with the non-selective beta-receptor agonist, isoproterenol, relative to mice that received no agonist (Szabo et al., 1997). There also is evidence to suggest that downregulation of SNS activity is associated with reduced IL-6 production. In a study that was conducted with rats, Huang and colleagues reported that norepinephrine depletion subsequent to treatment with 6-hydroxydopamine (6-OHDA) was associated with an attenuated IL-6 response to intravenous administration of recombinant human IL-1β, a known stimulus for IL-6 release in vivo (Huang, Takaki, & Arimura, 1997).

In addition to moderating the inflammatory response to an immunogenic stimulus, there also is evidence to suggest that SNS activation in the absence of antigenic stimulation may be sufficient to elicit IL-6 secretion from tissue-derived as opposed to hematopoietic (T-lymphocytes, macrophages) sources. Liao and colleagues found that injection of epinephrine into isolated perfused rat liver in vitro was associated with elevated basal IL-6 expression.

Moreover, the enhanced IL-6 production was blocked after addition of propranolol (Liao, Keiser, Scales, Kunkel, & Kluger, 1995). In a study of similar design, Gornekiewicz reported that addition of catecholamines to human dermal microvascular endothelium in vitro was associated with elevated basal IL-6 protein expression. Once again, this effect was attenuated after addition of a beta-receptor antagonist (Gornikiewicz et al., 2000). There also are several lines of evidence to suggest that beta-adrenergic stimulation upregulates the constitutional expression of IL-6 in adipose cells derived from mouse (Burysek & Houstek, 1997; Fasshauer, Klein, Lossner, & Paschke, 2003) and human (Mohamed-Ali et al., 2001; Path et al., 2001) populations. Adipose tissue is a major source of circulating IL-6 (Mohamed-Ali et al., 1997). Thus, insofar as IL-6 may be related causally to atherogenesis, upregulation of the constitutional secretion of IL-6 via beta-adrenergic stimulation may contribute substantially to the creation of a systemic proatherogenic milieu.

Given that IL-6 is the major determinant of CRP synthesis and release by the liver, it might be inferred that SNS activation influences levels of circulating CRP, indirectly, via associated effects on IL-6 production. To date, there are no known experimental studies that have evaluated whether SNS activation directly influences CRP expression, specifically. However, indirect support for an association between SNS activation and CRP is provided by a few observational studies that were conducted among hospital patients who were receiving beta-blocker therapy for treatment of CVD (Anzai et al., 2003; Doo et al., 2001; Jenkins, Keevil, Hutchinson, & Brooks, 2002; Joynt et al., 2004). All 4 studies reported an association between use of beta-blockers and lower mean CRP concentrations. Inference based on the results of these studies is limited due to the cross-sectional nature of the findings. However, the observed association between reduced adrenergic activity and CRP expression is compelling, and invites further exploration with prospective and experimental investigations.

Indirect pathway. It has been proposed that systemic infection or inflammation at sites distal to the artery wall can generate low-grade elevations in pro-inflammatory cytokines that may be sufficient to create pro-atherogenic conditions (J. Danesh et al., 1999). Thus, it might be proposed that stress could indirectly influence circulating levels of pro-atherogenic substances such as IL-6 and CRP by increasing individuals' susceptibility to infection. Evidence from several lines of research have indicated that exposure to stress is associated with decreases in functional immunity, as indicated by proliferative response to mitogen and natural killer cell

activity (Herbert & Cohen, 1993). These cellular-level associations also have been demonstrated at the clinical level, with self-reported severe chronic stress being associated with a greater risk of developing cold symptoms following experimental inoculation with common cold viruses (Cohen et al., 1998). That stress-associated infectious status might influence inflammatory marker levels has been suggested by a recent study that examined the association between vital exhaustion and (a) pathogen burden (i.e., the aggregated seropositivity to antibodies for multiple common herpes viruses) and (b) pro-inflammatory cytokines (van der Ven et al., 2003). Results showed that healthy men with high vital exhaustion scores evidenced greater pathogen burden relative men with low scores. Moreover, high vital exhaustion in combination with high pathogen burden was associated with elevated circulating IL-6 (van der Ven et al., 2003).

Another hypothesized route by which stress might influence circulating inflammatory markers involves the release of endogenous LPS from the gut into systemic circulation (Black & Garbutt, 2002). A substantial proportion of norepinephrine outflow is directed toward the mesenteric organs (Aneman et al., 1996). Moreover, application of norepinephrine to the mesenteric artery is associated with significant contractile response (Smyth, Bobalova, Ward, Keef, & Mutafova-Yambolieva, 2000). Intestinal ischemia has been found to enhance paracellular permeability of gut epithelium to LPS (Drewe, Beglinger, & Fricker, 2001). Thus, it is possible that stress-associated vasoconstriction of splanchnic vasculature may promote release of LPS into the systemic circulation, which then may elicit the release of pro-inflammatory cytokines.

1.4 SUMMARY AND CONCLUSIONS BASED ON PREVIOUS RESEARCH

The following conclusions can be drawn from the above review of previous research: (1) chronic stress, variously defined, is associated with risk of clinical CVD outcomes; (2) SNS activation appears to mediate the association between chronic stress and cardiovascular pathology among laboratory animals; (3) the atherogenic component of CVD is an inflammatory process; and (4) SNS activation may upregulate certain pro-inflammatory processes relevant to the development and progression of atherosclerosis. Given these data, it seems reasonable to propose that chronic stress may be related causally to cardiovascular pathogenesis via a

mechanism involving inflammation (see Figure 1). However, the plausibility of this model as it relates specifically to humans depends upon demonstration that (1) chronic stress is associated with early stages in the cardiovascular disease process, and (2) chronic stress is associated with elevations in inflammatory activity.

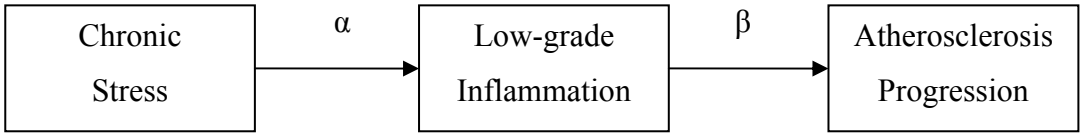


Figure 1: Proposed mediation model

1.4.1 Potential moderating influences of cardiovascular reactivity

Though existing research provides suggestive evidence for a link between chronic stress and CVD, reported correlations generally are modest at best. The small size of these statistical associations suggests the presence of (a) persons who have not been exposed to chronic stress but who nevertheless develop CVD; and (b) persons who have been exposed to chronic stress but who fail to develop CVD. That chronic stress should predict disease outcomes in some persons but not others suggests a potential role of moderating influences.

Cardiovascular reactivity (CVR) to psychological stress is one factor that may moderate the association between chronic stress and CVD. CVR is conceptualized as a trait characteristic, and is measured in terms of changes in cardiovascular activity in response to physical or psychological stimuli (Manuck, Kamarck, Kasprowicz, & Waldstein, 1993). A number of processes contribute to individual differences in CVR, with activity of the SNS assumed to be one of the most influential (Dunlap & Pfeifer, 1989). Accordingly, it might be inferred that a dispositional tendency toward exaggerated CVR may be an indirect marker for underlying dysregulation in SNS function. Thus, insofar as the association between stress and CVD may be accounted for, to some extent, by upregulated SNS activity, it follows that the link between stress and CVD may be stronger among persons who show high CVR (see Figure 2).

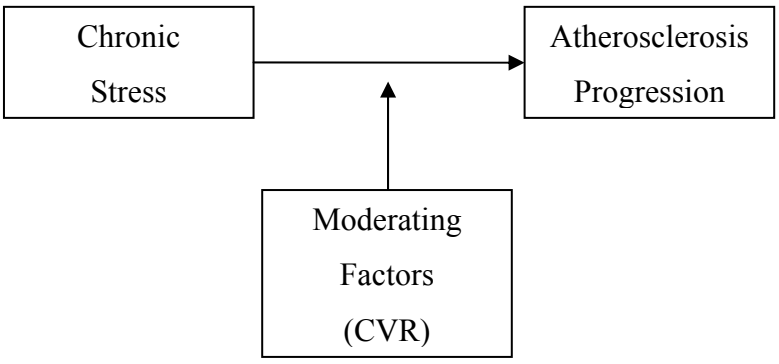


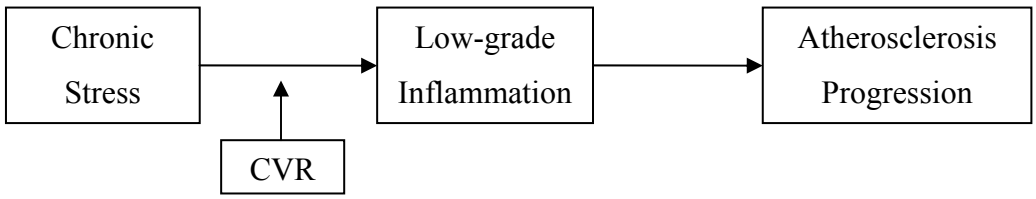
Figure 2: Proposed moderation model

CVR may influence the association between chronic stress and CVD at various points along the proposed mediated pathway (c.f. Figure 1). First of all, CVR may moderate the association between stress and inflammation (see Figure 3a). More specifically, given the evidence to suggest that beta-adrenergic activity may influence circulating IL-6 and CRP levels (see above), it might be expected that the SNS-mediated effects of chronic stress on inflammatory marker levels would be exacerbated among persons who tend to show high CVR to stress. Insofar as inflammation contributes causally to atherogenesis, it seems reasonable to propose that exacerbations in stress-associated increases in IL-6 and CRP levels may strengthen the association between chronic stress and CVD.

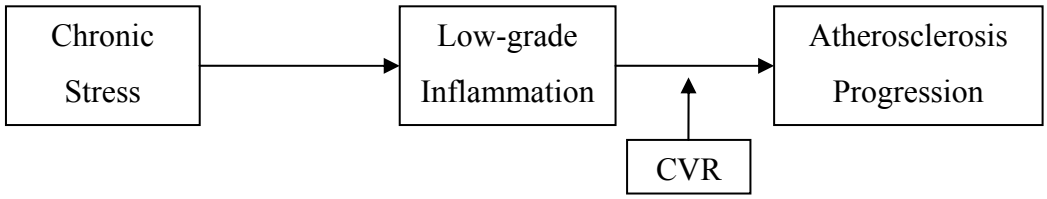
Alternatively, it is possible that CVR influences the pathway linking stress and CVD by moderating the association between inflammation and CVD outcomes (see Figure 3b). This moderating effect of CVR might be conferred by stimulating proatherogenic activities associated with IL-6 and CRP via a pathophysiologic mechanism involving changes in hemodynamic patterns. Atherosclerotic lesions tend to develop at regions where arterial blood flow becomes nonlaminar, such as the outer aspects of vessel bifurcations and curved portions of arteries (Frangos, Gahtan, & Sumpio, 1999). Fluid shear stress, or the frictional force per unit area exerted by blood flow on the arterial endothelium, is significantly reduced in these anatomically predisposed regions. Vulnerable regions further are characterized by relative blood flow stasis, which contributes to a "recirculation zone" wherein the adjacent luminal surface gains prolonged exposure to pro-atherogenic blood constituents such as damaged red blood cells and platelets (Markovitz & Matthews, 1991). Endothelial dysfunction, even in the absence of apparent denudation, is sufficient to trigger an inflammatory response (Ross, 1999). Thus, it is likely that

conditions created in these "recirculation zones" promote the recruitment of inflammatory mediators, such as IL-6 and CRP.

The reductions in shear stress that are thought to initiate the above-described processes are most apparent during systole; during diastole the comparatively reduced hemodynamic force permits reorganization of blood flow in vulnerable regions. Elevated heart rates, as might be observed during periods of increased stress, reduce the proportion of the cardiac cycle that is spent in diastole. Thus, during periods of increased heart rate, lesion-prone regions of the arteries are exposed to pathogenic conditions for a larger proportion of the cardiac cycle (Markovitz & Matthews, 1991). If increased heart rate during stress is associated with intra-arterial conditions with the potential to exacerbate atherogenesis, it might be argued that individuals who show dispositionally larger heart rate responses to stress (i.e., high cardiovascular reactors) would be at higher risk for CVD (Manuck et al., 1995).



a. CVR as marker of dysregulated autonomic activity.



b. CVR as facilitator of inflammatory process.

Figure 3: CVR as moderator of the association between chronic stress and CVD

In addition to creating pro-inflammatory conditions at vulnerable artery regions, low shear stress also may contribute to atherosclerotic lesion development via permissive effects on specific inflammatory-proliferative activities associated with certain cytokines (Ni, Hsieh, Chao,

& Wang, 2004; Rectenwald, Moldawer, Huber, Seeger, & Ozaki, 2000; Yamawaki, Lehoux, & Berk, 2003). For example, Ni and colleagues found that addition of differing concentrations of IL-6 to bovine aortic endothelial cells in culture was associated with a dose-response increase in phosphorylation of the STAT3 protein, which had the ultimate effect of increasing endothelial cell proliferation. Exposure of cultured cells to experimentally-induced shear stress, however, attenuated this pro-inflammatory effect of IL-6 by approximately 60% (Ni et al., 2004). Experimental shear stress also has been found to attenuate the pro-atherogenic effects associated with pretreatment of cultured endothelium with the pro-inflammatory cytokines, TNF-α and IL-1 (Rectenwald et al., 2000; Yamawaki et al., 2003). Thus, it might be argued that the lack of this protective effect of shear stress against the pro-atherogenic activities of inflammatory cytokines may contribute further to the site-specific distribution of atherosclerotic lesions.

To summarize: (1) Atherosclerotic lesions tend to develop at arterial sites that are subject to low shear stress; (2) reduced shear stress results in the generation of pro-inflammatory conditions at vulnerable regions of the artery wall; (3) effects associated with reduced shear stress are most evident during systole; (4) elevated heart rate is associated with more time spent in systole; and (5) exposure to shear stress may suppress the inflammatory-proliferative effects associated with IL-6. Given these findings, it might be expected that individual differences in CVR to stress will moderate the association between chronic stress and CVD risk.

1.5 PRESENT STUDY

The Pittsburgh Healthy Heart Project (PHHP) is a longitudinal investigation of the effects of psychosocial and biological risk factors on subclinical CVD among a healthy, older adult community sample. Features of the larger study that are directly relevant to the questions posed here include the following: ultrasound measurement of carotid artery intima-media thickening (IMT) and plaque at baseline and 3-year follow-up; baseline serological measures of IL-6 and CRP; and laboratory testing for cardiovascular reactivity (CVR). Also relevant to the present study are PHHP participants' reports of baseline chronic life stress as assessed by the Chronic Stress Scale (CSS) (Norris & Uhl, 1993), a self-report questionnaire measure of chronic stress across 7 life domains.

The primary objective of the present study was to examine whether: (1) higher levels of chronic stress predicted greater changes in carotid artery IMT and plaque; and (2) the association between chronic stress and changes in these surrogate CVD endpoints is mediated by low-grade inflammatory activity (see Figure 1). In exploratory analyses, we examined whether these associations differed as a function of sex and age. Given the findings of previous research on job strain and CVD risk that suggest that stress at work may be a stronger correlate of CVD risk among men relative to women, we expected that the association between the occupational stress subscale of the CSS and IMT and plaque progression would be stronger among male relative to female participants. Similarly, as both the marital stress and caregiving stress literatures suggest that these family stressors may be especially relevant to CVD risk among women, we expected that the 2 family stress subscales of the CSS (marital stress and filial stress; see below) would be stronger predictors of IMT and plaque progression among women relative to men. A secondary objective of the present study was to investigate whether individual differences in cardiovascular reactivity to stress moderates the association between chronic stress and IMT and plaque progression (see Figure 1.2). Specifically, we expected that the association between stress and progression would be stronger among high relative to low reactors.

1.5.1 Advantages of PHHP data for examining the questions posed here

1.5.1.1 Healthy, older adult sample

Recruitment of a healthy sample has the advantage of reducing the confounding influence of symptomatic disease on psychosocial assessments. The employ of an older adult population (range: 50 to 70 years) has advantages as well. Persons in this age range are at comparatively high risk of developing clinically manifest CVD. Thus, found associations between chronic life stress and subclinical CVD progression may have important implications for this group in terms of the future development of clinical outcomes.

1.5.1.2 Ultrasound measures of carotid artery IMT and plaque

Measures of carotid artery IMT and plaque have been identified as independent risk factors for symptomatic cardiovascular disease (Kuller et al., 1995). IMT, for example, has been found to be an independent correlate of future myocardial infarction and stroke among older adults

(O'Leary et al., 1999). The ability to evaluate carotid atherosclerosis in healthy persons who are at risk for CVD allows for the potential to identify the presence of modifiable risk factors that may be amenable to preventive intervention. Moreover, examination of subclinical disease markers among persons without a history of CVD avoids potential confounds of biased reporting of chronic stress that may arise when conducting research among patient populations. For example, relative to persons with known disease, PHHP participants' reports of chronic stress are unlikely to be influenced by knowledge of pre-existing disease and measured outcomes are less likely to be influenced by medication use. It should be noted, however, that a small portion of the sample began taking blood pressure (17.0%; n = 47) or cholesterol-lowering (18.5%; n = 51) medication at some time during the 3 years of follow-up. Medication use, as well as hormone replacement therapy (HRT) among women (49.6% of female participants; n = 68) will be examined for their effects on physiologic outcomes, and will be included as covariates in relevant analyses if necessary.

2.0 METHOD

2.1 PARTICIPANTS

Participants were 276, healthy older persons (mean age = 60.5; range = 50.0 to 70.8), who represented a subset of PHHP participants. The PHHP received approval of the institutional review board at the University of Pittsburgh, and participants provided written informed consent to all procedures. Recruitment strategies included targeted mailings and media postings in the Pittsburgh metropolitan area. Major inclusion criteria were age (50-70 years) and menopausal status (women were required to be peri- or post-menopausal, defined as absence of menstruation during the 6 months prior to enrollment). Individuals were excluded from the larger study if they reported a history of CVD or other chronic disease (including diabetic persons who were taking insulin), use of antihypertensive or lipid lowering medication, or pre-menopausal status. Persons with screening blood pressures $\geq$180/110, as well as persons with a pattern of excessive alcohol consumption ($\geq$ 5 portions 3 or more times a week) also were excluded from the PHHP.

Participants from the larger investigation were selected for inclusion in the present study if they (a) had completed the self-report measure of chronic stress at baseline (see below); (b) provided usable IL-6 or CRP data from the baseline blood draw (see below); and (c) had complete data from ultrasound assessment for carotid atherosclerosis.

2.2 MEASURES

2.2.1 Chronic stress

Chronic stress was assessed with a multidimensional self-report questionnaire that was designed by Norris and Uhl for use in their research on mechanisms explaining the development of psychopathology following exposure to a natural disaster (Hurricane Hugo) (Norris & Uhl, 1993). The choice of the Norris and Uhl chronic stress scale (CSS) largely was influenced by the fact that the CSS is one of only a few available comprehensive measures of chronic stress. The 27-item CSS is comprised of 7 multi-item subscales: marital stress (MAR; 3 items); parental stress (PAR; 4 items); filial stress (FIL; 4 items; assesses stress that arises from respondents' relationships with their own parents or other older relatives); financial stress (FIN; 4 items); occupational stress (OCC; 4 items); ecological stress (ECO; 5 items); and physical stress (PHYS; 3 items). Individual CSS items are displayed in Appendix A. All items are presented following the question stem "In the past 6 months, how often…", and responses are based on the following 5-point scale: never (0), almost never (1), sometimes (2), fairly often (3), very often (4). Subscale scores are computed as the summed ratings of the component items. In the original Norris and Uhl sample, test-retest stability of the CSS over 6 months was .65 for the total scale. Subscale retest reliabilities ranged from .65 for the FIN and PHYS subscales, to .63 for the PAR subscale, to .58 for the FIL and OCC subscales, to .43 for the MAR subscale (F.H. Norris, personal communication, February 9, 2005). The somewhat modest retest correlations may be due in part to the fact that Norris and Uhl conducted these analyses on a sample of recent natural disaster victims, whose lives likely were characterized by significant upheaval during the period of retest.

As originally designed, the CSS does not include a "total" score that is summed across subscales. The authors provided no explanation for their choice not to examine the cumulative effects of chronic stress across life domains (Norris & Uhl, 1993). As we were interested in examining whether scores on a comprehensive measure of life stress would correlate with IMT and plaque progression, we created an aggregate chronic stress score that was computed as the

weighted average stress score across all subscales with non-missing data[4]. Thus, for the purposes of the present study, we operationalized chronic stress in terms of (a) scores on individual subscales and (b) the aggregate chronic stress score. Preliminary analyses indicated that the aggregate CSS score, as well as scores on the individual subscales, correlated in expected directions with scores on relevant established stress measures (see Appendix B).

Two features of the CSS contribute to this scale's being an appropriate tool for assessing chronic stress in the present study: (1) the measurement of chronic stress exposure across several life domains; and (2) reference period of previous 6 months. Use of a multidimensional measure of chronic stress may be preferable to unidimensional assessments because focus on a single dimension of individuals' daily experience (e.g., work life) presumes that the chosen stressor is either (a) specifically relevant to cardiovascular pathogenesis; or (b) representative of individuals' total stress experience. Insofar as either of these assumptions is not met, studies of psychosocial correlates of CVD that measure stress in only a single life domain may fail to detect associations between disease outcomes and stress that occur in other areas of life. That the CSS asks respondents to report on their experience of life stress during the previous 6 months may provide a more accurate estimation of individuals' "typical" chronic stress exposure relative to instruments that ask participants to report on a comparatively shorter time period. Given the slowly progressing nature of CVD, it is likely that enduring chronic stress is likely to play a more important role in the developing disease process than short-term, and potentially atypical stress.

2.2.2 Carotid artery IMT and plaque

Ultrasound images of the carotid arteries were obtained using Toshiba SSA-270A and SSA-140A scanners (Toshiba American Medical Systems, Tustin, CA). The use of ultrasound technology to examine the carotid arteries has been found to be a valid and reliable method for detecting arterial changes that may be indicative of future disease (Salonen & Salonen, 1993; Sutton-Tyrrell, Wolfson, Thompson, & Kelsey, 1992) and the noninvasive nature of ultrasound scanning makes it an appropriate procedure for use in asymptomatic populations. Optimal images of the first 1.0 cm of the right and left common (CCA) and internal (ICA) carotid

[4] The weighted average score was computed by averaging across mean subscale scores divided by the associated standard deviations.

arteries, as well as all measurable areas of the right and left carotid bifurcations (i.e., the bulb), were digitized for a period of 10 seconds for later scoring of IMT and presence of eccentric plaque. An automated edge-detection system (Automated Measurement System (AMS), Goteborg University, Gothenburg, Sweden) was used to measure IMT across the carotid artery segments described above. The automated system is preferable to manual methods as it has been shown to be associated with less variability in measurements of ultrasound IMT (Wendelhag, Liang, Gustavsson, & Wikstrand, 1997). Overall mean IMT was derived by taking the average of the 6 far-wall loci across both right and left carotid arteries. IMT progression was computed as the arithmetic difference between 3-year follow-up and baseline IMT scores.

Plaque was defined as a visually distinct area of the vessel that protrudes into the lumen with a thickness that is at least 50% greater than surrounding area. Extent of plaque at each locus was indicated on a 4-point scale: 0 = no plaque; 1 = one small plaque (< 30% of vessel diameter); 2 = one medium plaque (30-50% of vessel diameter or multiple small plaques); and 3 = one large plaque (>50% of vessel diameter or multiple plaques with at least one medium plaque). Plaque also was described in terms of the number of visible lesions at each of the 6 loci. For the purposes of the present study, overall carotid plaque was represented as the total number of visible lesions, summed across the 6 loci. Change in plaque was computed as the arithmetic difference between the number of lesions present at 3-year follow-up less the number of lesions present at baseline.

2.2.3 Inflammatory markers

Blood samples for inflammatory marker assays were sent to the Department of Pathology, School of Medicine, at the University of Vermont. It should be noted that this laboratory was responsible for the development of the high-sensitivity assay for CRP, which is the current gold standard (Ridker, Hennekens et al., 2000).

Serum CRP was measured using a particle enhanced immunonephelometric assay (BN II nephelometer; Dade Behring). Polystyrene particles are coated with monoclonal CRP antibodies. In the presence of CRP, the coated particles agglutinate and cause an increase in the intensity of scattered light, with the increase in scatter being proportional to the amount of CRP in the sample. Standardization was performed using the WHO CRP reference standard. The

assay range is 0.175 to 1100 mg/L. Expected values for CRP in normal, healthy individuals are ≤ 3 mg/L, with the expected normal range being 0.18 to 5.05 ug/mL. At the University of Vermont, intra-assay CVs associated with this method range from 2.3 to 4.4% and inter-assay CVs range from 2.1 to 5.7%.

Serum IL-6 was measured by ultra-sensitive ELISA (R&D Systems, Minneapolis, MN). The lower detection limit was <0.10 pg/mL, and the detection range 0.156 to 10.0 pg/mL. A monoclonal anti-IL6 antibody is coated on the plastic support, and a polyclonal anti-IL6 antibody is used as the sandwich antibody. The amount of IL-6 bound is determined by a color reaction. At the University of Vermont, this method is associated with a routine CV of 6.3 % (Harris, Ferrucci, Russell, & al., 1999).

CRP and IL-6 assays were performed on samples obtained from the baseline and 3-year blood draws. The present study examined baseline levels of CRP and IL-6 as potential mediators of the association between stress and progression of IMT and plaque.

2.2.4 Cardiovascular reactivity

Participants were recruited for a 3-hour laboratory testing session. Five standardized laboratory tasks were presented to each of the participants, interspersed with resting baseline periods. The first 4 tasks were computer-based tasks, programmed using QBASIC and administered on an IBM 486 PC. These tasks previously have been used as part of a standardized testing battery, and have been found to evoke significant and reliable changes in cardiovascular activity (Kamarck et al., 1992). Tasks were presented in a standardized format and performed without verbalization. Responses were executed by microswitch or miniature manipulandum, which required minimal motoric activity. Thus, potential confounding by physical movement was minimized. A program routine adjusted task difficulty commensurate with performance, equating level of challenge for each subject throughout each task (Debski et al., 1991). The 4 tasks included a marksmanship task (Target), a visual short-term memory task (Scanning), a psychomotor task (Tracking), and a version of the Stroop Color-Word Conflict Test using recorded color words as an auditory distractor. Each task was 9 minutes in duration.

The fifth task was an evaluated speaking task that involved the preparation and presentation of a story under video-recorded conditions. Participants were presented with

written guidelines around which to organize their presentations (participants were instructed to defend themselves in traffic court), followed by a 4-minute speech preparation period, and the speech itself (4 minutes). Speech preparation and presentation were scored as separate task periods. Participants were informed that they would be evaluated based upon their poise, articulation, and the content of their speech. This evaluated speaking task has been found to elicit moderately reliable cardiovascular responses on retest (Kamarck, Debski, & Manuck, 2000).

Baseline periods. Preceding each laboratory task was a 10-minute resting baseline period, during which subjects were presented with a simple standardized display (Jennings, Kamarck, Stewart, Eddy, & Johnson, 1992).

Cardiovascular measures. During the laboratory session, the following cardiovascular measures were collected at rest and during the tasks: (1) Systolic (SBP) and diastolic (DBP) blood pressures were collected at 90-second intervals, using an Accutracker DX monitor; (2) Continuous measures of heart rate (HR) were assessed using a 2-lead EKG; signals were digitized, ensemble-averaged, and scored using operator-assisted software (Debski et al., 1991); (3) Impedance cardiography also was collected in the laboratory. CVR was defined as residualized change scores. For each parameter (SBP, DBP, HR), readings were averaged separately across each task and across all resting baselines. Each task measure was regressed on the corresponding baseline measure, and resulting residualized change scores were standardized and averaged across tasks to enhance reliability for individual assessments (Kamarck et al., 1992).

2.3 PROCEDURE

2.3.1 Overview

Figure 4 presents a flow diagram depicting the temporal sequence of procedures followed by PHHP participants. Following informed consent, baseline data collection procedures for the larger study involved 10 laboratory sessions that were scheduled over a 5-month period. These sessions included: a medical screening visit (enrollment); 3 visits involving training and

feedback for an initial 3-day ambulatory monitoring period; a visit for cardiovascular reactivity testing; a visit for baseline carotid artery ultrasound assessment; a second visit for additional ultrasound measures (not reported here); and 3 visits associated with a second 3-day ambulatory monitoring interval. As data from the ambulatory monitoring component of the PHHP are not relevant to research questions being addressed by the present study, details of the associated procedure will not be described.

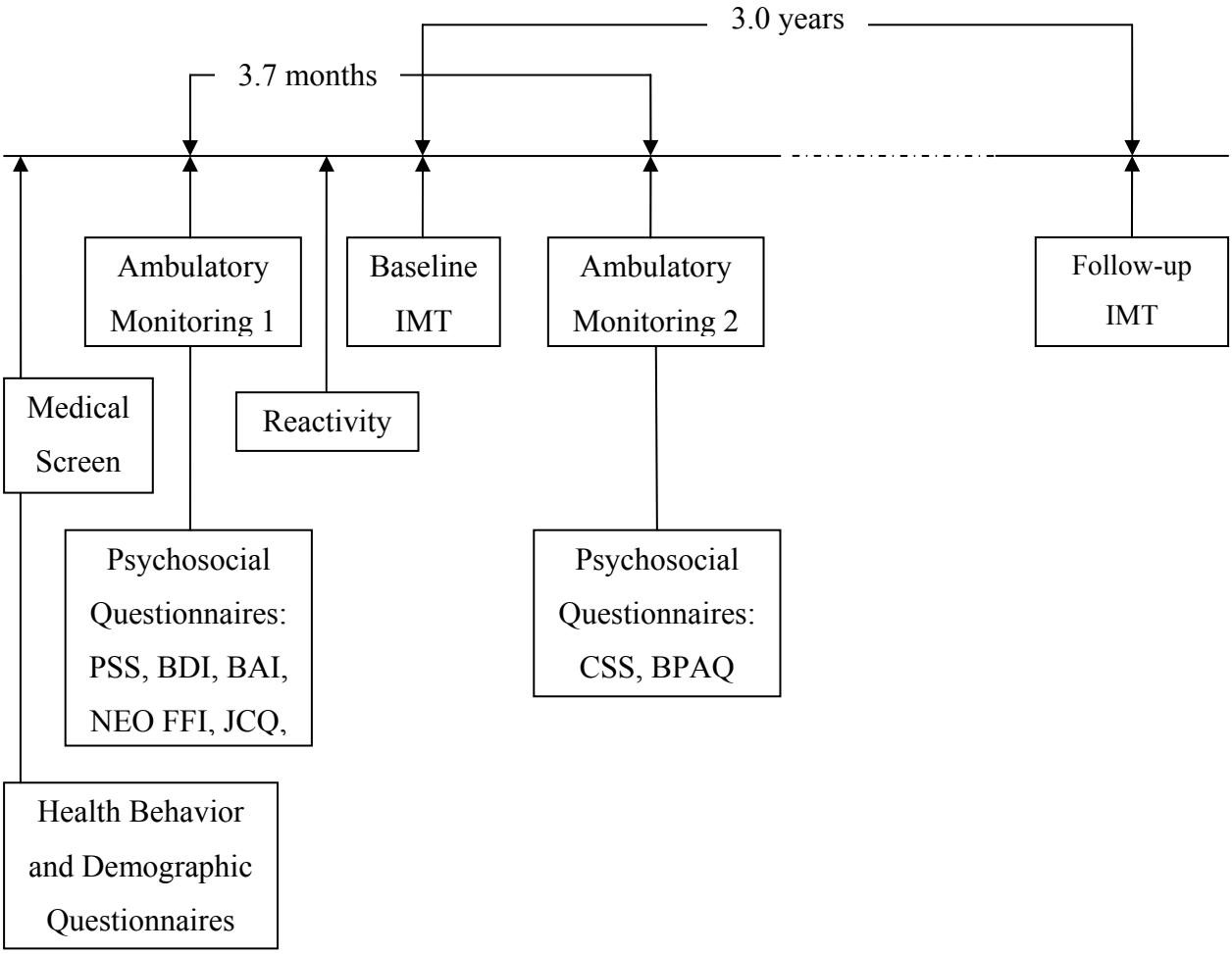


Figure 4: Procedure timeline

2.3.2 Medical screening visit

Prior to attending the medical screening visit, participants were instructed to fast for 12 hours. Following informed consent procedures, participants provided a thorough medical history that included a review of past medical conditions such as heart-related problems (high blood pressure, congestive heart failure, arrhythmia, heart murmur/valve problem, need for pacemaker, and Rose Questionnaire to evaluate angina), stroke/TIA/CVA, epilepsy/seizures, neurological or neuromuscular conditions (multiple sclerosis, Parkinson's disease, Alzheimer's), diabetes, cancer, endocrine, rheumatologic, lung, liver, kidney disease/acute renal failure, HIV/AIDS, amputations, allergies, surgeries and any other diseases or medical conditions. Participants also were asked about medication (prescription, non-prescription, hormone replacement therapy and aspirin) and vitamin use, and were screened for excessive alcohol use, smoking status, and menopausal status. Family history (1st degree relative) of heart condition, stroke, high blood pressure and nervous/emotional condition also were reviewed

Physical examination included the following: casual clinic blood pressures; height, weight, and caliper measures (body fat); blood glucose finger stick; and blood draw for measurement of insulin, cholesterol, triglycerides, catecholamines, and inflammatory proteins (interleukin-6, C-reactive protein). Blood was drawn from the right or left antecubital vein, and labeled with PHHP subject ID number, date and time of sample. Blood samples were stored at room temperature for 40 minutes prior to assay.

Following the physical exam, participants completed a series of demographic and health behavior questionnaires. Demographic data collected by these questionnaires included the following: participant marital and employment status and spouse employment status (when relevant); information about participants' family ties, such as number of children and grandchildren, and number of persons living in the home; and socioeconomic information such as annual household income and highest level of educational attainment (participant and spouse). Data on health behaviors included use of tobacco and alcohol products, usual physical activity and exercise, and sleep habits[5].

[5] Not an exhaustive list.

2.3.3 Data collection for psychosocial variables

Approximately one month following the baseline medical screening (mean of 35.6 days, range of 0 to 257 days), participants returned to the laboratory to train for ambulatory monitoring (not discussed further), and to complete a battery of psychosocial questionnaires. These instruments included the Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983); the Job Content Questionnaire (JCQ) (R.A. Karasek, 1985); the Beck Depression Inventory (BDI) (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961); the Beck Anxiety Inventory (BAI) (Beck, Epstein, Brown, & Steer, 1988); the NEO Five-Factor Index (NEO FFI) (Costa & McCrae, 1989); the Cook-Medley Hostility Scale (CMHS) (Cook & Medley, 1954); and the Dyadic Adjustment Scale (DAS) (Spanier, 1976). Individual questionnaire items were presented on a computer screen, and a light pen (or keyboard, on occasion) was used to select responses. Questionnaires were formatted such that each item required a response before the participant was permitted to proceed to the next item. This procedure ensured that no items were skipped, and that participants were unable to change previous responses.

Additional psychosocial questionnaires were completed when participants returned to the lab approximately 4 months later to train for the second ambulatory monitoring period (mean of 149.3 days after medical screening, range of 72 to 527 days). These measures included the CSS (Norris & Uhl, 1993); the Buss-Perry Aggression Questionnaire (BPAQ) (Buss & Perry, 1992); and the Marlowe-Crowne Social Desirability Questionnaire (MCSD) (Crowne & Marlowe, 1964). These questionnaires were administered using the computer-based procedure described above.

2.3.4 Cardiovascular reactivity testing

About 2 months following enrollment (mean of 69 days, range of 12 to 246 days), participants attended a 3-3.5-hour testing session for the assessment of cardiovascular reactivity. Testing was conducted in a small sound-attenuated and temperature-controlled chamber wherein participants were fitted with instrumentation for psychophysiologic measurement. Throughout the session, participants were seated in a Lumex reclining chair (Grahamfield, Inc., Atlanta, GA, USA), locked in the upright position and equipped with head and body bolsters to standardize and

maintain posture during the testing sessions. Task response manipulanda were attached to a tray table that was placed across the arms of the chair. The video monitor for task presentation was situated on an adjustable platform immediately in front of the participant. Audiotaped instructions for each baseline and task were presented over a speaker. Preceding each computer task, written instructions and practice trials were presented on the video monitor. Participants were required to meet specific performance criteria during practice trials before they were allowed to proceed, and performance feedback was provided at the end of each task. Order of task presentation (Target, Scanning, Tracking, Stroop, Speech Preparation, Speech Presentation) was invariant across subjects.

2.3.5 Carotid artery ultrasound

About 2.5 months after enrollment (mean of 72 days, range of 14 to 312 days), participants went to the ultrasound laboratory for a baseline scan of the carotid arteries. On an average of about 39 months post-enrollment, (mean of 1178 days, range of 972 to 1686 days), participants returned for a follow-up ultrasound scan and additional medical screening to update risk factor status.

3.0 STATISTICAL POWER

Given the primary aims of the present study, 6 separate analyses were conducted to compute the power of the present sample to detect the following: (1) a significant association between chronic stress and 3 year IMT progression; (2) a significant association between chronic stress and 3 year change in plaque lesion number; (3) a significant association between chronic stress and IL-6; (4) a significant association between chronic stress and CRP; (5) a significant moderating influence of CVR on the association between chronic stress and IMT progression; and (6) a significant moderating influence of CVR on the association between chronic stress and plaque progression.

Four pieces of information are included in a power analysis: anticipated effect size, desired power, alpha level, and sample size (J. Cohen, 1988). From any 3 of these variables, the fourth variable can be computed. With regard to the present power analysis, the sample size was known ($\underline{n}$ = 276) and the 2-tailed alpha level was set to $\underline{p} < .05$. Anticipated effect sizes were obtained from existing research. To date, no known studies have examined the CSS as a predictor of CVD-relevant outcomes, or of inflammation. Thus, approximate effect sizes were derived from investigations that focused on other psychosocial factors as predictors of (a) IMT and or plaque progression, or (b) circulating markers of inflammation.

Effect sizes for relevant studies were computed from published means and standard deviations when available, and then aggregated across related studies into a single, average effect as per procedures described by Rosenthal (Rosenthal, 1991). It should be noted that one study did not include standard deviation information, but did provide $\underline{R}^2$-change values (Gallo et al., 2003). In this case, $\underline{f}^2$ values were derived from $\underline{R}^2$ data, and then converted to Pearson's $\underline{r}$ using procedures described by Cohen (J. Cohen, 1988). Two studies did not provide sufficient data to compute effect sizes based on the above procedures (Everson, Kaplan, Goldberg, Salonen, &

Salonen, 1997; Paterniti et al., 2001), and thus did not contribute to aggregate effect sizes.[6] Calculated power for the present study to detect significant findings is presented in Table 1. As indicated by the third column of Table 1, the present study is moderately well-powered to detect changes in plaque of the magnitude reported here, but may be limited in power to detect effects when IMT change is examined as the outcome. Power to detect significant findings involving inflammatory markers is moderate to good. By comparison, the power to detect a significant moderating effect of CVR on the association between chronic stress and IMT and plaque progression, respectively, is small.

Table 1: Results of Power Analyses Based on Effect Sizes Reported in Previous Research

Model	Effect Size* (Pearson's r)	Power (n = 276)	References
Stress → Change in mean IMT	.11	.47	(Gallo et al., 2003; Julkunen et al., 1994; J. Lynch, Kaplan et al., 1997; J. Lynch, Krause et al., 1997; Paterniti et al., 2001)
Stress → Change in plaque	.15	.71	(Gallo et al., 2003; J. Lynch, Kaplan et al., 1997; J. Lynch, Krause et al., 1997)
Stress → Average IL-6 levels	.40	1.00	(Appels et al., 2000; Lutgendorf et al., 1999; Maes et al., 1999; Sutherland et al., 2003; van der Ven et al., 2003)
Stress → Average CRP levels	.15	.69	(Hapuarachchi et al., 2003; Steptoe & Marmot, 2003)
Stress–by-CVR → change in mean IMT	.06	.17	(J. W. Lynch et al., 1998)
Stress–by-CVR → change in plaque	.04	.10	(J. W. Lynch et al., 1998)

[6] Paterniti et al. included data sufficient to compute an effect for mean IMT but not for plaque.

4.0 DATA ANALYSIS

4.1 MAIN ANALYSIS

All statistical analyses were conducted using the SAS statistical software package, (Version 8; SAS Institute, Cary, NC). The GLM procedure in SAS was used to compute main analyses with IMT change as the outcome. Inspection of the carotid plaque data revealed a skewed, kurtotic distribution, with the mode and median 3-year change in number of plaque lesions being 0. Thus, change in lesion number was converted to a binary variable: 1 = increase in lesion number, and 0 = no change or negative change in lesions. All analyses with plaque change as the outcome were computed with the LOGISTIC procedure in SAS.

Preliminary analyses were conducted to determine which demographic and biobehavioral variables should be included in the proposed models as covariates. Candidate variables included the following: age, sex, race (white vs. non-white), clinic blood pressure (SBP, DBP, and pulse pressure[7]), total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglycerides, body mass index (BMI)[8], tobacco use, alcohol use, physical activity, history of diabetes or rheumatologic conditions, and use of anti-hypertensive, cholesterol-lowering, anti-inflammatory (including aspirin), or hormone replacement medications during follow-up. The following procedure was used to determine which of the candidate variables would be included as covariates in the final analyses: First, we conducted univariate correlations between the candidate variables and each of the four outcomes of interest (IMT change, plaque change, CRP, IL-6). Next, baseline measures (for IMT and plaque change) and any demographic variables that were significantly correlated with a given outcome were entered into a regression model to predict that outcome. Then, individual candidate variables

[7] Pulse pressure (PP) = SBP-DBP.
[8] Three-year change in clinic blood pressures, cholesterol fractions, triglycerides and BMI were examined as covariates in addition to baseline levels of each of these variables.

that were significantly correlated with the outcome in univariate analyses were included with the demographic variables and examined as predictors of the outcome. All variables that remained independent predictors of the outcome when examined with baseline IMT or plaque (where relevant) and the demographic variables were then entered simultaneously in a single regression equation. If the effect associated with a potential covariate failed to reach significance when examined with the other candidate variables in multivariate analyses, then that variable was excluded from the final model.

4.2 POTENTIAL MODERATING EFFECT OF CVR

We examined the potential moderating effect of CVR on the association between CSS variables and cardiovascular outcomes by including, in separate models, interaction terms that consisted of (a) average CSS or CSS subscale scores, and (b) systolic, diastolic, or heart rate reactivity, respectively. Significant interaction effects were explored by examining the simple effect of stress on IMT or plaque change at one standard deviation above or below the centered mean of reactivity, as per the recommendation of Aiken and West (Aiken & West, 1991).

5.0 RESULTS

5.1 SAMPLE CHARACTERISTICS

From the original PHHP sample (n = 464), 376 participants received ultrasound scans of the carotid arteries at baseline, with 375 providing usable data. Of these, 361 received follow-up scans, with 359 providing usable data from both baseline and follow-up. As administration of the CSS was initiated after the PHHP was already in progress, only 294 of the participants with complete baseline and follow-up ultrasound data also provided data on this measure. From this subsample, 18 participants were excluded due to missing CRP or IL-6 data, thus resulting in the present sample of n = 276. Participants who were administered the CSS did not differ from those who were not administered the measure on most risk factor variables, inflammatory marker levels, baseline IMT and plaque, and IMT and plaque change. The 2 groups did differ, however, on HDL cholesterol level (present sample lower; t = -3.44, p < .001) and waist-to-hip ratio (present sample larger; t = 3.77, p < .001).

The present sample (n = 276) was 49.6% female, and 17.0% nonwhite. Participants represented all levels of educational attainment, with 26.4% of the sample having completed high school or less, and 48.9% having completed a bachelor's or more advanced degree. This distribution of educational attainment indicates that the sample is, in general, more highly educated than the population as a whole. 74.6% of the sample reported being married or having a spousal equivalent; 58.3% were employed at least part-time; and 88.0% reported having at least one child.

Table 2 displays descriptive data on baseline risk factors and inflammatory markers for the entire sample and separately by sex. As indicated by the table, the sample was, on average, slightly overweight (normal BMI: 18.5-24.9), had slightly elevated SBP (normal SBP: < 120

mmHg), slightly elevated total and LDL cholesterol levels (desirable: < 200 mg/dL, < 100 mg/dL, respectively), and slightly low HDL cholesterol levels (desirable: ≥ 60 mg/dL). On average, triglyceride levels were in the desirable range (< 200 mg/dL), and glucose values were normal (< 100 mg/dL). As participants were excluded from the present report if CRP or IL-6 values exceeded 10μg/mL and 10pg/mL, respectively, measured values for these 2 inflammatory markers were within normal limits. Distributions of inflammatory markers for the entire sample and by sex are displayed in Figures 5-10.

Table 2: Sample descriptive data

	Total Sample (n = 276)	Men (n = 139)	Women (n = 137)	Sex Difference p
Age	60.5 (5.0)	60.4 (5.3)	60.6 (4.7)	.76
% Female (n)	49.6 (137)	--	--	--
% Non-white (n)	16.7 (46)	15.1 (21)	18.3 (25)	.48
% High school or less (n)	26.5 (73)	16.6 (23)	36.5 (50)	< .001
% Current smokers (n)	10.9 (30)	13.7 (19)	8.0 (11)	.13
BMI	27.9 (4.6)	28.0 (3.5)	27.8 (5.6)	.73
Waist Circum cm	100.6 (11.3)	101.6 (9.9)	99.6 (12.6)	.15
Waist-to-hip ratio	.98 (.07)	1.02 (.05)	.94 (.07)	<.001
Total Cholesterol mg/dL	213.6 (35.1)	209.4 (31.2)	217.9 (38.3)	<.05
LDL mg/dL	132.7 (32.6)	132.6 (28.1)	132.8 (36.7)	.95
HDL mg/dL	53.2 (15.0)	46.9 (11.7)	59.7 (15.3)	<.001
Triglycerides mg/dL	137.4 (76.6)	147.8 (87.8)	126.9 (61.7)	<.02
Glucose	92.8 (14.9)	95.4 (16.5)	90.3 (12.5)	<.01
SBP mmHg	129.6 (15.7)	129.4 (15.4)	129.8 (15.9)	.82
DBP mmHg	80.0 (9.2)	80.8 (8.6)	79.2 (9.6)	.14
PP mmHg	49.6 (12.6)	48.6 (12.6)	50.7 (12.6)	.18
CRP ug/mL	2.92 (3.3)	2.2 (2.6)	3.6 (3.9)	<.001
IL-6 pg/mL	1.81 (1.3)	1.8 (1.5)	1.8 (1.0)	.82

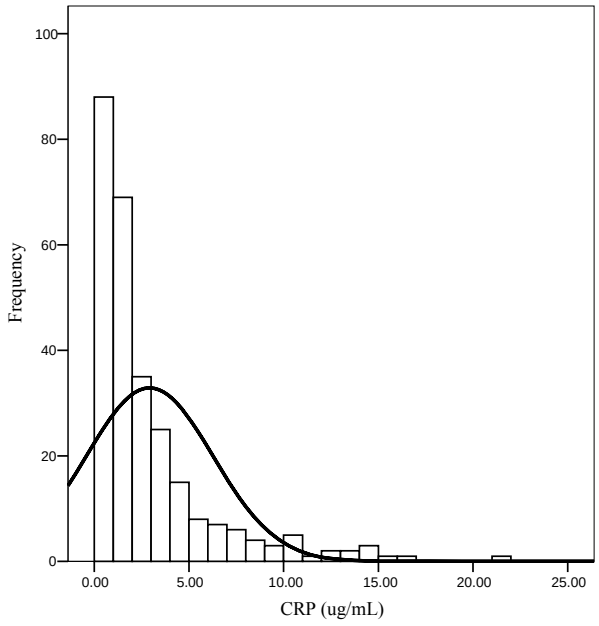


Figure 5: Distribution of CRP Concentrations--Entire Sample

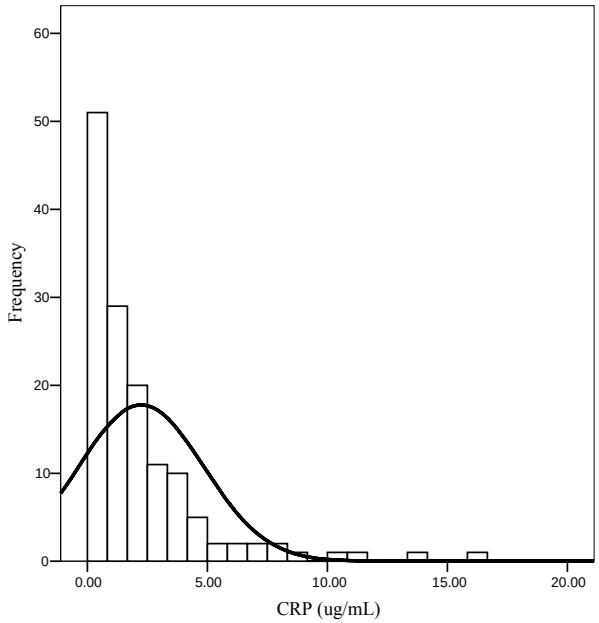


Figure 6: Distribution of CRP Concentrations—Men

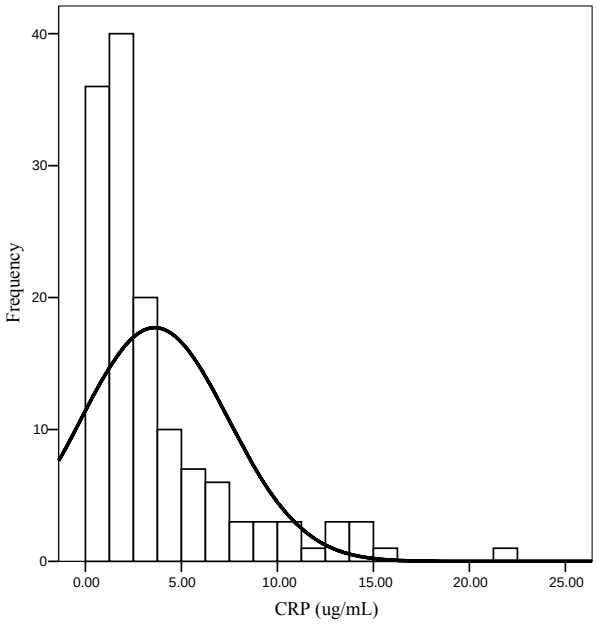


Figure 7: Distribution of CRP Concentrations—Women

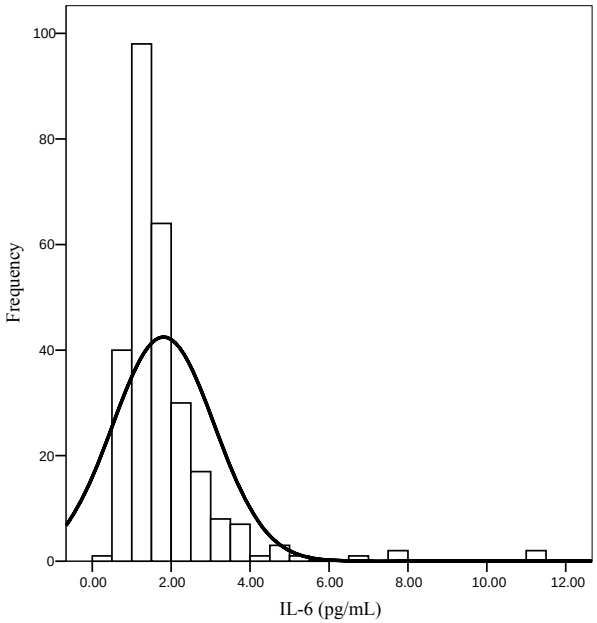


Figure 8: Distribution of IL-6 Concentrations--Entire Sample

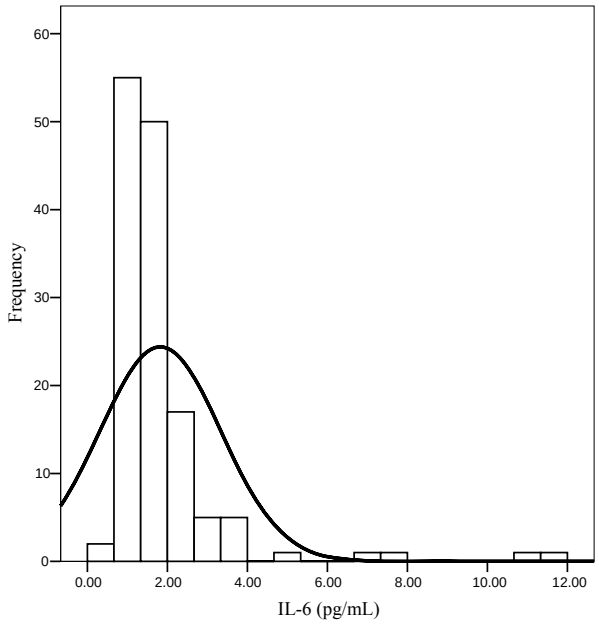


Figure 9: Distribution of IL-6 Concentrations—Men

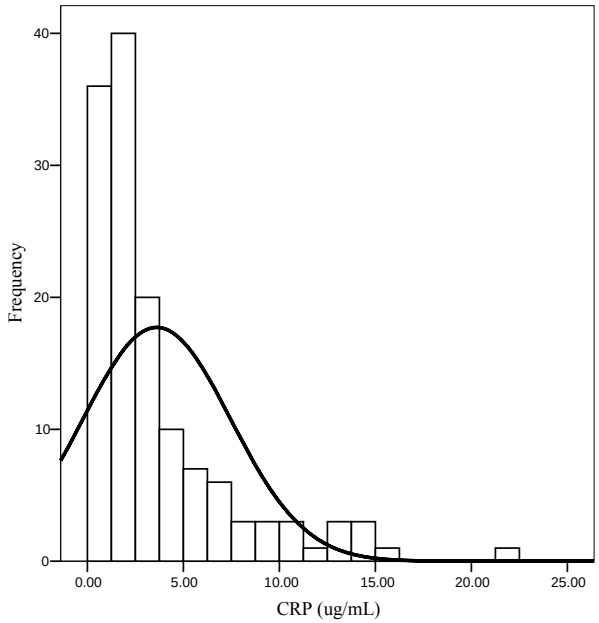


Figure 10: Distribution of IL-6 Concentrations--Women

Table 3 displays means and standard deviations for average CSS subscale scores for the entire sample and by sex. Men and women differed on reports of occupational and financial

stress, with men reporting significantly higher levels of each of these two dimensions of chronic stress.

Table 3: Means and Standard Deviations of CSS Scores for Entire Sample and by Sex

	Total Sample (n = 276)	Men (n = 139)	Women (n = 137)	Sex Difference p
CSS Average	1.24 (.55)	1.29 (.56)	1.19 (.54)	.12
Marital stress	3.52 (2.76)	3.76 (2.43)	3.28 (3.04)	.15
Parental stress	4.91 (3.40)	4.92 (3.31)	4.90 (3.50)	.96
Filial stress	5.87 (4.65)	6.04 (4.40)	5.70 (4.90)	.55
Occupational stress	2.93 (2.78)	3.35 (2.95)	2.51 (2.53)	.01
Financial stress	3.43 (3.05)	4.13 (3.24)	2.74 (2.66)	.0001
Ecological stress	3.51 (3.33)	3.63 (3.35)	3.39 (3.33)	.56
Physical Stress	1.23 (1.66)	1.15 (1.66)	1.31 (1.66)	.39

Table 4 displays means and standard deviations for average baseline, follow-up and change values for IMT. Distributions of baseline IMT and IMT change scores for the entire sample and by sex are displayed in Figures 11 through 16. Participants showed modest carotid intima-media thickening during the 3 years of follow-up, with men showing greater progression than women. Normative data on IMT progression have been collected from the Atherosclerosis Risk in Communities (ARIC) study (Chambless et al., 2002). Average IMT for men and women from the present sample are larger than analogous data reported in ARIC (.66-.69 mm and .59-.63 mm for men and women, respectively). Annualized progression rates from the present sample (.03 mm/yr) also are somewhat larger than those shown in ARIC (.007-.01 mm/yr). It should be noted that ARIC employed a comparatively younger sample (ages 45-64), and examined only the common carotid artery (i.e., internal carotid artery and carotid bulb

measurements were not included in the ARIC IMT index). Comparable common carotid data from the present sample show an annualized change of .01 mm (men: .014 mm/yr; women: .006 mm/yr), which falls within the upper range of the ARIC data.

40.6% ($\underline{n}$ = 112) of the present sample showed evidence of visible plaque at baseline, and 45.7% ($\underline{n}$ = 126) showed an increase in the number of plaque lesions from baseline to follow-up. The proportion of men and women, respectively, with plaque at baseline did not differ significantly (men: 43.2%; women: 38.0%; $chi^2 = .78$, $\underline{p} = .38$). However, a greater proportion of men than women showed an increase in lesion number during the follow-up period (men: 51.8%; women: 39.4%; $chi^2 = 4.26$, $\underline{p} < .05$). Distributions of baseline plaque lesion number and change in lesions for the entire sample and by sex are shown in Figures 17 through 22.

Baseline IMT score was positively correlated with the presence of plaque lesions at baseline ($\underline{r} = .46$, $p \leq .001$). IMT change was positively correlated with increase in number of plaque lesions during the follow-up ($\underline{r} = .31$, $p \leq .001$).

Table 4: Average baseline, follow-up and change values for IMT

	Sample ($\underline{n}$ = 276)	Men ($\underline{n}$ = 139)	Women ($\underline{n}$ = 137)	Sex Difference $\underline{p}$
Baseline IMT	.77 (.15)	.79 (.16)	.74 (.14)	< .02
Follow-up IMT	.85 (.22)	.91 (.24)	.80 (.19)	< .001
IMT Change	.09 (.14)	.12 (.16)	.05 (.11)	< .001

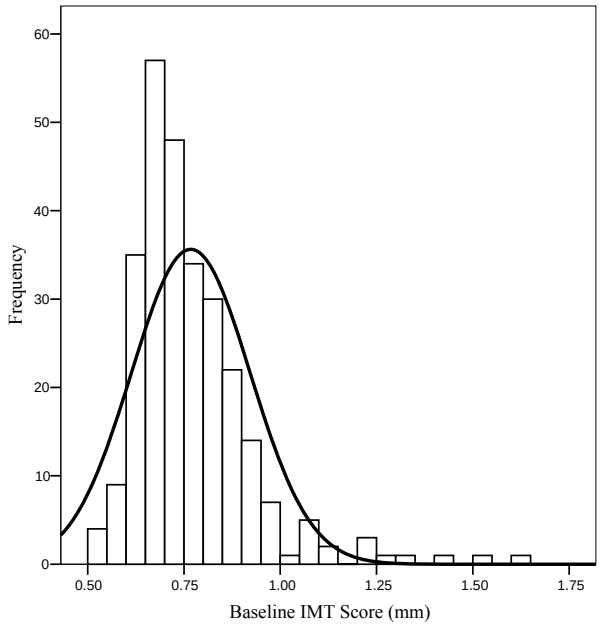


Figure 11: Distribution of Baseline IMT Scores—Entire Sample

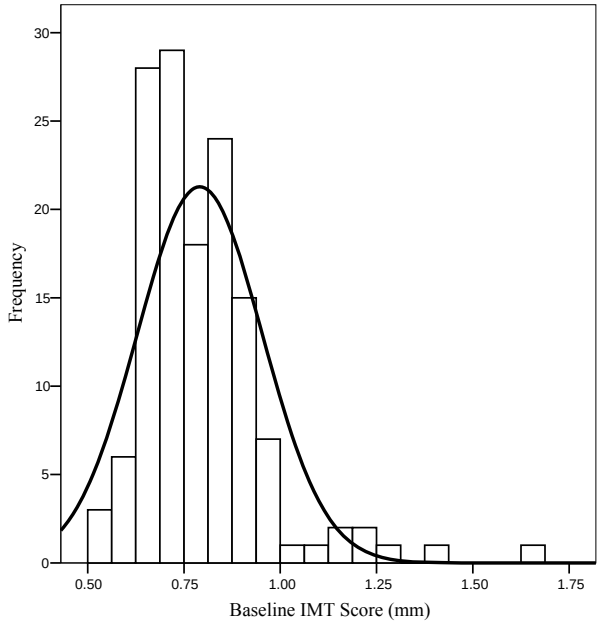


Figure 12: Distribution of Baseline IMT Scores—Men

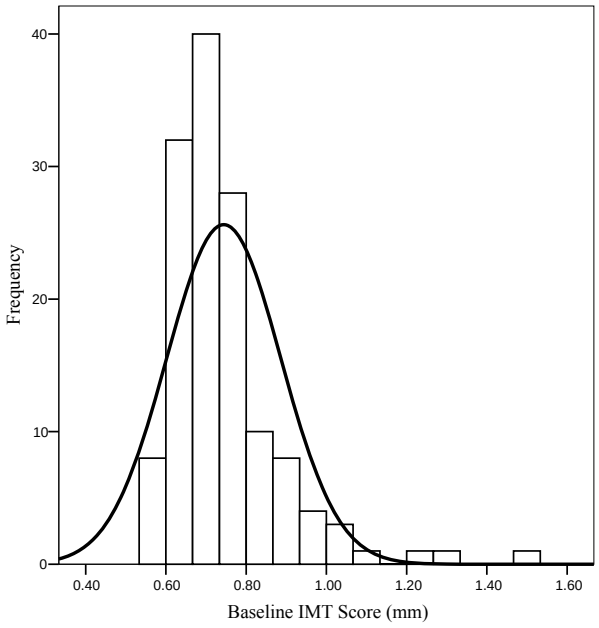


Figure 13: Distribution of Baseline IMT Scores—Women

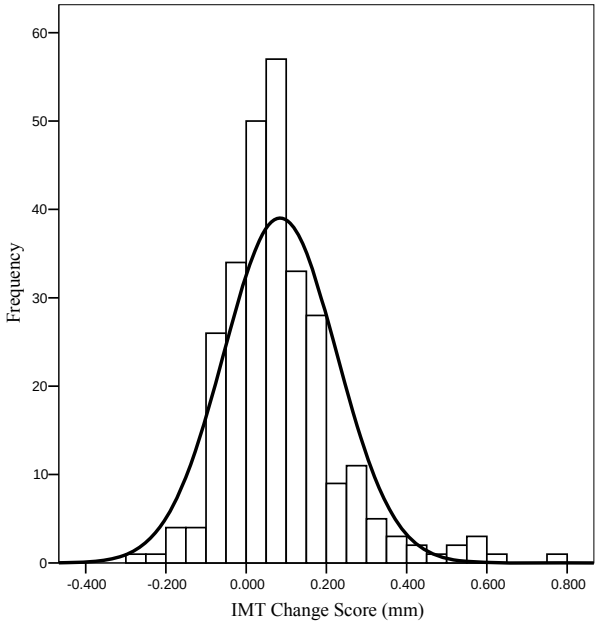


Figure 14: Distribution of IMT Change Scores—Entire Sample

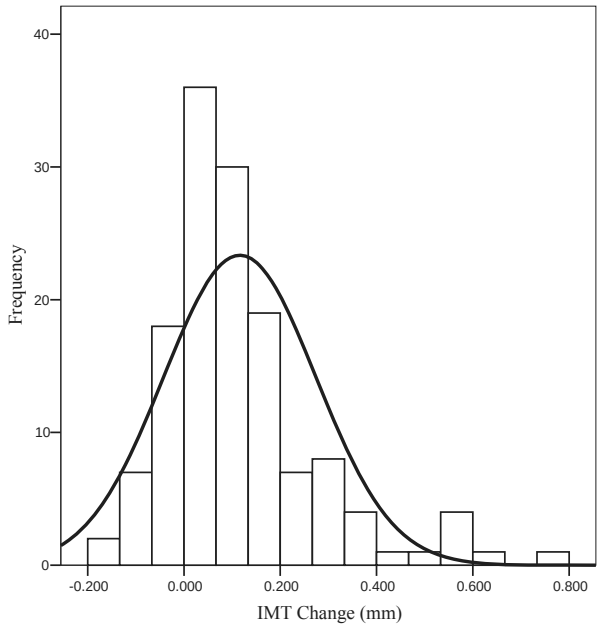


Figure 15: Distribution of IMT Change Scores—Men

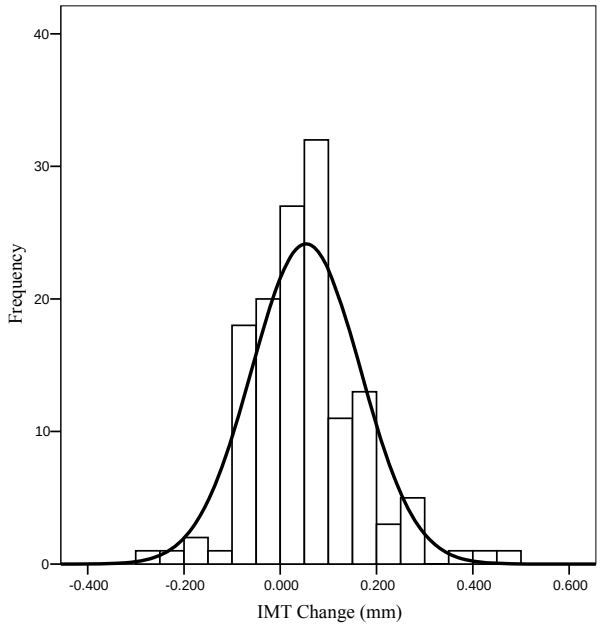


Figure 16: Distribution of IMT Change Scores—Women

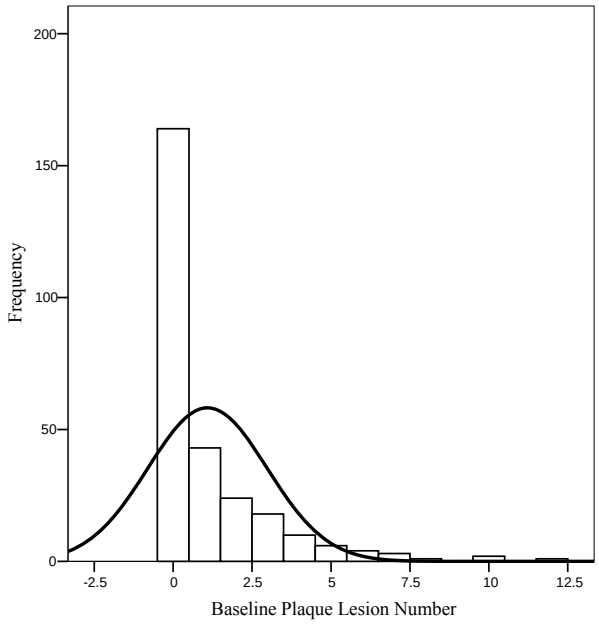


Figure 17: Baseline Plaque Lesion Number--Entire Sample

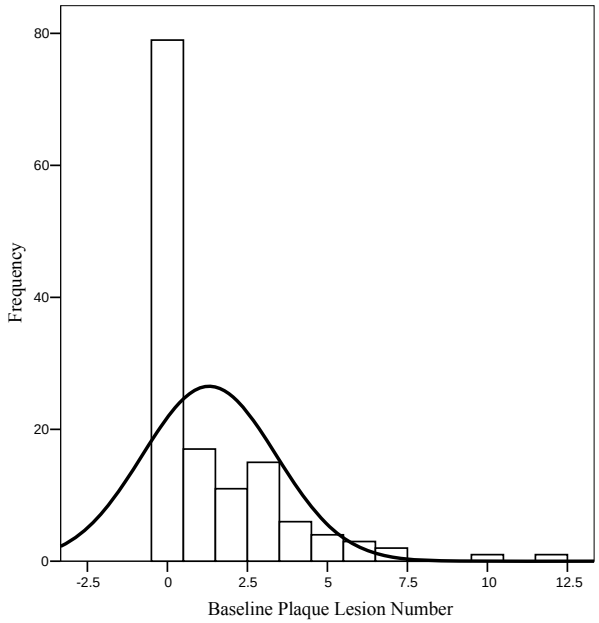


Figure 18: Baseline Plaque Lesion Number—Men

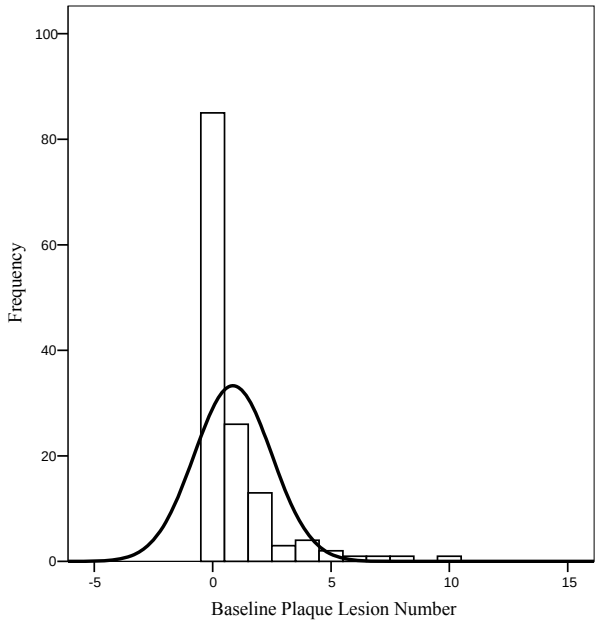


Figure 19: Baseline Plaque Lesion Number—Women

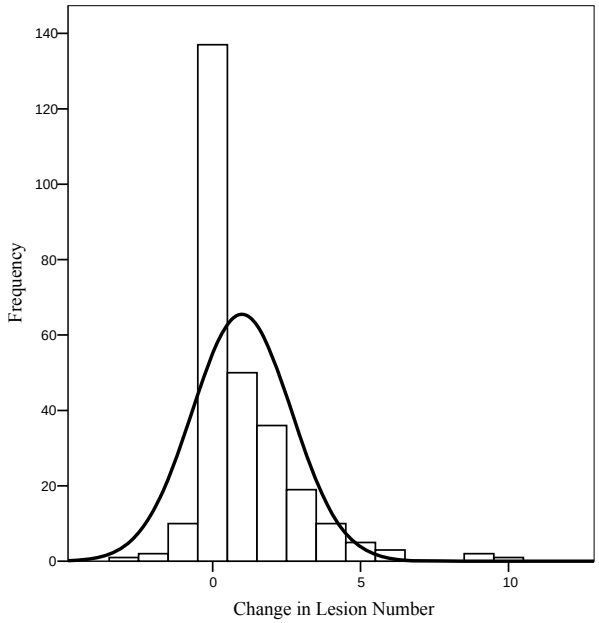


Figure 20: Change in Plaque Lesion Number--Entire Sample

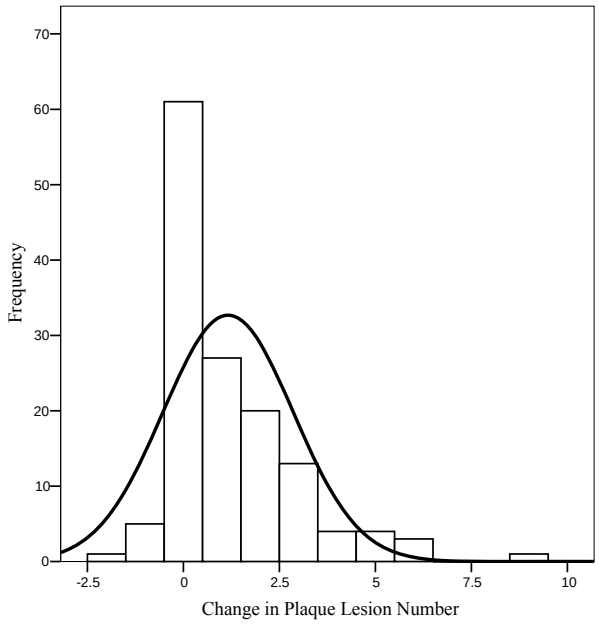


Figure 21: Change in Plaque Lesion Number—Men

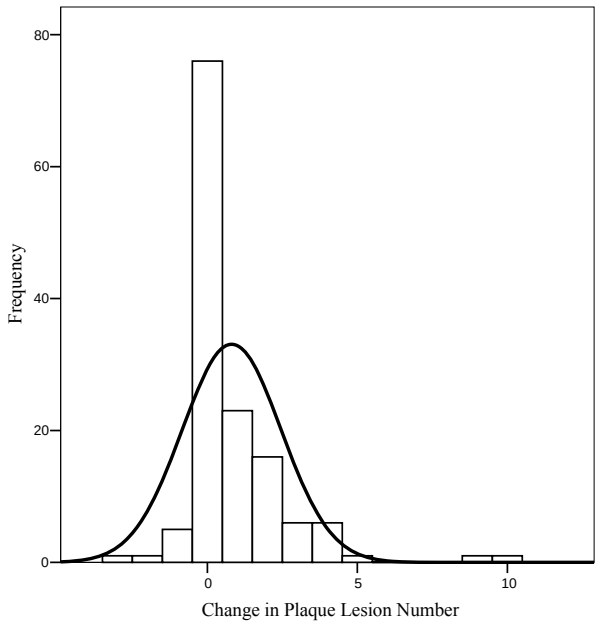


Figure 22: Change in Plaque Lesion Number--Women

5.2 COVARIATE SELECTION

Table 5 displays results of univariate Pearson correlation analyses of IMT and plaque change with potential baseline covariates. Tables 6 and 7 display results from analyses for men and women, respectively. Correlations of baseline IMT and plaque with candidate variables are displayed in Tables 5 through 7 as well. As indicated by Table 5, sex, HDL, alcohol consumption, and prescription pain medication use were significantly related to IMT change, with male sex, alcohol, and pain medication being related to greater IMT progression, and higher HDL being related to less progression. IMT change also was positively correlated with baseline IMT (r=.15, p<.05). Significant correlates of plaque change were sex, race, SBP, pulse pressure, HDL, and waist circumference. Male sex, non-Caucasian race, higher SBP, greater pulse pressure, and larger waist circumference each were related to increase in plaque lesion number, whereas higher HDL was associated with lack of or negative change in lesions. More baseline plaque also was related to greater likelihood of plaque increase over time (r=.22, p<.001).

When correlations were examined separately by sex, weight-related factors (BMI, waist circumference, and insulin resistance) emerged as additional correlates of plaque change among men. Among women, use of pain medication emerged as an additional correlate of both IMT and plaque change. Also among women, LDL rather than HDL correlated with plaque change.

Although several covariates correlated significantly with IMT and plaque when examined in univariate analyses, many failed to reach significance when entered into multivariate regression, thus resulting in only a few covariates being included in each of the final models. Using the procedure described in the Methods, baseline IMT, age, sex, and the age-by-sex cross-product[9] were selected as covariates for the models predicting IMT change. By comparison, analyses for plaque progression resulted in number of baseline plaque lesions, pulse pressure, and HDL being selected as covariates. Table 8 displays beta coefficients and associated significance levels for each of the selected covariates of IMT change.[10] Table 9 displays odds ratios and corresponding confidence intervals for selected covariates of plaque change.[11]

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[9] Previous analysis showed that the age-by-sex cross-product was an independent predictor of IMT change.
[10] Covariates for IMT analyses by sex: men = baseline IMT and age; women = baseline IMT, age, pain medication
[11] Covariates for plaque analyses by sex: men = baseline plaque, pp, and BMI; women = baseline plaque, LDL, and pain medication

Table 5: Pearson Correlations of IMT and Plaque with Candidate Baseline Covariates

	IMT		Plaque	
	Baseline	Change	Baseline	Change
Age	.25***	.03	.22***	.11†
Sex (0 = male, 1 = female)	-.15*	-.22***	-.05	-.12*
Race (1 = white, 2 = non-white)	-.06	-.11†	-.05	-.14*
Education	-.05	.02	-.17**	-.04
Systolic Blood Pressure (mmHg)	.13*	-.01	.09	.17**
Diastolic Blood Pressure (mmHg)	-.03	.01	-.01	.02
Pulse Pressure (SBP-DBP)	.18**	-.02	.11†	.19**
Total Cholesterol	.01	-.01	.00	.10†
LDL	.08	.04	.05	.11†
HDL	-.20**	-.14*	-.14*	-.15*
Triglycerides	.06	.06	.05	.15*
Insulin Resistance (log-transformed)	.01	.03	.02	.11†
Smoking (1 = yes, 2 = no)	.09	.10†	.16**	.03
Alcohol Use	.09	.14*	.04	.03
Physical Activity	-.04	.06	.02	.06
BMI	.07	.00	.04	.06
Waist Circumference	.13*	.01	.09	.14*
Diabetes Diagnosis	.06	.07	.04	.07
Rheumatologic Condition	.03	.10	.08	-.05
Allergies	-.06	.08	-.00	.07
Corticosteroid Drugs for Asthma	-.04	-.04	-.09	-.03
Prescription Pain Medication	.06	.19*	.10	.11†
Frequency of Aspirin Use	-.02	-.09	.04	-.05

† $p < .10$. * $p < .05$. ** $p < .01$. ***$p < .001$

Table 6: Pearson Correlations of IMT and Plaque with Candidate Baseline Covariates—Men

	IMT		Plaque	
	Baseline	Change	Baseline	Change
Age	.27**	.17*	.34*	.24**
Race (1 = white, 2 = non-white)	-.16†	-.15†	-.17†	-.16†
Education	-.10	-.04	-.20*	-.08
Systolic Blood Pressure (mmHg)	.13	-.08	.17*	.23**
Diastolic Blood Pressure (mmHg)	-.03	-.04	-.04	.03
Pulse Pressure (SBP-DBP)	.17*	-.07	.23**	.27**
Total Cholesterol	.01	.01	.09	.07
LDL	.09	.04	-.02	.02
HDL	-.13	-.06	-.11	-.04
Triglycerides	-.02	.01	-.05	.13
Insulin Resistance (log-transformed)	.03	.02	.04	.20*
Smoking (1 = yes, 2 = no)	.02	.04	.16†	.01
Alcohol Use	.07	.07	.08	-.03
Physical Activity	-.16†	.05	-.04	.11
BMI	.10	.05	.11	.19*
Waist Circumference	.11	.04	.14	.20*
Diabetes Diagnosis	.06	.03	-.01	.06
Rheumatologic Condition	.06	.19*	.14†	-.05
Allergies	-.03	.15†	.03	.18*
Corticosteroid Drugs for Asthma	-.05	-.04	-.11	-.13
Prescription Pain Medication	.08	.13	.07	.03
Frequency of Aspirin Use	-.04	-.15†	.01	-.05

† $p < .10$. * $p < .05$. ** $p < .01$. ***$p < .001$

Table 7: Pearson Correlations of IMT and Plaque with Candidate Baseline Covariates—Women

	IMT		Plaque	
	Baseline	Change	Baseline	Change
Age	.25**	.17†	.09	-.03
Race (1 = white, 2 = non-white)	.06	-.04	.06	-.11
Education	-.09	-.04	-.19*	-.08
Systolic Blood Pressure (mmHg)	.15†	.09	.00	.18*
Diastolic Blood Pressure (mmHg)	-.06	.03	-.00	.10
Pulse Pressure (SBP-DBP)	.23**	.09	.00	-.01
Total Cholesterol	.05	.04	.09	.14
LDL	.07	.04	.10	.20*
HDL	-.17*	-.06	-.16†	.17†
Triglycerides	.14	.06	.18*	.13
Insulin Resistance (log-transformed)	-.02	.05	.01	.03
Smoking (1 = yes, 2 = no)	.16†	.16†	.16†	.04
Alcohol Use	-.02	.06	-.06	-.01
Physical Activity	.05	-.01	.07	-.03
BMI	.05	-.05	-.00	-.04
Waist Circumference	.12	-.06	.04	.08
Diabetes Diagnosis	.02	.15†	.19*	.08
Rheumatologic Condition	.01	.02	.02	-.05
Allergies	-.05	.08	-.02	.01
Corticosteroid Drugs for Asthma	-.03	-.06	-.07	.11
Prescription Pain Medication	.04	.31***	.13	.19*
Frequency of Aspirin Use	-.04	-.06	.06	-.08
Hormone Replacement Therapy	-.06	-.05	-.05	-.14

† $p < .10$. * $p < .05$. ** $p < .01$. ***$p < .001$

Table 8: Results of Multivariate Regression of IMT Change on Selected Covariates

	b	t	p
Baseline IMT	.11	1.92	.06
Age	.01	2.61	.01
Sex	.49	2.43	.02
Age*Sex	-.01	-2.72	.007

Table 9: Results of Multivariate Logistic Regression of Plaque Change on Selected Covariates

Stress	Odds Ratio	Confidence Interval	p
Baseline Lesion Number	1.24	1.06, 1.44	.000
HDL	.98	.96, .995	.012
Pulse Pressure	1.03	1.01, 1.06	.002

Tables 10 through 12 display correlations of CRP and IL-6 with the candidate variables for the entire sample and separately by sex. Higher levels of CRP were associated with female sex, less education, higher triglyceride levels, greater insulin resistance, greater likelihood of being a smoker, less physical activity, greater BMI, larger waist circumference, and use of prescription pain medication. By comparison, higher IL-6 concentrations were associated with greater insulin resistance, less physical activity, greater BMI, larger waist circumference, greater likelihood of having a rheumatologic condition, and pain medication use.

When correlations were examined separately by sex, education emerged as a significant predictor of IL-6 among men. Among women, age emerged as a significant correlate of CRP. Contrary to what might be expected, older age was associated with lower CRP levels among the women in the present sample.

Table 10: Pearson Correlations of Inflammatory Markers with Candidate Baseline Covariates

	CRP	IL-6
Age	.12†	-.01
Sex (0 = male, 1 = female)	.20**	.02
Race (1 = white, 2 = non-white)	.07	.08
Education	-.22***	-.11†
Systolic Blood Pressure (mmHg)	.05	.06
Diastolic Blood Pressure (mmHg)	.09	.11†
Pulse Pressure (SBP-DBP)	-.00	-.01
Total Cholesterol	.12†	-.08
LDL	.05	-.11†
HDL	.02	-.09
Triglycerides	.14*	.11†
Insulin Resistance (log-transformed)	.20**	.21**
Smoking (1 = yes, 2 = no)	.16*	.17**
Alcohol Use	-.01	.04
Physical Activity	-.17**	-.12*
BMI	.33***	.34***
Waist Circumference	.29***	.29***
Diabetes Diagnosis	.05	.00
Rheumatologic Condition	.08	.20**
Allergies	.08	.03
Corticosteroid Drugs for Asthma	-.06	-.05
Prescription Pain Medication	.24***	.16**
Frequency of Aspirin Use	-.03	.03

† $p < .01$. * $p < .05$. ** $p < .01$. ***$p < .001$

Table 11: Pearson Correlations of Inflammatory Markers with Candidate Baseline Covariates—Men

	CRP	IL-6
Age	-.05	.02
Race (1 = white, 2 = non-white)	.07	.09
Education	-.20*	-.19*
Systolic Blood Pressure (mmHg)	.12	.08
Diastolic Blood Pressure (mmHg)	.06	.09
Pulse Pressure (SBP-DBP)	.11	.04
Total Cholesterol	.15†	-.09
LDL	.14	-.13
HDL	-.17†	-.09
Triglycerides	.13	.09
Insulin Resistance (log-transformed)	.15†	.09
Smoking (1 = yes, 2 = no)	.24**	.21*
Alcohol Use	.15†	.09
Physical Activity	-.10	-.08
BMI	.31***	.20*
Waist Circumference	.29**	.17*
Diabetes Diagnosis	.08	-.02
Rheumatologic Condition	.19*	.33***
Allergies	.03	.07
Corticosteroid Drugs for Asthma	-.13	-.07
Prescription Pain Medication	.32***	.24**
Frequency of Aspirin Use	.02	.05

† $p < .01$. * $p < .05$. ** $p < .01$. ***$p < .001$

Table 12: Pearson Correlations of Inflammatory Markers with Candidate Baseline Covariates-Women

	CRP	IL-6
Age	-.23**	-.05
Race (1 = white, 2 = non-white)	.06	.07
Education	-.16†	-.01
Systolic Blood Pressure (mmHg)	-.03	.04
Diastolic Blood Pressure (mmHg)	.15†	.14
Pulse Pressure (SBP-DBP)	-.15	-.06
Total Cholesterol	.05	-.08
LDL	-.03	-.09
HDL	-.02	-.12
Triglycerides	.25**	.16†
Insulin Resistance (log-transformed)	.28**	.33***
Smoking (1 = yes, 2 = no)	.11	.12
Alcohol Use	-.02	-.00
Physical Activity	-.19*	-.18*
BMI	.40***	.48***
Waist Circumference	.36***	.42***
Diabetes Diagnosis	.10	.12
Rheumatologic Condition	-.02	.06
Allergies	.06	-.03
Corticosteroid Drugs for Asthma	.04	-.02
Prescription Pain Medication	.16†	.07
Frequency of Aspirin Use	-.06	.01
Hormone Replacement Therapy	.08	-.19*

† $p < .01$. * $p < .05$. ** $p < .01$. ***$p < .001$

Covariate selection analyses identified sex, baseline BMI, baseline triglyceride levels, baseline tobacco use and baseline use of prescription-strength analgesic drugs as variables that should be included in analyses to predict CRP in the entire sample. By comparison, baseline BMI, rheumatologic conditions, and tobacco use were selected as covariates for IL-6 analyses. Beta coefficients and associated significance levels for each of the selected covariates of CRP and IL-6 are displayed in Tables 13 and 14, respectively.[12] [13]

Table 13: Results of Multivariate Regression of CRP Concentration on Selected Covariates

Stress	b	t	p
Sex	.13	4.75	<.001
BMI	.02	2.61	<.001
Triglycerides	.12	2.11	.04
Tobacco Use	.15	3.49	.001
Prescription Pain Med.	.19	2.91	.004

Table 14: Results of Multivariate Regression of IL-6 Concentration on Selected Covariates

Stress	b	t	p
BMI	.01	6.09	<.001
Rheumatologic Cond.	.11	2.94	.004
Tobacco Use	.08	3.39	.001

Because of the important effects of age on IMT, plaque, and inflammatory markers, all correlations involving these measures and potential covariates were re-run with additional control for age (data not shown). Results of these partial correlations did not differ to any notable extent from the univariate results reported here.

[12] Covariates for CRP analyses by sex: men =BMI, smoking, pain medication; women = age, BMI, triglycerides.
[13] Covariates for IL-6 analyses by sex: men = BMI, smoking, rheumatologic condition; women = BMI.

5.3 MAIN ANALYSES

5.3.1 CSS as a predictor of 3-year change in IMT and plaque

Table 15 displays univariate correlations between CSS subscale scores and measures of carotid artery IMT and plaque. As shown in the table, neither the average CSS score nor any of the individual subscales were correlated with baseline IMT, baseline plaque, or plaque change. However, marital stress showed a significant inverse correlation with IMT change such that reports of higher marital stress were associated with less IMT progression. Visual inspection of IMT change by quintile of marital stress score revealed that the significant negative correlation appeared to be due primarily to comparatively low IMT change among participants whose marital stress scores were in the highest quintile and comparatively high IMT change among participants in the second lowest quintile. By comparison, a smooth but subtle increase in IMT change can be seen with increasing levels of physical stress (see Appendix C).

Table 15: Pearson correlations between CSS scores and carotid artery measures

	Baseline IMT	IMT Change	Baseline Plaque	Plaque Change
Average CSS	-.05	.05	-.01	-.04
Marital	-.08	-.16*	-.07	-.02
Parental	-.12†	-.06	-.05	-.06
Filial	-.05	-.01	-.07	-.03
Occupational	-.02	.10	-.05	-.09
Ecological	-.03	.05	.04	-.06
Financial	.02	.08	-.03	-.02
Physical	-.01	.11†	.10	-.01

* < .05. † < .10.

Table 16 displays results from multivariate linear regression analyses with IMT change as the criterion variable. After controlling for baseline IMT, age, sex, and the age-by-sex interaction, the association between marital stress and 3-year IMT change failed to achieve statistical significance. By comparison, addition of the above covariates resulted in the emergence of physical stress as an independent predictor of IMT change, such that higher levels of physical stress were associated with greater progression. No other associations were significant.

Table 16: CSS as predictor of 3-year IMT change*

Stress	b	t	p
Average CSS	.01	.84	.40
Marital	-.007	-1.91	.06
Parental	-.001	-.49	.63
Filial	-.0001	-.08	.94
Occupational	.003	1.09	.28
Ecological	.002	1.02	.31
Financial	.002	.84	.40
Physical	.02	2.13	.03

*Includes controls for baseline IMT, age, sex, age-by-sex

Table 17 displays results from multivariate logistic regression analyses with change in plaque as the criterion variable. Additional statistical control for significant covariates (number of plaque lesions at baseline, HDL cholesterol, and pulse pressure) did not reveal any significant associations between chronic stress and 3-year plaque change.

Table 17: CSS as predictor of 3-year plaque change

Stress	Odds Ratio	Confidence Interval	p
Average CSS	.88	.55, 1.39	.58
Marital	1.02	.90, 1.14	.80
Parental	.97	.89, 1.06	.50
Filial	1.00	.95, 1.06	.97
Occupational	.93	.82, 1.05	.24
Ecological	.97	.90, 1.05	.41
Financial	.97	.90, 1.06	.51
Physical	1.02	.88, 1.18	.81

*Includes controls for baseline plaque, HDL cholesterol, pulse pressure

5.3.2 Inflammatory markers as predictors of IMT and plaque change

Table 18 displays univariate correlations between the 2 measured inflammatory markers (CRP, IL-6), and measures of carotid artery IMT and plaque. As indicated by the table, neither inflammatory marker was related to baseline or change variables. In multivariate analyses that controlled for baseline IMT, age, sex, and the interaction of age with sex, neither CRP nor IL-6 emerged as a significant predictor of 3-year IMT change (CRP: b = -.03, t = -1.07, p = .29. IL-6: b = -.001, t = -.01, p = .99). Similarly, in multivariate logistic regression analyses that controlled for baseline lesion number, HDL cholesterol, and pulse pressure, CRP failed to emerge as a significant predictor of 3-year change in plaque (OR = .84, CI = .29, 2.48). By comparison, IL-6 did emerge as an independent predictor of plaque change, but in a direction that was opposite to that which was predicted with higher levels of IL-6 being associated with a decreased likelihood of plaque progression during the follow-up period (OR = .10, CI = .01, .80).

Table 18: Correlations between inflammatory markers and carotid artery measures

	Baseline IMT	IMT Change	Baseline Plaque	Plaque Change
CRP	.04	-.07	.03	-.01
IL-6	.003	.001	.08	-.10

* < .05. † < .10.

5.3.3 CSS as predictor of baseline inflammatory marker levels

Table 19 displays univariate correlations between CSS subscales and each of the measured inflammatory markers. As shown in the table, only the physical stress subscale was significantly correlated with CRP, such that higher levels of stress were associated with higher CRP levels. None of the CSS subscales were significantly correlated with levels of IL-6. Visual inspection of CRP levels by zero, low, middle, and high physical stress groups reveals an apparently linear increase in CRP levels with increasing physical stress (see Appendix D).

Tables 20 and 21 display results from multivariate regression analyses for CRP and IL-6, respectively. After controlling for sex, BMI, triglycerides, smoking status, and use of prescription pain medications, physical stress failed to emerge as an independent predictor of CRP. Further analysis indicated that BMI and anti-inflammatory medication use each independently accounted for the majority of the reduction in effect associated with physical stress. None of the remaining CSS subscales emerged as significant predictors of either CRP or IL-6.

Table 19: Correlations between CSS scores and inflammatory markers

Stress	CRP	IL-6
Average CSS	.11†	.05
Marital	.09	.07
Parental	.02	-.08
Filial	-.01	-.01
Occupational	-.04	.01
Ecological	.10	.07
Financial	.05	.06
Physical	.14*	.10†

* < .05. † < .10.

Table 20: CSS as predictor of baseline CRP*

Stress	b	T	p
Average CSS	.019	.79	.43
Marital	.005	.87	.39
Parental	-.002	-.49	.62
Filial	.002	.60	.55
Occupational	-.005	-.75	.45
Ecological	.003	.76	.45
Financial	.003	.72	.47
Physical	.0005	.05	.96

* Includes controls for sex, BMI, triglycerides, smoking status, use of prescription pain medication

Table 21: CSS as predictor of baseline IL-6*

Stress	b	t	p
Average CSS	-.002	-.13	.90
Marital	.001	.22	.83
Parental	-.003	-1.26	.21
Filial	.001	.56	.58
Occupational	-.001	-.35	.73
Ecological	.001	.61	.54
Financial	.0003	.13	.90
Physical	-.0002	-.04	.97

* Includes controls for BMI, current rheumatologic condition, smoking status

5.3.4 Examination of mediation model

The primary aim of the present study was to examine whether inflammatory marker levels mediate the association between chronic stress and progression of carotid artery IMT and plaque. As indicated above, the only positive predictive association between stress and the outcome variables was that reported for physical stress and IMT change. However, neither CRP nor IL-6 predicted change in IMT. Moreover, none of the CSS variables were independently related to CRP or IL-6. Thus, it would be of little benefit to test the proposed mediation model.

5.3.5 CVR as moderator of the association between chronic stress and IMT and plaque change

Although the majority of CSS variables were unrelated to change in IMT or plaque in the entire sample, it remains possible that stress may yet be a significant predictor of the these 2 outcomes among persons who show higher CVR. In other words, even though the main effects of most

CSS subscales were not significant, the interactive effect of stress with CVR may be. Tables 22 through 24 display results of analyses that examined interactions of stress with systolic, diastolic, or heart rate reactivity as predictors of change in IMT; Tables 23 through 25 display results of analogous analyses that were conducted for change in plaque. Neither systolic nor heart rate reactivity appeared to moderate the association between stress and IMT change. However, as indicated by Table 23, diastolic reactivity emerged as a significant moderator of the association between filial stress and change in IMT. We explored this interaction effect by examining the association between filial stress and IMT at one standard deviation above and below the centered mean of diastolic reactivity. Results showed that the direction of association between filial stress and IMT differed depending on whether assessed among those exhibiting high or low diastolic reactivity. Specifically, more filial stress was associated with less IMT change when examined at high reactivity and with greater IMT change when examined at low reactivity. In neither analysis, however, did the effect of filial stress achieve statistical significance (high reactivity: $\underline{b}$ = -.003, $\underline{t}$ = -1.56, $\underline{p}$ = .12; low reactivity: $\underline{b}$ = .003, $\underline{t}$ = 1.39, $\underline{p}$ = .17). In no instance was CVR a significant moderator of the effect of stress on plaque change.

Table 22: CSS-by-systolic reactivity on IMT change*

Stress	$\underline{b}$	$\underline{t}$	$\underline{p}$
Average CSS x $CVR_{systolic}$	-.007	-.44	.66
Marital x $CVR_{systolic}$	-.002	-.36	.72
Parental x $CVR_{systolic}$	.001	.51	.61
Filial x $CVR_{systolic}$	.0001	.05	.96
Occupational x $CVR_{systolic}$	.0003	.08	.94
Ecological x $CVR_{systolic}$	-.0005	-.19	.85
Financial x $CVR_{systolic}$	-.001	-.39	.70
Physical x $CVR_{systolic}$	-.0007	-.13	.90

* Includes controls for baseline IMT, age, sex, age-by-sex

Table 23: CSS-by-diastolic reactivity on IMT change*

Stress	b	t	p
Average CSS x $CVR_{diastolic}$	-.02	-1.06	.29
Marital x $CVR_{diastolic}$	.0007	.12	.91
Parental x $CVR_{diastolic}$	-.003	-.87	.39
Filial x $CVR_{diastolic}$	-.005	-2.11	.04
Occupational x $CVR_{diastolic}$	-.0005	-.11	.91
Ecological x $CVR_{diastolic}$	-.002	-.58	.57
Financial x $CVR_{diastolic}$	-.0003	-.07	.95
Physical x $CVR_{diastolic}$	-.0001	-.02	.98

* Includes controls for baseline IMT, age, sex, age-by-sex

Table 24: CSS-by-heart rate reactivity on IMT change

Stress	b	t	p
Average CSS x $CVR_{heart\ rate}$	-.01	-.91	.36
Marital x $CVR_{heart\ rate}$	.002	.53	.60
Parental x $CVR_{heart\ rate}$	-.0009	-.30	.76
Filial x $CVR_{heart\ rate}$	-.005	-.26	.79
Occupational x $CVR_{heart\ rate}$	.003	.61	.55
Ecological x $CVR_{heart\ rate}$	-.002	-.66	.51
Financial x $CVR_{heart\ rate}$	-.004	-1.20	.23
Physical x $CVR_{heart\ rate}$	-.002	-.43	.67

* Includes controls for baseline IMT, age, sex, age-by-sex

Table 25: CSS-by-systolic reactivity on plaque change*

Stress	B	S.E.	Wald Chi-Square	Sig.
Average CSS x $CVR_{systolic}$	-.23	.29	.61	.43
Marital x $CVR_{systolic}$	-.05	.08	.44	.51
Parental x $CVR_{systolic}$	-.04	.05	.71	.40
Filial x $CVR_{systolic}$	.04	.04	1.02	.31
Occupational x $CVR_{systolic}$	-.02	.08	.03	.85
Ecological x $CVR_{systolic}$	-.05	.05	.95	.33
Financial x $CVR_{systolic}$	.03	.06	.21	.65
Physical x $CVR_{systolic}$	-.15	.10	2.24	.13

*Includes controls for baseline plaque, HDL cholesterol, pulse pressure

Table 26: CSS-by-diastolic reactivity as predictor of 3-year plaque change*

Stress	B	S.E.	Wald Chi-Square	Sig.
Average CSS x $CVR_{diastolic}$	-.20	.32	.37	.54
Marital x $CVR_{diastolic}$	-.05	.10	.25	.62
Parental x $CVR_{diastolic}$	-.09	.06	2.36	.12
Filial x $CVR_{diastolic}$	.003	.04	.008	.93
Occupational x $CVR_{diastolic}$	-.02	.08	.09	.76
Ecological x $CVR_{diastolic}$	-.06	.06	1.28	.26
Financial x $CVR_{diastolic}$	.05	.07	.45	.50
Physical x $CVR_{diastolic}$	-.01	.12	.01	.91

* Includes controls for baseline plaque, HDL cholesterol, pulse pressure

Table 27: CSS-by-heart rate reactivity as predictor of 3-year plaque change*

Stress	B	S.E.	Wald Chi-Square	Sig.
Average CSS x $CVR_{heart\ rate}$	-.02	.29	.003	.96
Marital x $CVR_{heart\ rate}$	-.007	.08	.008	.93
Parental x $CVR_{heart\ rate}$	.03	.05	.22	.64
Filial x $CVR_{heart\ rate}$	.001	.04	.0004	.98
Occupational x $CVR_{heart\ rate}$	-.02	.09	.08	.78
Ecological x $CVR_{heart\ rate}$	.06	.05	1.18	.28
Financial x $CVR_{heart\ rate}$	-.005	.06	.006	.94
Physical x $CVR_{heart\ rate}$	-.17	.11	2.51	.11

* Includes controls for baseline plaque, HDL cholesterol, pulse pressure

5.4 EXPLORATORY ANALYSES

5.4.1 Moderating effects of sex and age

In exploratory analyses, we investigated whether the present study hypotheses might be supported if examined separately (a) by sex; and (b) among older and younger participants. We explored the effects of sex and age first by examining each as a moderating variable (i.e., included interaction terms involving (a) stress and (b) sex or age, respectively).[14]

5.4.1.1 Moderating effects of sex

Tables 28 and 29 display results from analyses that examined whether sex moderated the association between stress and IMT and plaque progression, respectively. As shown in the

[14] Results of sex-stratified analyses are presented in Appendix E.

tables, none of the CSS-by-sex interactions emerged as significant effects for either cardiovascular outcome measure.

Table 28: CSS-by-sex as predictor of 3-year IMT change*

Stress	b	t	p
Average CSS x Sex	-.03	-1.17	.24
Marital x Sex	-.003	-.44	.66
Parental x Sex	-.004	-.75	.45
Filial x Sex	-.004	-1.42	.16
Occupational x Sex	-.004	-.58	.56
Ecological x Sex	-.006	-1.37	.17
Financial x Sex	.006	1.18	.24
Physical x Sex	-.008	-.92	.36

* Includes controls for baseline IMT, age

Table 29: CSS-by-sex as predictor of 3-year plaque change*

Stress	Odds Ratio	Confidence Interval	p
Average CSS x Sex	1.58	.62, 4.02	.33
Marital x Sex	1.18	.93, 1.51	.18
Parental x Sex	1.04	.88, 1.23	.68
Filial x Sex	1.02	.92, 1.14	.69
Occupational x Sex	.93	.72, 1.19	.55
Ecological x Sex	1.04	.89, 1.21	.62
Financial x Sex	1.15	.97, 1.37	.12
Physical x Sex	1.02	.76, 1.38	.88

* Includes controls for baseline plaque, HDL cholesterol, pulse pressure

Tables 30 and 31 display results from analyses that examined whether sex moderated the association between stress and CRP and IL-6, respectively. Inspection of Table 30 reveals a significant moderating effect of sex on the relation between financial stress and CRP. We explored the significant financial stress-by-sex interaction by examining the simple effect of financial stress on CRP in separate models for men and women. Results for men showed a nonsignificant trend for greater financial stress to be associated with higher levels of CRP (b = .01, t = 1.82, p = .07). Among women, financial stress was unrelated to CRP levels (b = -.01, t = -.80, p = .43). No other stress-by-sex effects were significant when CRP was examined as the outcome.

Table 30: CSS-by-sex as predictor of baseline CRP

Stress	b	t	P
Average CSS x Sex	.01	.21	.83
Marital x Sex	-.01	-.53	.59
Parental x Sex	.01	1.37	.17
Filial x Sex	.01	1.46	.15
Occupational x Sex	.01	.90	.37
Ecological x Sex	-.00	-.00	.99
Financial x Sex	-.02	-2.12	.04
Physical x Sex	-.01	-.87	.39

* Includes controls for sex, BMI, triglycerides, smoking status, use of prescription pain medication

Inspection of Table 31 reveals a significant moderating effect of sex on the relation between parental stress and IL-6. Examination of the simple effect for men showed a significant inverse association between parental stress and IL-6, such that greater parental stress was associated with lower levels of IL-6 (b = -.01, t = -2.09, p < .05). By comparison, parental stress was unrelated to IL-6 levels in women (b = .002, t = 0.68, p = .50). No other stress-by-sex effects were significant when IL-6 was examined as the outcome.

Table 31: CSS-by-sex as predictor of baseline IL-6*

Stress	b	t	p
Average CSS x Sex	.01	.19	.85
Marital x Sex	.01	2.52	.13
Parental x Sex	.01	2.24	.03
Filial x Sex	-.0001	-.03	.98
Occupational x Sex	-.01	-1.20	.23
Ecological x Sex	-.0001	-.04	.97
Financial x Sex	-.0005	-.28	.78
Physical x Sex	-.01	-1.15	.25

* Includes controls for BMI, current rheumatologic condition, smoking status

5.4.1.2 Moderating effects of age

Tables 32 and 33 display results from analyses that examined whether age moderated the association between stress and IMT and plaque progression, respectively. As shown in the tables, none of the CSS-by-age interactions emerged as significant effects for either cardiovascular outcome measure.

Tables 34 and 35 display results from analyses that examined whether age moderated the association between stress and CRP and IL-6, respectively. Inspection of Table 34 reveals significant moderating effects of age on the relation between 2 of the CSS subscales and levels of CRP: filial stress and occupational stress. We explored these significant interaction effects by examining the respective effects of these 2 stress domains at one standard deviation above and below the mean age of the sample (60.5 years). As might be expected, more filial stress was associated with higher levels of CRP among older participants (b = .01, t = 2.75, p < .01). Unexpectedly, filial stress was associated with lower CRP levels among younger participants (b = -.02, t = -2.45, p < .05). By comparison, the simple effect of occupational stress on CRP levels did not reach significance when examined among older participants (b = .01, t = 1.07, p = .29).

Among younger participants, however, occupational stress was associated with lower CRP levels (b = -.02, t = -1.98, p < .05).

Inspection of Table 35 reveals a significant moderating effect of age on the association between parental stress and levels of IL-6. Analysis of simple effects revealed a significant inverse association between parental stress and IL-6 among younger participants such that more parental stress was associated with lower IL-6 levels (b = -.01, t = -2.54, p < .05). Among older participants, parental stress was unrelated to IL-6 (b = .003, t = .76, p = .45). No other stress-by-age interaction effects reached significance.

Because both sex and age appeared to moderate the effect of parental stress on IL-6, we explored the sex-by-age-by-parental stress 3-way interaction. This complex interaction was not significant (b = .0001, t = .11, p = .91). However, when analyses were conducted separately by sex, the moderating effect of age was significant only among the men (men: b= -.009, t = -2.25, p < .05; women: b = .003, t = 0.84, p = .40). Examination of simple slopes revealed that more parental stress was associated with lower IL-6 levels among younger men (b = -.02, t = -3.04, p < .01). Among older men, parental stress was unrelated to IL-6 (b = -.001, t = -.29, p =.77).

Table 32: CSS-by-age as predictor of 3-year IMT change*

Stress	b	t	p
Average CSS x Age	.002	.63	.53
Marital x Age	-.0002	-.22	.82
Parental x Age	.0003	.53	.60
Filial x Age	-.00003	-.09	.93
Occupational x Age	.0002	.26	.79
Ecological x Age	-.00001	-.04	.97
Financial x Age	-.0002	-.41	.68
Physical x Age	-001	1.41	.16

* Includes controls for baseline IMT, sex

Table 33: CSS-by-age on 3-year plaque change

Stress	Odds Ratio	Confidence Interval	p
Average CSS x Age	.98	.90, 1.08	.71
Marital x Age	.99	.97, 1.01	.40
Parental x Age	.99	.97, 1.00	.12
Filial x Age	1.00	.98, 1.01	.38
Occupational x Age	.99	.96, 1.02	.52
Ecological x Age	1.00	.99, 1.02	.63
Financial x Age	1.01	.99, 1.02	.52
Physical x Age	1.01	.98, 1.04	.51

* Includes controls for baseline plaque, HDL cholesterol, pulse pressure

Table 34: CSS-by-age as predictor of baseline CRP*

Stress	b	t	p
Average CSS x Age	.01	1.64	.10
Marital x Age	.0004	.32	.75
Parental x Age	.001	1.16	.25
Filial x Age	.002	3.71	< .001
Occupational x Age	.003	2.08	.04
Ecological x Age	-.001	-1.04	.30
Financial x Age	.0003	.35	.72
Physical x Age	.001	.82	.41

* Includes controls for sex, BMI, triglycerides, smoking status, use of prescription pain medication

Table 35: CSS-by-age as predictor of baseline IL-6*

Stress	b	t	p
Average CSS x Age	.001	.32	.75
Marital x Age	-.0004	-.53	.60
Parental x Age	.001	2.32	.02
Filial x Age	.001	1.61	.11
Occupational x Age	.0001	-.14	.89
Ecological x Age	< .0001	-.70	.49
Financial x Age	< .0001	.26	.80
Physical x Age	-.0001	-.69	.49

* Includes controls for BMI, current rheumatologic condition, smoking status

5.5 ANCILLARY ANALYSES

To further clarify the present results, we conducted a set of supplementary analyses. These analyses included examination of (1) baseline IMT as a moderator of the association between CSS subscales and (a) inflammatory markers and (b) IMT change; (2) use of antihypertensive or lipid-lowering medication as a moderator of the association between CSS subscales and (a) inflammatory markers and (b) IMT and plaque change; (3) the utility of a "threshold model" of chronic stress[15] as a predictor of (a) IMT and plaque change and (b) inflammatory markers; (4) inflammatory marker levels as moderators of the association between CSS subscales and IMT and plaque change; and (5) interactions between CSS subscales as predictors of (a) IMT and

[15] CSS subscales were re-scored such that individuals who scored at or above the sample median on a given subscale were assigned a score of "1" on that subscale, whereas persons who scored below the median were assigned a score of "0." The re-scored total CSS score was computed as the sum of the rescored subscales. A second set of analyses was conducted wherein 95th percentile scores rather than median scores were used to define the threshold.

plaque change and (b) inflammatory markers. As results from most of these analyses did little to clarify the present report's major findings, associated data will not be presented here. However, it should be noted that baseline IMT was a significant moderator of the association between physical stress and IMT change ($b = .07$, $t = 2.49$, $p < .05$) such that physical stress predicted greater IMT change among persons with higher levels of baseline IMT ($b = .02$, $t = 3.68$, $p < .001$) but not among those with lower baseline IMT levels ($b = -.0003$, $t = -.04$, $p = .97$).

6.0 DISCUSSION

The primary aim of the present study was to examine whether (a) self-reported chronic life stress predicts 3-year change in carotid artery IMT and plaque, and (b) the association between stress and these 2 markers of subclinical CVD is explained by a mediational pathway involving elevations in circulating inflammatory markers (IL-6 and CRP). Specifically, we anticipated that more life stress would be associated with (a) greater IMT and plaque progression; and (b) higher levels of circulating inflammatory markers. Furthermore, we expected that the elevated inflammatory marker levels associated with high chronic stress would explain, in part, the association between stress and the cardiovascular outcome variables. A secondary aim of the present study was to examine whether individual differences in cardiovascular reactivity to laboratory challenge (CVR) moderates the association between life stress and progression of IMT and plaque. Our expectation was that the association between stress and progression would be stronger among high reactors relative to low reactors. Results did not provide strong support for the above hypotheses. Furthermore, exploratory analyses by sex and age failed to reveal support for the predicted associations among subgroups of the sample population.

6.1 CHRONIC LIFE STRESS AS A PREDICTOR OF IMT AND PLAQUE CHANGE

In contrast to our expectations, the aggregated CSS score was not associated with 3-year change in IMT or plaque. When individual CSS subscales were examined, physical stress emerged as an independent predictor of IMT change, such that higher reports of physical stress were associated with a greater increase in IMT during the follow-up. This finding is difficult to interpret as it is uncertain to what extent psychological versus purely physical dimensions of this type of stress

are contributing to greater IMT progression. It is possible that frustration associated with, for example, having trouble getting around, could be associated with chronic activation of the HPA and SNS which might have implications for early atherogenesis. Alternatively, the association between reports of greater physical stress and greater IMT progression could be due to generally less healthy persons showing more early vascular pathology. In exploratory analyses (not shown here), we attempted to isolate the effect of the subjective, or psychological dimension of physical stress by examining the single item "Did any physical disabilities place an extra burden on you?" Scores on this single item were not significantly associated with IMT change. This lack of statistical association may be due to a true lack of association between the psychological dimension of physical stress and IMT change, or it may be due to the fact that we evaluated the association using only a single-item measure.

Interestingly, ancillary analyses revealed that the association between physical stress and IMT change was moderated by baseline IMT. Specifically, physical stress predicted IMT change when examined among persons whose baseline IMT values were at least one standard deviation above the sample mean but not among those whose IMT values were at least one standard deviation below the mean. Given that physical stress was unrelated to baseline IMT in the present sample, these findings suggest that physical stress may be a better predictor of advanced lesion progression than of initial lesion development.

Also notable is a significant inverse univariate correlation between scores on the marital stress subscale and IMT change, such that more marital stress was associated with less IMT progression. This association was attenuated, however, when marital stress was examined in a model that included covariates for age and sex. In the present sample, women and younger persons reported more marital stress relative to men and older persons. Women and younger persons also showed less progression compared to their male and senior counterparts. Thus, the significant inverse univariate correlation between marital stress and IMT change likely reflected the fact that higher marital stress was reported by persons who because of their sex and age, showed relatively less progression. It is important to note that while the inverse association between marital stress and IMT progression no longer was statistically significant following control for age and sex, a marginal trend remained apparent. Two of the 3 items that comprise the marital stress subscale measure frequency of disagreements between the respondent and his or her spouse over (a) monetary spending and (b) balance of give-and-take. It is possible that

individuals who report more frequent disagreements with their spouses over these issues tend to be the member of the couple who takes greater responsibility for the financial and physical wellbeing of the household and family. In this way, a higher score on the marital stress subscale might be an indirect marker of a generally conscientious personality. Conscientious persons might take better care of their own health, and thus show less IMT progression.

Neither aggregate CSS scores nor scores on any of the individual subscales predicted 3-year change in plaque. Plaque measures are thought to provide a more precise index of true atherosclerotic disease relative to IMT which may be influenced by processes unrelated to atherogenesis. Thus, that physical stress failed to predict change in plaque suggests that the association between physical stress and IMT may be due to a relation with vascular aging rather than atherogenic processes. It should be kept in mind however, that IMT is scored more objectively than plaque. Furthermore, IMT measurement is associated with a sizable body of validation evidence, whereas plaque measurement is not. It is possible that the association between physical stress and plaque change was negatively influenced by the use of a comparatively unreliable dependent variable measure.

The general lack of positive association between chronic stress and the cardiovascular outcomes examined in the present study stands in contrast to existing research that has identified a link between chronic stress and cardiovascular disease risk. One explanation for this apparent discrepancy is that the majority of research on chronic stress and CVD has focused on clinical disease outcomes. It is possible that chronic stress is a more reliable predictor of clinical events than of changes in vascular morphology that may be indicative of earlier, subclinical disease progression. Clinically manifest CVD (i.e., myocardial ischemia, infarction, or sudden cardiac death) is triggered by transient pathologic alterations in cardiovascular physiology such as increased cardiac demand, increased hemoconcentration, coronary constriction, arrhythmia, and plaque rupture. It is possible that the condition of being chronically stressed is associated with exposure to phenomena that elevate the probability of experiencing such pathologic changes. For example, experiences of chronic difficulty have been found to give rise to severe acute life stressors (Brown & Harris, 1989). Research has shown that acute mental stress may act as a trigger for transient ischemic episodes (Gullette et al., 1997), lower the threshold for malignant tachycardia (Verrier & Lown, 1984), and influence plasma volume and density (Patterson, Krantz, & Jochum, 1995). Thus, the association between chronic stress and clinical CVD risk

that has been reported in previous research might be contingent upon the experience of frequent acute stressors. In other words, the condition of chronic stress may elevate the likelihood of experiencing an acute stressor, which then could act as a trigger for an acute coronary event. Future research might investigate the role of acute stressors in the association between chronic stress exposure and CVD risk.

To propose that chronic psychosocial stress may be a better predictor of clinical events than of early, subclinical disease progression is not to suggest that psychosocial factors are unrelated to early cardiovascular pathogenesis. The present study, however, may not have had sufficient power to detect such an association. Six known prospective studies have examined associations between psychosocial factors and IMT and/or plaque progression (Everson, Kaplan et al., 1997; Gallo et al., 2003; Julkunen et al., 1994; J. Lynch, Kaplan et al., 1997; J. Lynch, Krause et al., 1997; Paterniti et al., 2001). The average length of follow-up among these studies was 3.6 years (range 2.0 to 5.2 years), which is comparable to the follow-up time employed by the present study. The average sample size, by comparison, was $\underline{n}$ = 657, with four studies employing sample sizes in excess of $\underline{n}$ = 700. Although five of these six studies reported significant associations between psychosocial factors and selected outcomes, corresponding effect sizes were very small. As reported in an earlier section (see Methods, Table 1), the aggregate effect size calculated across these studies for the association between psychological factors and IMT change was $\underline{r}$ = .11 and between psychological factors and plaque change was $\underline{r}$ = .15. Thus, the corresponding power of the present study to detect these effects was .47 and .71, respectively. It is possible that the present data might have supported the study hypotheses had our sample size been larger.

It should be noted that three of the five studies that reported significant associations between psychosocial factors and IMT and plaque progression were derived from the KIHD study, which recruited participants from a region of Finland that is characterized by residents with high CVD risk factor profiles (Julkunen et al., 1994; J. Lynch, Kaplan et al., 1997; J. Lynch, Krause et al., 1997). It is possible that the effects of psychological factors, such as chronic stress, on subclinical disease progression are more influential among persons who are at high biological or behavioral risk for CVD. In exploratory analyses (data not shown) we examined whether baseline risk factors moderated the association between chronic stress and IMT and plaque progression. For example, we explored whether smokers and persons with higher

baseline cholesterol levels were more likely to show an association between chronic stress and IMT and plaque progression relative to non-smokers and persons with low baseline cholesterol. The results of these analyses provided no significant findings, which largely may have been due to the fact that few participants in the present sample showed an especially high risk factor profile. Nevertheless, our finding that physical stress appeared to influence IMT progression only among persons with higher baseline IMT scores is consistent with the possibility that psychosocial stress may have an exacerbating effect on early CVD progression among persons already at risk.

Another explanation for the discrepancy between the present findings and those reported by previous research involves the employed measure of chronic stress. Preliminary analyses indicated that scores on the CSS correlate in expected directions with scores on established measures of stress and personality. However, the CSS may lack construct validity with regard to CVD risk. In other words, the CSS may not measure the "toxic" features of chronic stress that are relevant to physiologic processes that contribute to IMT and plaque progression. For example, the CSS occupational stress subscale correlated with the demand and workplace social support dimensions of the Karasek Job Content Questionnaire (R.A. Karasek, 1985) but not with the decisional control dimension. Both low job control (Bosma et al., 1997) and high demand/control imbalance (R.A. Karasek et al., 1988) have been associated with elevated CVD risk. Perhaps additional information on participants' perceived level of workplace control would have provided a measure of occupational stress that is more closely associated with subclinical disease progression.

A second issue pertaining to the CSS that may explain the lack of significant findings concerns the reliability of the CSS as a measure of chronic stress. By design, the CSS is a retrospective measure in that it asks respondents to reflect on their experience of stress during the 6-month reporting period. Retrospective reports often are biased by conditions at the time of reporting (A. A. Stone et al., 2000). Said differently, participants' interpretations of CSS items, when encountered on 2 or more occasions, might differ depending on factors present at the time of CSS administration. It also is possible, however, that the phenomena being assessed by the CSS are not stable over the course of a 6-month reporting period. In this way, participants' reports of stress during the preceding 6 months might have been unduly influenced by current stress levels, which may or may not reflect stress experienced during the previous 6 months.

Insofar as current stress is atypical, CSS scores might represent a high or low stress "episode" rather than a chronic stress condition. It has been suggested that episodic stressors might more likely be associated with the transition from subclinical to clinical CVD rather than long term progression of early subclinical disease as might be represented by change in IMT and plaque (Kop, 1997). Thus, if CSS scores reflect episodic rather than chronic stress, it is unlikely that they would correlate with changes in the 2 cardiovascular outcome measures examined in the present study. A replication of the present study might include multiple administrations of the CSS to gain a more reliable measure of participants' experiences of chronic life stress.

In sum, with the exception of the physical stress finding, the present data do not support an association between chronic stress and IMT and plaque progression. This lack of association contrasts with findings of previous research which suggest a link between psychosocial factors and these two markers of early cardiovascular pathogenesis. Two features of the present study likely compromised the ability to detect significant associations: (1) the relative good health of the employed sample; and (2) the limited power of the present study to detect small effect sizes of the kind reported in the extant literature (see Table 1). The small sizes of the effects reported by previous research, in combination with the general lack of effect reported here, suggest that IMT and plaque change may be relatively insensitive indices of the influence of psychosocial factors on cardiovascular health. Given that the average follow-up time employed by existing studies of psychosocial factors and early CVD progression (including the present study) was 3-4 years, it is possible that the apparent insensitivity of IMT and plaque measures to psychosocial influences is due to an insufficient amount of time being allowed to detect a sizable effect. The effects of chronic psychosocial stress, for example, may not be apparent until reaching a certain "temporal threshold." Future research might examine the association between chronic stress and early CVD progression during a longer period of follow-up, and with multiple measures of chronic stress during that time. Future studies also might investigate whether psychosocial stress plays a more influential role in the progression of early cardiovascular pathogenesis among vulnerable or at-risk populations. Potential risk categories might include poor health behaviors, family history of CVD, diabetes, or rheumatic disease, low socioeconomic status, and unhealthy childhood environment.

6.2 INFLAMMATORY MARKERS AS PREDICTORS OF IMT AND PLAQUE CHANGE

CRP was unrelated to 3-year change in either IMT or plaque. IL-6 was unrelated to IMT change. Unexpectedly, higher levels of IL-6 were associated with a reduced probability of showing an increase in plaque lesion number during the follow-up. As already discussed, suggestive evidence from a large and growing literature supports a prospective association between CRP (and to a lesser extent, IL-6) and future CVD risk among initially healthy persons (J. Danesh et al., 2004). A considerably smaller body of research has explored whether inflammatory markers are associated with intima-media thickening or the appearance of eccentric plaque (Cao et al., 2003; Chapman, Beilby, McQuillan, Thompson, & Hung, 2004; Folsom, Aleksic, Catellier, Juneja, & Wu, 2002; Folsom et al., 2001; Hak et al., 1999; Hulthe, Wikstrand, & Fagerberg, 2001; Sitzer et al., 2002; Tracy et al., 1997; van der Meer et al., 2002; T. J. Wang et al., 2002; Willeit et al., 2000). In general, findings from this latter group of studies have been mixed. For example, Tracy and colleagues detected no association between CRP and IMT when examined cross-sectionally among participants in the Cardiovascular Health Study (CHS) (Tracy et al., 1997). By comparison, van der Meer and colleagues found CRP, but not IL-6, to be associated cross-sectionally with IMT among participants in the Rotterdam Study (van der Meer et al., 2002). Higher CRP levels also were associated with a higher plaque score among these individuals (van der Meer et al., 2002). The single prospective study reported a positive univariate association between baseline CRP and appearance of new atherosclerotic lesions 5 years later (Willeit et al., 2000). CRP was unrelated, however, to progression of existing lesions during the 5-year follow-up. Furthermore, when analyses included covariate terms for traditional risk factors, the effect of CRP on new lesion development no longer was significant (Willeit et al., 2000).

One explanation for the inconsistency of this literature concerns the multiple operational definitions of plaque and IMT that have been employed across studies. Plaque, for example, has been defined in terms of protrusion into lumen and wall texture (Willeit et al., 2000), the proportion of sites with detectable plaque (van der Meer et al., 2002), focal thickening of $\geq$ 1 mm (Chapman et al., 2004), and a distinct area > 50% thicker than surrounding regions (Hulthe et al., 2001). Recall that the present study operationalized plaque change as a binary variable that

indicated whether the number of visually apparent plaque lesions increased during the 3-year follow-up period. By comparison, carotid IMT scores have been derived by averaging near and far wall measurements (Cao et al., 2003; Hak et al., 1999; Tracy et al., 1997; van der Meer et al., 2002; T. J. Wang et al., 2002), or like the present study, by examining the far wall only (Chapman et al., 2004; Hulthe et al., 2001; Sitzer et al., 2002). Moreover, carotid IMT measures have been computed as the average of common, internal, and bulb regions (Folsom et al., 2002; Folsom et al., 2001; Sitzer et al., 2002; T. J. Wang et al., 2002), common and internal regions (Cao et al., 2003; Tracy et al., 1997), common and bulb regions (Hulthe et al., 2001), and the common region alone (Hak et al., 1999; van der Meer et al., 2002). One recent study found that traditional cardiovascular risk factors may differentially influence IMT in the common, internal, and bulb segments of the carotid artery (Schott et al., 2004). It is possible that CRP and IL-6 also may have differential effects on the various segments of the carotid artery. Given the heterogeneity in outcome measures, the extant literature on inflammatory markers and subclinical CVD is difficult to interpret. Accordingly, it is difficult to assess how well the present findings fit with that research. Future work might focus on the standardization of IMT and plaque measures.

Another explanation for the lack of a consistent association between inflammatory markers and surrogate CVD endpoints concerns the extent to which IMT and plaque measures reflect disease processes in which inflammation is thought to play a role, namely, atherogenesis and thrombosis. A criticism of IMT as an index of subclinical CVD is that the measure reflects properties of the vasculature other than atherosclerosis. Specifically, it has been proposed that non-atherosclerotic thickening of the intima and media may occur as an adaptive response to mechanical stress or aging (Glagov, Zarins, Giddens, & Ku, 1988). Thus, insofar as adaptive processes contribute substantially to 3-year IMT change in the present sample, it is not unreasonable that CRP and IL-6 should fail to predict that change.

By comparison, plaque measures are thought to provide a fairly specific index of atherosclerosis. The present study failed to detect an association between CRP and the likelihood of change in plaque lesion number during the 3-year follow-up period. A lack of independent association between CRP and plaque has been reported in 4 of 5 previous cross-sectional studies as well (Chapman et al., 2004; Folsom et al., 2001; Hulthe et al., 2001; Willeit et al., 2000). Unexpectedly, the present study found IL-6 to be inversely associated with plaque

change, such that higher levels of IL-6 predicted a decreased likelihood of change in lesion number. As there is no reason to expect that higher IL-6 levels would have a protective effect on plaque progression, this association likely reflects a chance occurrence. To date, only 2 other known studies have examined the association between IL-6 and carotid plaque (Chapman et al., 2004; van der Meer et al., 2002). In neither study was IL-6 an independent predictor of prevalent plaque measures. Taken together, the findings of the present study, in combination with those from previous research suggest that inflammation is an unlikely correlate of early, subclinical atherosclerosis. However, it is possible that the relation between inflammation and atherosclerosis is not one that can be estimated by examining quantitative plaque measures as outcomes. It has been suggested that the association between inflammation and risk for clinical cardiovascular events may be due, in part, to the contribution of inflammation toward development of unstable plaques (Willerson & Ridker, 2001). Risk of plaque rupture has been found to correlate poorly with degree of stenosis (Fuster & Lewis, 1994). Thus, it is possible that inflammatory processes may influence the structural integrity of early atherosclerotic lesions without affecting to the quantity of lesion produced.

Another factor that might have influenced the ability of the present study to detect a significant association between inflammatory markers and IMT and plaque change in the present sample involves the relative distributions of the predictor and outcome variables among men and women. CRP levels tend to be higher in women relative to men (McConnell et al., 2002). By comparison, IMT and plaque change tend to be greater in men relative to women (Chambless et al., 2002). Inspection of Figures 6, 7, 9, and 10 (inflammatory markers) and Figures 15 and 16 (IMT) demonstrate that these sex differences are apparent in the present sample. Thus, it is unsurprising that analyses among the entire sample should result in null findings. Analyses stratified by sex (see Appendix E, Tables E-7 and E-8), however, similarly resulted in a lack of correlation between inflammatory markers and IMT and plaque change. Given the reduction in sample size associated with sex-specific analyses, it is possible that we simple did not have sufficient power to detect significant correlations. Additionally, among the women in our sample, age was negatively rather than positively associated with CRP concentrations. As IMT increases with age, the respective influences of CRP and age on IMT progression were counter to one another, and thus likely contributed to the lack of effect for CRP.

In sum, the data presented here fail to support an association between circulating inflammatory marker levels and 3-year progression of IMT and plaque. This failure of the present study to detect a significant association is not inconsistent with the existing research in this area, which is comprised largely of mixed outcomes. The cloudiness of the research on inflammation and IMT and plaque contrasts remarkably with the literature supporting a prospective association between CRP and incidence of clinical CVD events (Danesh et al., 2004). One might infer from the difference in consistency between these two literatures that inflammation plays a larger role in later rather than earlier stages of cardiovascular pathogenesis. Findings from experimental animal research, however, suggest a potential causal role of IL-6 and CRP in early atherogenesis (Rus et al., 1996; Torzewski et al., 2000). Thus, investigation of these two inflammatory markers as predictors of IMT and plaque progression in humans is not unreasonable. Given the biologic plausibility of inflammatory factors playing a causal role in atherosclerosis progression, one direction that future research might take is to examine the prospective association of inflammation with markers of early cardiovascular pathogenesis (e.g., IMT, plaque, endothelial function) among children over the course of several years. As children likely would show negligible vascular pathology, researchers might more easily be able to identify early associations between inflammation and CVD. Additionally, future research might investigate the interplay of inflammation and subclinical disease markers in the prediction of future clinical events. No known published prospective studies of CRP and clinical CVD have examined baseline levels of atherosclerosis. It is possible that inflammatory activity influences the integrity of pre-existing plaques such that they are more prone to rupture, and thus initiating acute events.

6.3 CSS AS PREDICTOR OF BASELINE INFLAMMATORY MARKER LEVELS

The present study failed to detect a significant independent association between CSS scores and baseline inflammatory marker levels. It should be noted that the physical stress subscale of the CSS did show a positive univariate correlation with CRP. However, this association lost significance when examined in a model that included adjustment for relevant covariates. Specifically, BMI and use of prescription pain medications each accounted for a substantial

proportion of the variance in CRP associated with physical stress. Both the physical stress subscale of the CSS and prescription pain medication use may be indirect markers of biological aging. Biological age is a construct that is used to describe an individual's functional status relative to his or her chronological peers (Borkan & Norris, 1980). Findings from human and comparative research have shown that biological age does not always parallel chronological age (Anstey, Lord, & Smith, 1996; Collier & Coleman, 1991). Chronological aging is known to be associated with chronic, low-grade inflammatory activity (Krabbe, Pedersen, & Bruunsgaard, 2004). However, it is unknown whether age-related alterations in inflammation arise as a function of an internal "clock" mechanism or whether age-related low grade inflammatory activity simply reflects the sum total of age-related physical burden. It is possible that among the present sample, high scores on the CSS physical stress subscale, as well as positive endorsement of pain medication use may be identifying individuals who are undergoing more rapid biological aging. Insofar as increased IMT may be a structural marker of vascular aging, the observed association between physical stress and IMT in the present sample lends further support to this interpretation. Future research might investigate whether associations between stress and physical health outcomes is moderated by individual differences in rates of biological aging.

The present null findings for CRP are consistent with those reported by a previous study that found no significant association between scores on a multidimensional "perceived adversity" scale and levels of circulating CRP (Steptoe & Marmot, 2003). The present results contrast, however, with findings from the only other known study to examine whether self-reported chronic stress in multiple life domains predicts inflammatory marker levels. Hapuarachchi and colleagues examined associations between scores on several unidimensional stress instruments and levels of CRP. The authors found that CRP correlated positively with measures of occupational strain, perceived stress, and psychological stress (General Health Questionnaire, GHQ-12), and inversely with measures of job satisfaction and social support (Hapuarachchi, Chalmers, Winefield, & Blake-Mortimer, 2003). It should be noted, however that the average age of the sample employed by Hapuarachchi and colleagues was 46 years. It is possible that any effects of stress on CRP levels are more pronounced among younger relative to older persons.

To date, no known studies have examined the association between multidimensional chronic life stress and circulating IL-6 levels. Two studies, however, have examined IL-6 levels

among persons who were acting as the primary caregiver for a relative with dementia (Kiecolt-Glaser et al., 2003; Lutgendorf et al., 1999). In the earlier study, IL-6 levels were found to be higher among older female caregivers relative to (a) older women who were undergoing the stress of moving to a new residence; (b) older women who were neither caregivers nor movers; and (c) younger women who were neither caregivers nor movers (Lutgendorf et al., 1999). In the more recent study, male and female caregivers showed a larger age-related increase in IL-6 over 6 years of follow-up relative to men and women without caregiving responsibilities (Kiecolt-Glaser et al., 2003). Thus, it appears that the present IL-6 findings conflict with those reported by previous research. As suggested for the association between chronic stress and the present measures of IMT and plaque, it is possible that the CSS does not measure those features of chronic stress that are most likely to influence IL-6 levels. Findings from the 2 studies described above suggest that the burden of caregiving might be one type of stress that is associated with the activation of physiologic processes that regulate the level of IL-6 in circulation. Given the unpredictable behavior of persons with dementia, informal caregivers of dementia patients might spend their days in a constant state of "watchful waiting" that may be accompanied by chronic physiologic arousal. Furthermore, insofar as a caregiver lives with the care recipient, there may be limited opportunity to recover from daily psychological stress and associated physiologic perturbations.

Caregiver burden was not explicitly addressed by the CSS. It is interesting to note, however, that the present study detected a significant positive association between filial stress and CRP among older participants. It is possible that older participants might have been reflecting on caregiving experiences when responding to items included in the filial stress subscale (cf. Appendix A). By comparison, and contrary to what might be expected, filial stress was associated with lower CRP levels among younger participants. One explanation for this counterintuitive finding is that generally more healthy persons (with lower CRP levels) are more likely to attend to their families of origin, and thus report higher levels of familial stress. Similar unexpected associations were observed between (a) occupational stress and CRP among all younger participants; and (b) parental stress and IL-6 among younger men. Specifically, among younger participants, more occupational stress was associated with lower levels of CRP. Among younger men, more parental stress was associated with lower IL-6. It is possible that less healthy persons (with higher levels of CRP) select themselves out of high-stress jobs, and thus report less

occupational stress relative to their healthier counterparts. A similar explanation might be offered for the association between parental stress and IL-6: less healthy men (with higher levels of IL-6) might be less likely to involve themselves in their children's affairs, and thus report relatively lower levels of parental stress. Alternatively, assuming that parental stress is an indirect marker of parental involvement, it may be that being an active parent has a protective effect on IL-6 levels among the men in the present sample. That the above counterintuitive findings were observed only among younger participants might be explained by differential stress reporting with age. Findings from previous population research have shown that reports of stress tend to decrease with increasing age (Cohen & Williamson, 1988). Thus, it is possible that a generally lower level of stress reporting among older participants may have reduced the likelihood of detecting significant associations.

The lack of a consistent association between chronic stress and circulating inflammatory markers does not necessarily indicate that stress is not a correlate of inflammation. Multiple factors contribute to circulating inflammatory marker levels such as age, sex, stage in the menstrual cycle, infectious disease status, previous exposure to pathogens, and genetics. It is possible that effects of stress on circulating inflammatory marker levels exist in the present sample, but are small. It is the challenge of future research to determine whether the proportion of variance in inflammatory marker levels that may be accounted for by psychological factors has significant implications for health. Alternatively, the effects of stress on inflammation may depend on individuals' status on other factors associated with inflammatory processes. For example, one study found that persons who scored high on a measure of vital exhaustion showed higher levels of IL-6 relative to persons who scored low on the measure, especially among persons who were seropositive to multiple infectious agents (van der Ven et al., 2003). Another study found that vital exhaustion was associated with elevated levels of CRP only among persons who were carriers of the A allele of the TNF-alpha -308 G/A polymorphism (Jeanmonod, von Kanel, Maly, & Fischer, 2004). Taken together, the findings from these 2 studies suggest that vital exhaustion may further influence inflammatory marker levels that already are elevated due to other factors. Insofar as these findings for vital exhaustion might be extended to other psychosocial factors, such as chronic life stress, it may be important for future studies to consider the role of moderating influences when examining an association between stress and inflammation.

A few procedural issues also might have reduced the ability of the present study to detect a significant association between chronic stress and inflammation. First of all, participants with current infectious diseases were not excluded from the blood draw for CRP and IL-6. Inclusion of participants with concurrent infectious or inflammatory conditions potentially could have masked the effects of stress on inflammatory markers. However, use of CRP standard cut points should have reduced the prevalence of such individuals in the present sample. Another factor that might have influenced the ability of the present study to detect an association between chronic stress and inflammation concerns the timing of the administration of the CSS relative to the blood draw for inflammatory markers. Baseline blood draws were taken during the initial medical screening, whereas the CSS was administered, on average, 5.1 months later (range = 2.5 to 14.0 months). Although the average lag between inflammatory marker and stress measurements fell within the reporting period of the CSS (i.e., past 6 months), approximately 17% (n = 48) of the participants were administered the CSS more than 6 months after the blood draw. Insofar as CSS scores may have been biased toward more recent stress experiences, it is possible that the true association between inflammatory marker levels and concurrent stress might have been minimized.

In sum, the findings of the present study do little to clarify the small and largely inconclusive existing literature on chronic psychosocial stress and circulating markers of inflammation. A fundamental challenge to this area of study is the fact that inflammatory markers simultaneously index the influence of chronic and acute factors. For this reason, studies that measure inflammatory markers as outcomes must employ careful controls at multiple levels. Ideally, participants would be free of acute or chronic infectious or inflammatory disease, have suffered no recent physical injuries, be taking no medications with anti-inflammatory effects, be roughly the same age, and, among women, be at approximately the same phase of the menstrual cycle. Given the obvious difficulty in obtaining such an ideal sample, it is no surprise that relatively few human studies of chronic stress and inflammation have been conducted. Interestingly, there are no known published studies to date that have investigated the association between chronic stress and circulating inflammatory markers among laboratory animals. A number of studies have investigated the influence of chronic or repeated stress on the ability of stimulated cells to produce inflammatory cytokines in vitro (Avitsur, Kavelaars, Heijnen, & Sheridan, 2005; Bartolomucci, Sacerdote, Panerai, Peterzani, Palanza, & Parmigiani, 2003).

Whether increased production of inflammatory cytokines (or decreased production of cytokines with anti-inflammatory effects) by stimulated cells can lead ultimately to chronic elevations in circulating levels of these markers is unknown, and ought to be a focus of future research. Perhaps now is the time to take advantage of the control permitted in the conduct of infrahuman animal research to examine the effects of chronic stress on circulating inflammatory markers. Experimental animal studies would allow researchers to assess the effects of standardized chronic stressors on inflammatory outcomes. Further, transgenic animals that are bred to develop chronic inflammatory conditions (e.g., rheumatoid arthritis) can be exposed to varying levels of chronic stress to determine whether stressor exposure has an exacerbating effect among vulnerable individuals. Knowledge gained from such research would provide researchers with a better foundation on which to base human studies.

6.4 CVR AS A MODERATOR OF THE ASSOCIATION BETWEEN CHRONIC STRESS AND IMT AND PLAQUE CHANGE

A secondary aim of the present study was to examine whether trait CVR moderates the association between chronic stress and IMT and plaque change. As the main effect of stress on the cardiovascular outcome variables was not significant, we modified our original hypothesis that stress would be a stronger predictor of IMT and plaque change among high relative to low reactors. Rather, we examined the possibility that a significant main effect might emerge when assessed among persons who showed high levels of reactivity but not among those who showed low reactivity. We detected only one significant CVR-by-chronic stress interaction: diastolic reactivity moderated the association between filial stress and IMT change. Analysis of simple effects revealed a cross-over interaction such that more filial stress was associated with less IMT change when examined among high reactors and with greater IMT change when examined among low reactors. Neither association, however, achieved statistical significance (p's > .10). Given the number of CVR-by-chronic stress analyses that were conducted, this single significant interaction and associated counter-intuitive effects likely were a consequence of chance.

Interactive effects of psychosocial factors and CVR on IMT and plaque progression have been described in 2 reports from the Kuopio Ischemic Heart Disease Study (KIHD) (Everson et

al., 1997; J. W. Lynch, Everson, Kaplan, Salonen, & Salonen, 1998). The first report examined CVR as a moderator of the association between workplace demands and atherosclerosis progression (Everson et al., 1997), whereas the second examined the interactive effects of CVR and SES (i.e., income and education) on progression of atherosclerosis (J. W. Lynch et al., 1998). Everson and colleagues reported significant workplace demand-by-CVR interaction effects both for a model that included change in mean IMT as well as for a model that examined change in plaque height. This finding stands in contrast to the results of the present study, which showed no moderating effect of CVR on the association between the occupational stress subscale of the CSS and IMT and plaque progression. By comparison, Lynch and colleagues reported only marginal effects for the (a) income-by-CVR interaction, and (b) education-by-CVR interaction on progression of mean IMT (p's < .10), and no significant interaction effects when plaque height was examined as the outcome (J. W. Lynch et al., 1998). In combination, these 2 reports from the KIHD study suggest that the moderating effect of CVR on the association between chronic stress and IMT and plaque progression, although apparent, may be small. It is possible that the present study lacked sufficient power to detect a small-size moderating effect on a non-significant main effect association. As indicated by the power analyses presented in the Methods, the power of the present study to detect a significant moderating effect of CVR on the association of psychosocial factors with IMT and plaque change was .17 and .10, respectively. This explanation may be especially relevant to the present study's failure to find a significant moderating effect of CVR on the association between occupational stress and IMT and plaque progression, as only a subgroup of the present sample were employed at the time the CSS was administered (n = 161).

6.5 ADDITIONAL LIMITATIONS OF THE PRESENT STUDY

Beyond those already discussed, a few additional limitations of the present study need be addressed. First of all, the present sample was composed of healthy, older adults with no evidence of pre-existing CVD at baseline. As CVD of any type is not uncommon among persons in the recruited age range, it is possible that the older adults who comprised the present sample represent a comparatively protected group, and therefore may be less likely to demonstrate

robust evidence of our predicted associations. Another limitation of the present study is that participants received only one baseline blood draw for inflammatory markers. Previous research has reported fairly large intra-individual variation in serum CRP (Ledue & Rifai, 2003). Thus, it has been recommended that the mean of 2 independent CRP measurements, separated by at least 2 weeks, should be used to establish risk of future clinical CVD events (Pearson et al., 2003). That our single measurement may not have provided an accurate assessment of true baseline inflammatory marker levels could have weakened both the association between (a) stress and inflammation and (b) inflammation and IMT and plaque progression. A third disadvantage associated with the present study is that IMT and plaque were assessed on only 2 occasions. It is possible that IMT progression, for example, does not follow a linear trajectory. A non-linear IMT progression trajectory may have implications for the questions posed by the present report, as the association between (a) chronic stress and/or inflammatory markers and (b) IMT change may depend on the point in the trajectory of progression at which baseline is assessed. Extension of data collection to a third time-point would permit investigation of non-linear IMT growth trajectories, and thus provide and opportunity for these issues to be addressed. An additional IMT data-point also would increase the reliability of an observed linear trajectory. Another limitation of the present sample that was alluded to above concerns the modest sample size. This limitation was especially apparent when examining the predictions we made regarding the moderating effects of sex on the association between CSS subscales and IMT and plaque change. For example, contrary to expectations occupational stress was not a better predictor of IMT change among men relative to women. Given that only 161 (89 men; 72 women) of the 276 participants in the present study were employed at the time of enrollment, the power to detect a significant occupational stress-by-sex interaction was somewhat compromised. It is possible that the predicted effects might have been detected among a larger population. Finally, it should be reiterated that both atherogenic and vascular remodeling processes contribute to IMT, thus calling into question whether change in IMT is an accurate measure of true atherosclerotic progression. In this way, had we detected a significant association between CSS scores and IMT progression, it would have been unclear as to which aspect of disease progression chronic stress was influencing.

6.6 CONCLUSIONS AND FUTURE DIRECTIONS

Results from the present study did not show strong support for the proposed model that links chronic stress with IMT and plaque progression via a mediational pathway involving elevations in circulating inflammatory markers. One interpretation of the lack of significant findings is that the proposed model does not accurately describe the true association between chronic stress, inflammation, and progression of carotid artery IMT and plaque. Said plainly, inflammation may not be a physiologic mechanism by which chronic stress influences CVD risk. An alternative interpretation is that the expected associations do exist, but were not detected by the present methods. For example, it is possible that the employed measure of chronic stress did not capture specific features of psychosocial stress that are relevant to health outcomes, such as alterations in circulating inflammatory markers or progression of markers of subclinical CVD. Future work might explore whether various dimensions of stress, for example, elements of low control or interpersonal tension, relate differentially to health outcomes. It also is possible that continuous exposure to a single chronic stressor, as suggested by high scores on CSS subscales, is not as relevant to health outcomes as is serial exposure to multiple acute or episodic stressors. There is evidence to suggest that continuous exposure to a homotypic stressor may result in habituation of the norepinephrine response to that stressor (Terrazzino, Perego, & De Simoni, 1995). Insofar as SNS activation is fundamental to the production of stress-related physical pathology, habituation of sympathetic response to a chronic psychosocial stressor could have implications for the proposed association between chronic stress and CVD. Future studies might seek to examine whether sustained chronic stress from a single source or serial exposures to multiple acute or episodic stressors show differential associations with health outcomes.

Alternatively, assessment of stress alone may be insufficient to detect an effect on health outcomes. Individuals' appraisal of a stressor also may dictate whether that stressor is physiologically toxic (Lazarus & Folkman, 1984). For example, one recent study found that the severity of medical students' appraisals of one or more of 5 experienced stressful events (e.g., death of family member; financial problems) was inversely associated with reports of perceived health and health behaviors (Hojat, Gonnella, Erdmann, & Vogel, 2003). Trait factors, such as personality, also might influence whether the experience of a given stressor is associated with pathophysiologic sequelae. Hostility, for example, has been found to moderate the extent of

CVR during an anagram task with harassment such that high hostile persons showed more reactivity than low hostile persons (Suarez & Williams, 1989). The moderating role of individual differences in personality and coping was suggested by the nonhuman primate research conducted by Kaplan and colleagues (J. R. Kaplan et al., 1982). Results indicated that stress-associated atherosclerosis progression was especially marked in dominant males. Thus, it may be of interest to future studies to examine appraisal, coping, and personality in addition to chronic stress to determine whether these factors moderate the association between stress and disease.

It is possible that the present study did not employ appropriate methods for assessing the association between circulating inflammatory markers and early cardiovascular pathology. The histological composition of atherosclerotic lesions changes as CVD progresses from the initial subclinical stages to manifest clinical pathology (Stary et al., 1995). Moreover, lesion types differ in terms of the timing of earliest onset. Initial lesions, for example, that are characterized by isolated macrophages and foam cells, can appear as early as the first decade of life. By comparison, complicated lesions that are characterized by lipid involvement, fibrosis, and calcification tend to appear only during the fourth decade of life or later (Stary et al., 1995). It is likely that inflammatory factors have differential effects on lesion progression at earlier and later stages of CVD pathogenesis. Results from one recent comparative study suggest that inflammatory activities, such as the recruitment of white blood cells by endothelial adhesion molecules, might be more important during the initiation phase of lesion development than during later growth phases (Lessner, Prado, Waller, & Galis, 2002). Insofar as inflammation might be a mediating mechanism that links chronic stress and CVD, it might be beneficial for future studies to explore the associations examined here across the lifespan, thus permitting the examination of the proposed effects at various stages of disease development.

The importance of considering the role of maturational stages and processes was underscored by our findings for the physical stress subscale of the CSS. As discussed above, it is possible that the physical stress subscale is an indirect marker of biological aging. Just as stress might have differing effects on atherosclerosis development at various stages of disease development, so too might physiologic correlates of stress differ as a function of biological aging. The influence of biological aging on inflammatory response to stress was suggested by the findings of a recent study that compared changes in CRP levels following acute laboratory

stress among persons with (a) rheumatoid arthritis, and (b) osteoarthritis (Veldhuijzen van Zanten, Ring, Carroll, & Kitas, 2005). Following completion of mental arithmetic with harassment while tilted to a 64-degree head-up-tilt, rheumatoid arthritis patients showed a greater pre- to post-task increase in serum CRP relative to osteoarthritis patients (Veldhuijzen van Zanten et al., 2005). Autoimmune diseases, such as rheumatoid arthritis are associated with accelerated "immunosenescence" (Brod, 2000). In other words, younger persons with autoimmune conditions display an immunologic profile similar to what is typical of comparatively healthy older persons. Thus, it might be said that the immune systems of the rheumatoid arthritis patients included in the Veldhuijzen van Zanten et al. study had undergone more advanced biological aging relative to the osteoarthritis patients. Accordingly, group differences in CRP response to stressor exposure might have been accounted for by differences in biological aging.

In addition to potentially acting as a moderator of the physiologic response to stress, accelerated biological aging might be a mechanism by which stress influences disease risk. One recent study found that healthy women's reports of perceived stress were associated with markers of cellular aging, such as reduced telomere length, lower telomerase activity, and higher oxidative stress (Epel et al., 2004). As tissues from different organ systems age at different rates, the identification of a single marker of biological aging is unlikely. A more feasible direction for future research might be to identify reliable markers of biological aging for individual organ systems. Research of this nature currently is being conducted (Warner, 2004). Relevant system-specific markers of aging then could be incorporated into studies of stress and disease, and examined as mediators or moderators of the stress disease association.

APPENDIX A

Table 36: CSS subscale items

Subscale/Items
Marital stress
- Did your spouse expect more from you than he or she was willing to give back?
- Did your spouse spend money in ways you thought unwise?
- Did problems experienced by your spouse place an extra burden on you?
Parental stress
- Did you wonder if your children were trying hard enough to prepare for the life ahead of them?
- Did you have to give attention to your children failing to get along with others?
- Did your children seem to ignore your guidance and advice?
- Did problems experienced by your children place an extra burden on you?
Filial stress
- Was one of your parents or some other older relative complaining or critical of you?
- Did you feel responsible for the care and well-being of a parent or any older relative?
- Did you worry that a parent or some other older relative was declining in mental capacity?
- Did problems experienced by a parent or another older relative place an extra burden on you?

(continued)

Subscale/Items
Financial stress
- Did you not have enough money to afford the kind of clothing or food you or your family should have?
- Did you have trouble meeting the monthly payments on bills?
- Were you confident that your source of income was secure?*
- Did financial problems place an extra burden on you?
Occupational stress
- Did you feel your work was too dirty, noisy, or dangerous?
- Did you have more work than you could handle?
- Were you treated unfairly by others on the job?
- Did problems experienced by co-workers place an extra burden on you?
Ecological stress
- Did you feel crowded in your present housing situation?
- Did you worry about crime in your neighborhood?
- Did you worry about drugs in your neighborhood?
- Was your neighborhood excessively noisy?
- Did problems experienced by neighbors place an extra burden on you?
Physical Stress
- Did you have trouble getting around? I mean things like climbing stairs or getting outdoors?
- Did your health prevent you from doing things you wanted to do?
- Did any physical disabilities place an extra burden on you?

* Reverse scored.

APPENDIX B

Table 37: Pearson correlations of CSS subscales with other relevant stress measures

CSS Subscale	PSS	DAS	Income[a]	Education[b]	Psychological Demand[c]	Coworker Support[d]	Supervisor Support[e]	Social Support[f]	Skill Discretion[g]	Decision Authority[h]
CSSTOT	.36***	-.32***	-.12*	-.08	.14†	-.24**	-.25***	-.28***	-.13†	-.20**
CSSAVG	.36***	-.37***	-.18**	-.09	.13†	-.21**	-.24**	-.26***	-.13†	-.20**
MAR	.18**	-.53***	.13*	-.03	.14†	-.09	-.07	-.09	-.13†	-.14†
PAR	.11†	.15*	-.03	-.11†	-.03	-.07	-.03	-.05	-.14†	-.19*
FIL	.18**	-.05	.00	.05	.07	-.12	-.13†	-.15†	-.04	-.02
FIN	.22***	-.18**	-.22***	-.07	-.03	-.13†	-.16*	-.17*	-.10	-.07
OCC	.35***	-.12†	.07	.03	.36***	-.34***	-.21**	-.31***	.10	-.06
ECO	.19**	.19**	-.28***	-.12*	.01	-.09	-.22***	-.19*	-.06	-.13†
PHYS	.17**	-.17*	-.16**	-.08	-.01	-.05	-.10	-.09	-.17*	-.23**

† p < .10. * p < .05. ** p < .01. *** p < .001.

[a, b] = Income and education items included in PHHP demographic questionnaire; [c, d, e, f, g, h] = Subscales from Job Content Questionnaire (R.A. Karasek, 1985)

APPENDIX C: IMT AND PLAQUE CHANGE BY QUANTILES OF CSS SCORES

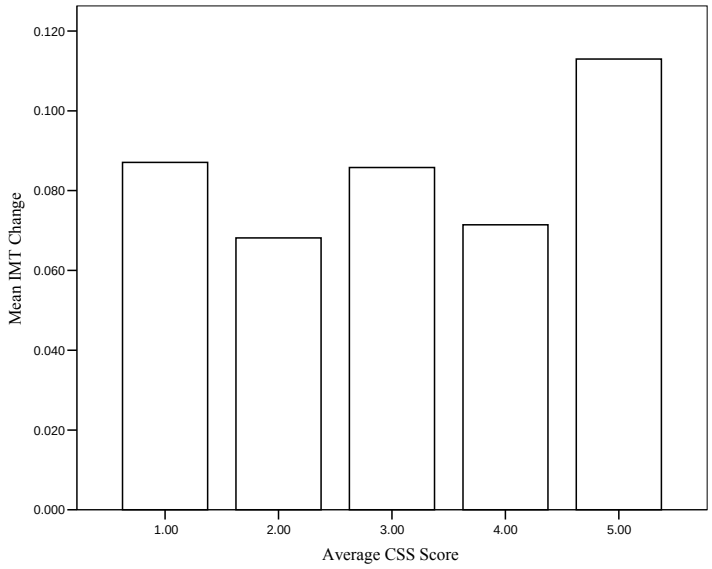

Figure 23: Mean IMT change by quintile of average CSS Score

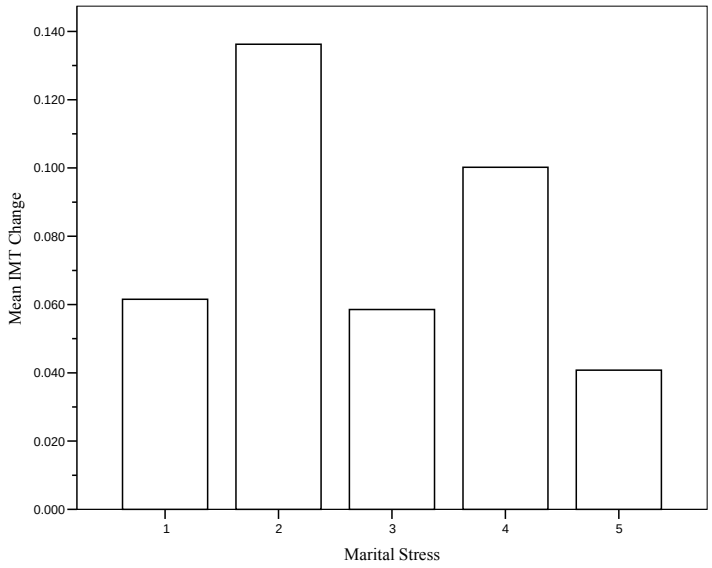

Figure 24: Mean IMT change by quintile of Marital Stress

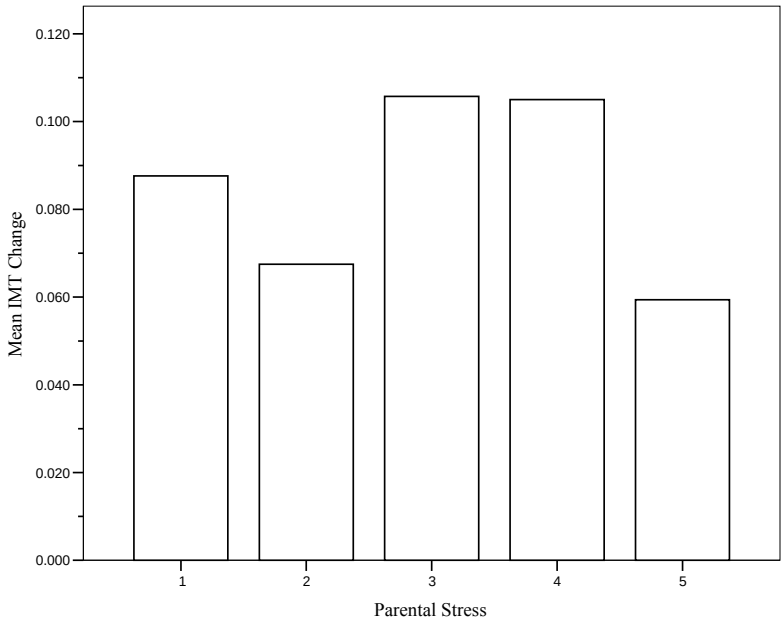

Figure 25: Mean IMT change by quintile of Parental Stress

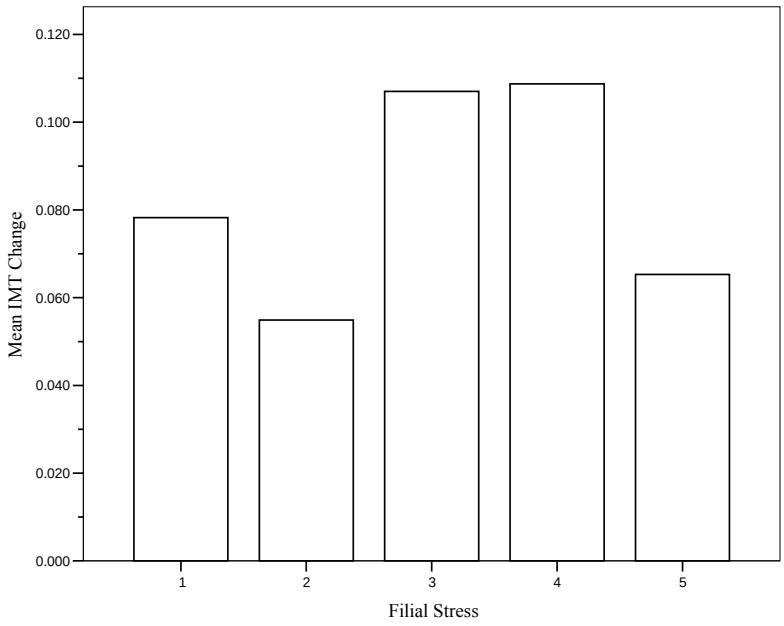

Figure 26: Mean IMT change by quintile of Filial Stress

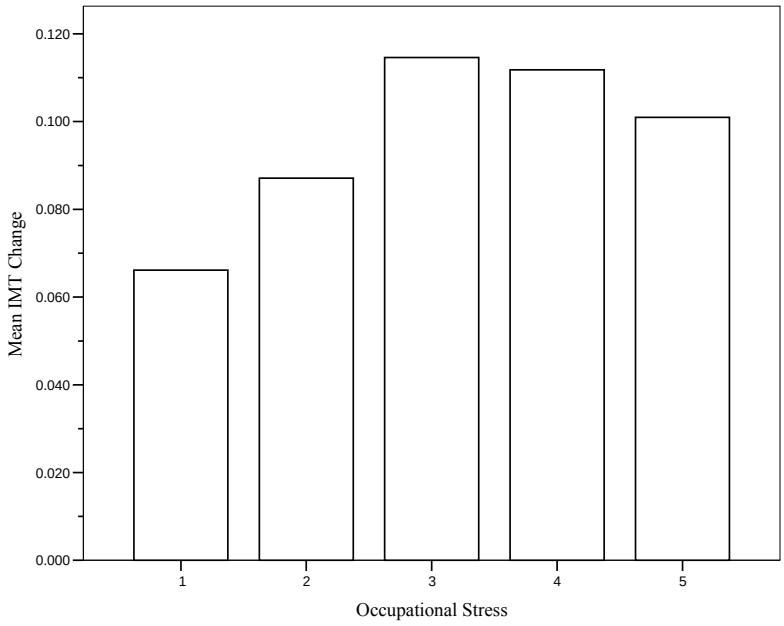

Figure 27: Mean IMT change by quintile of Occupational Stress

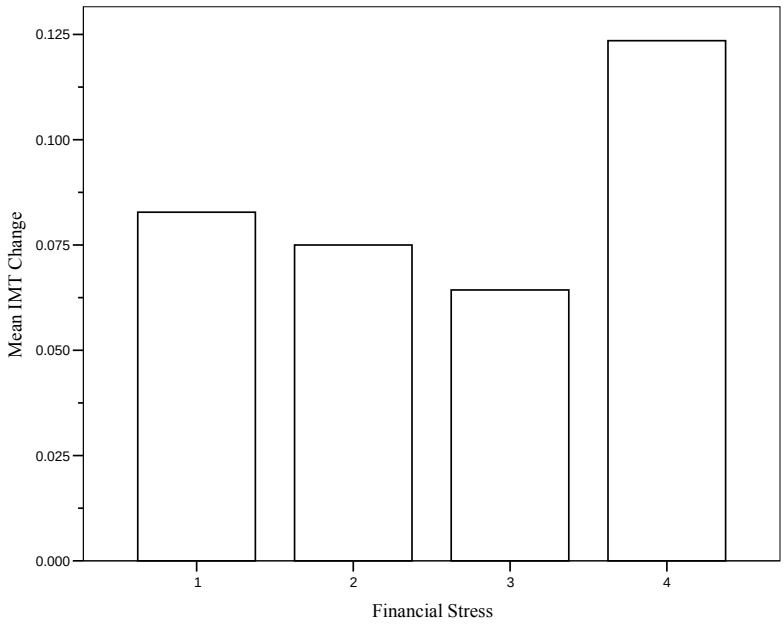

Figure 28: Mean IMT change by quartile of Financial Stress

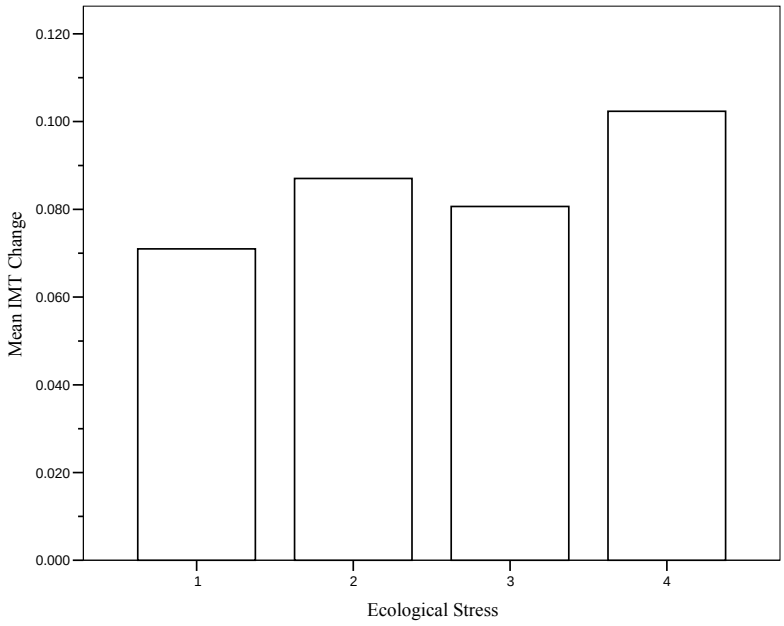

Figure 29: Mean IMT change by quartile of Ecological Stress

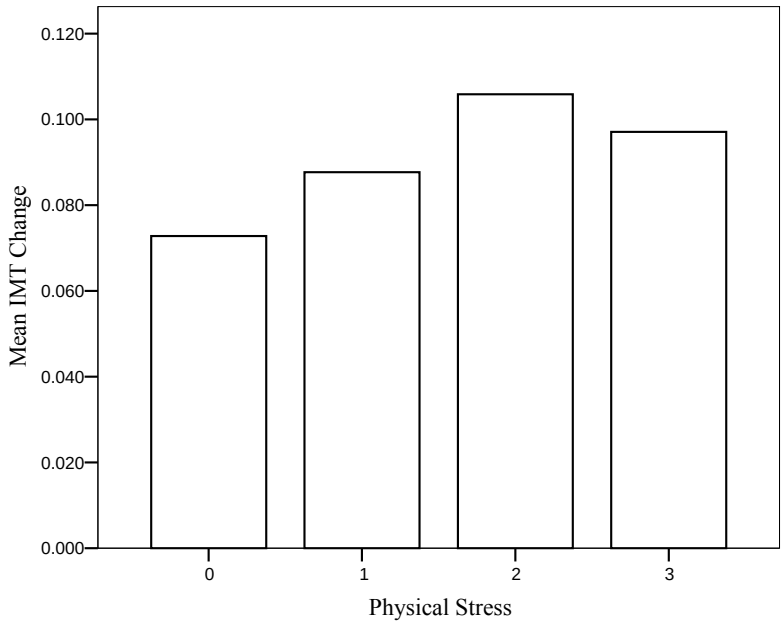

Figure 30: Mean IMT among zero, low, medium, and high Physical Stress groups[16]

[16] Fifty percent of the sample reported zero Physical Stress. Thus, for the purposes of presentation, participant data were separated into zero and non-zero stress groups. Bars 1, 2, and 3 represent the lowest, middle, and highest tertiles among the non-zero stress group.

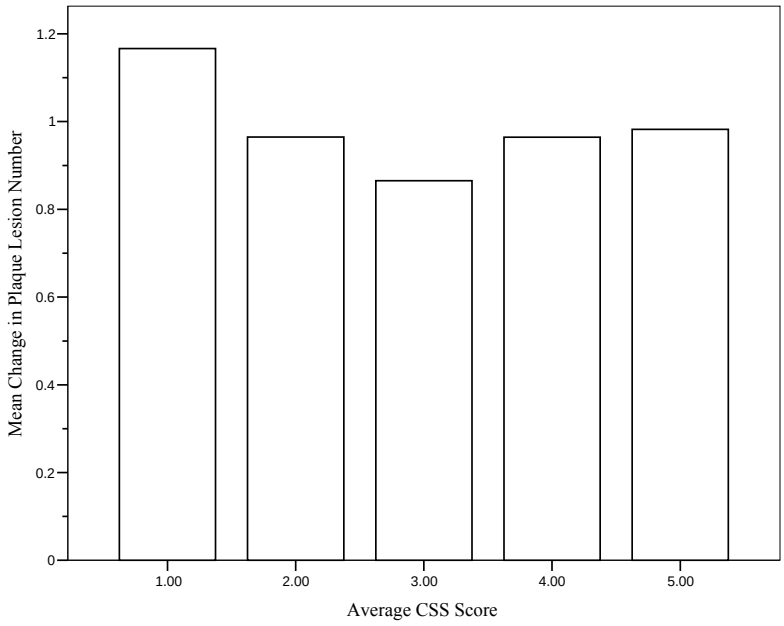

Figure 31: Mean change in plaque lesion number by quintile of average CSS score

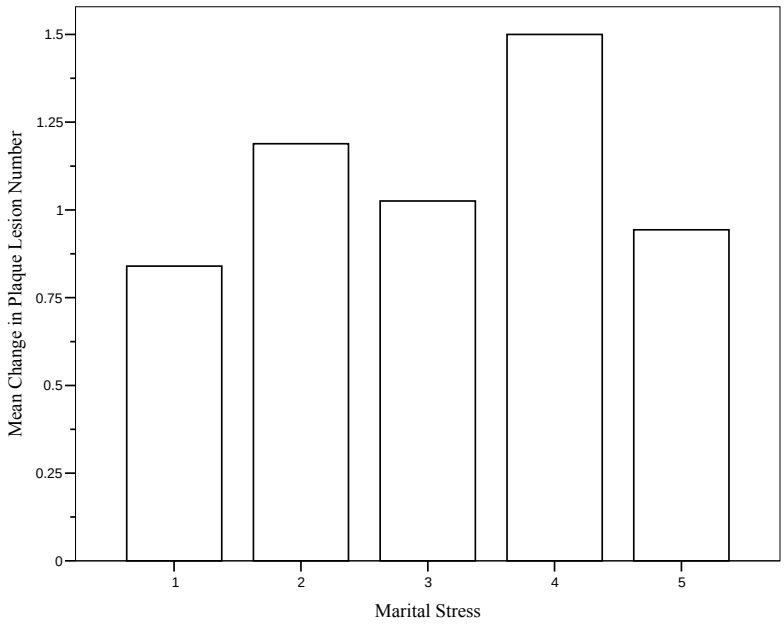

Figure 32: Mean change in plaque lesion number by quintile of Marital Stress

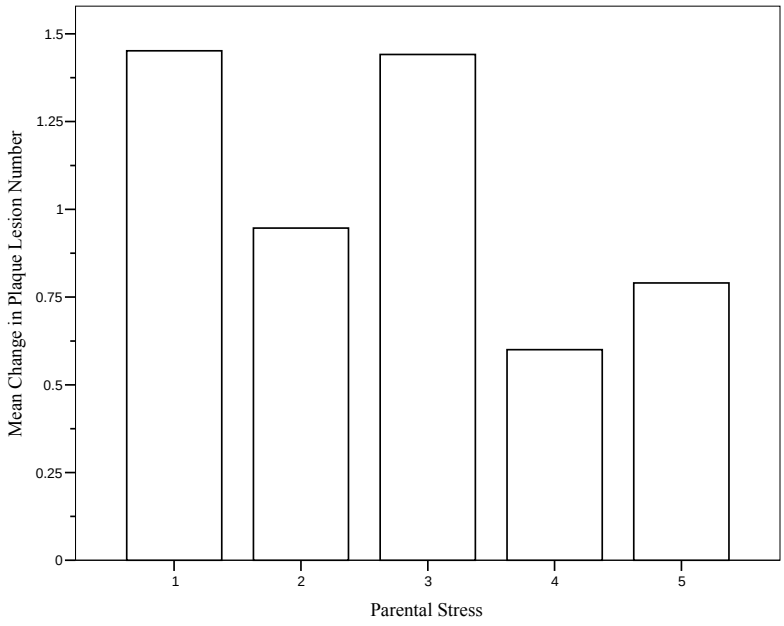

Figure 33: Mean change in plaque lesion number by quintile of Parental Stress

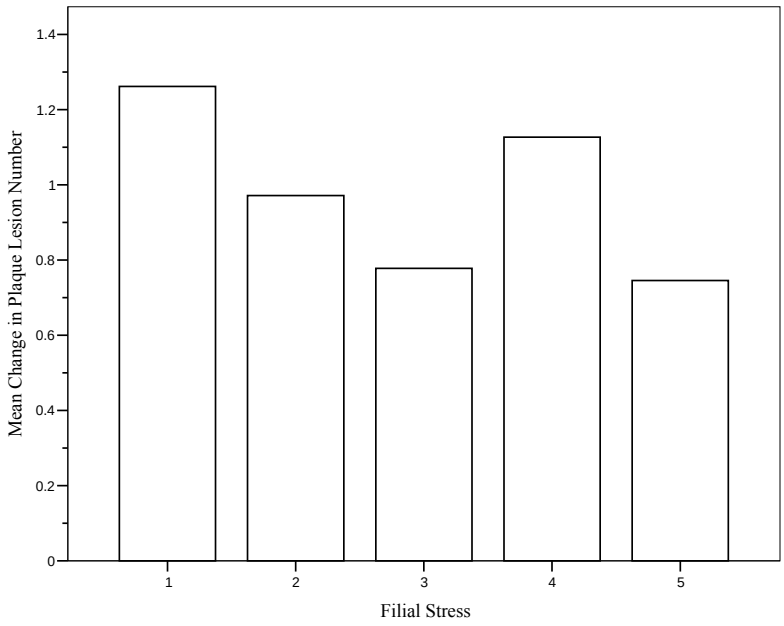

Figure 34: Mean change in plaque lesion number by quintile of Filial Stress

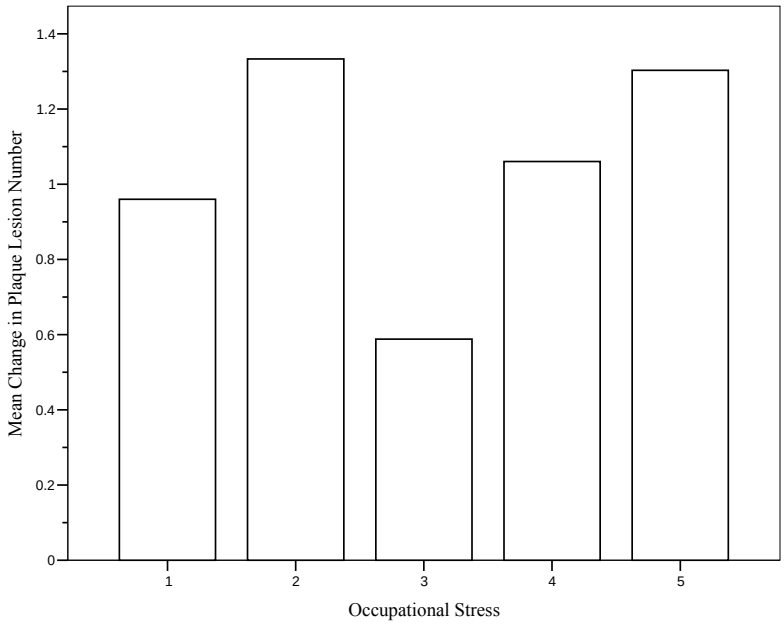

Figure 35: Mean change in plaque lesion number by quintile of Occupational Stress

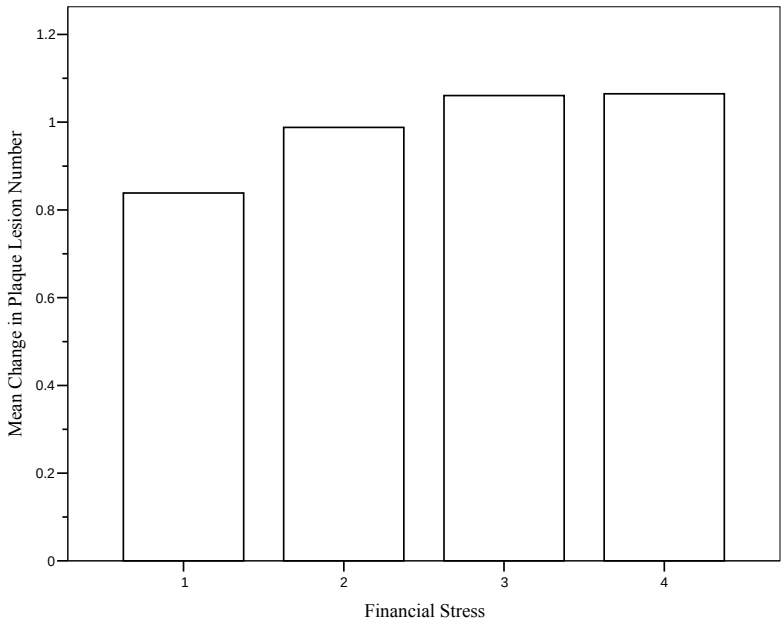

Figure 36: Mean change in plaque lesion number by quartile of Financial Stress

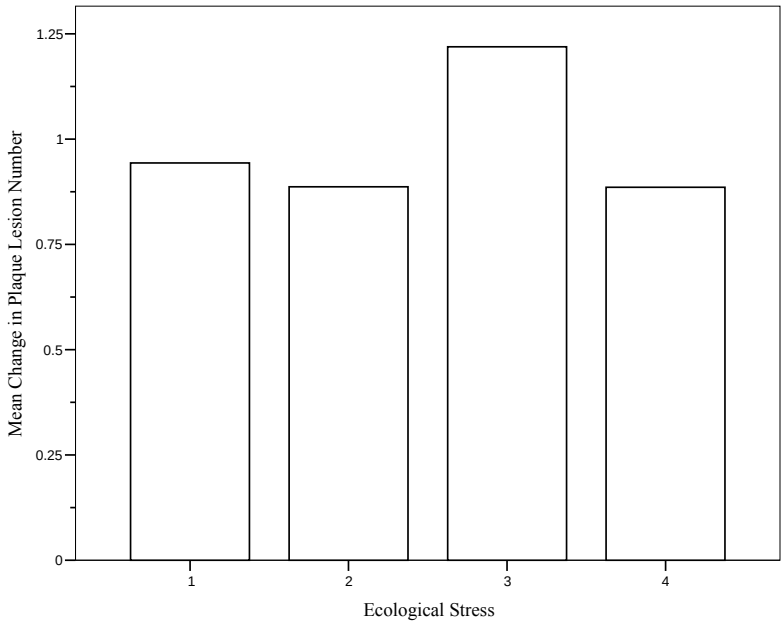

Figure 37: Mean change in plaque lesion number by quartile of Ecological Stress

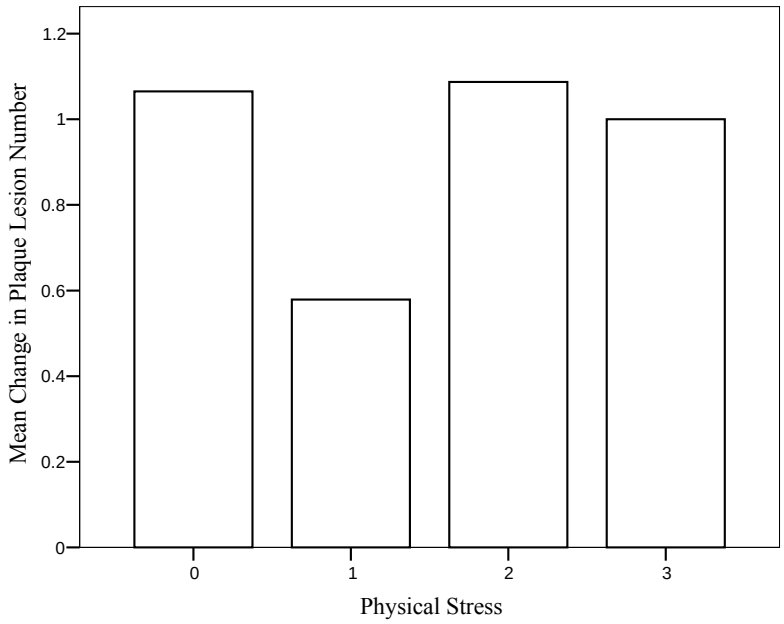

Figure 38: Mean change in plaque lesions by zero, low, middle, and high Physical Stress

APPENDIX D: MEAN CRP AND IL-6 LEVELS BY QUANTILES OF CSS SCORES

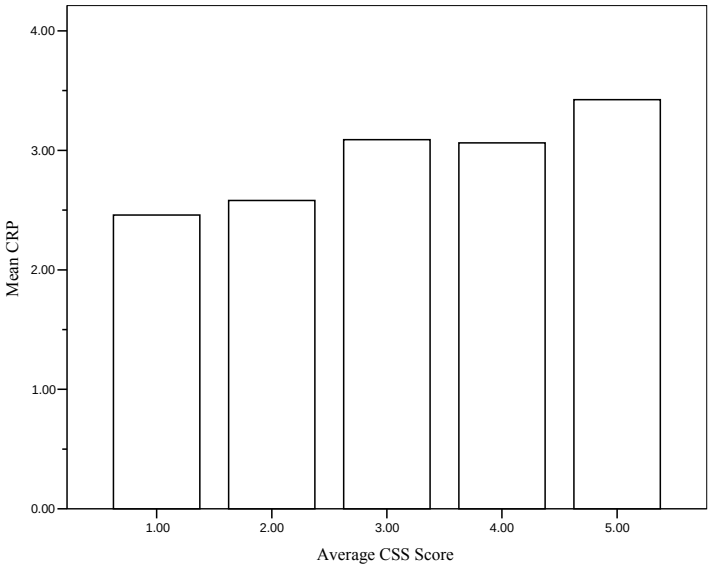

Figure 39: CRP concentration by quintile of average CSS score

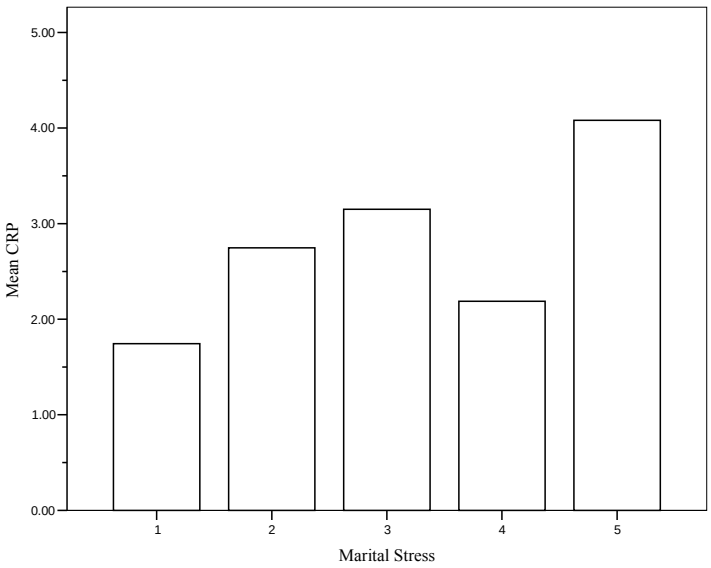

Figure 40: CRP concentration by quintile of Marital Stress

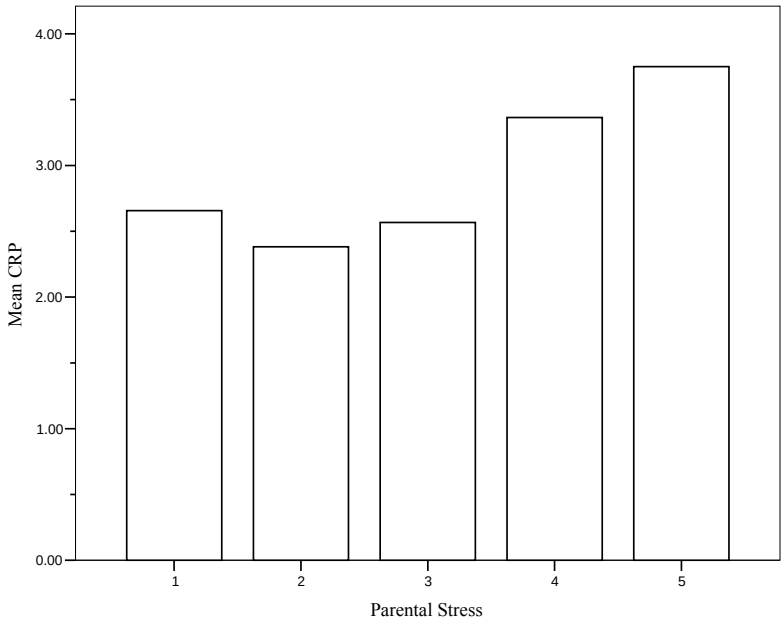

Figure 41: CRP concentration by quintile of Parental Stress

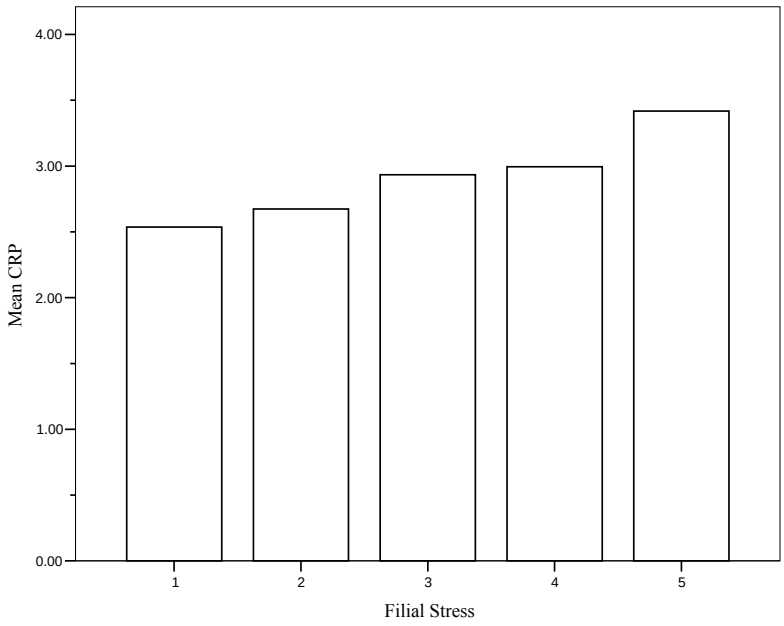

Figure 42: CRP concentration by quintile of Filial Stress

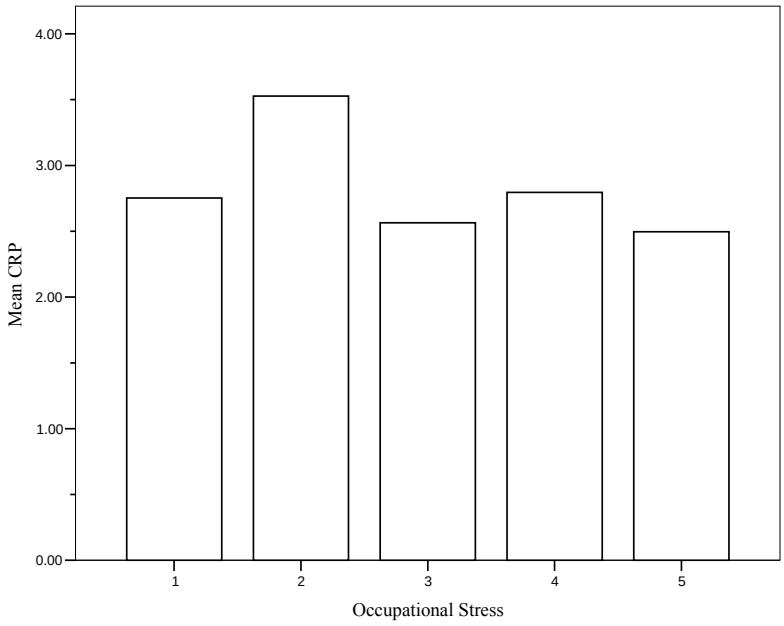

Figure 43: CRP concentration by quintile of Occupational Stress

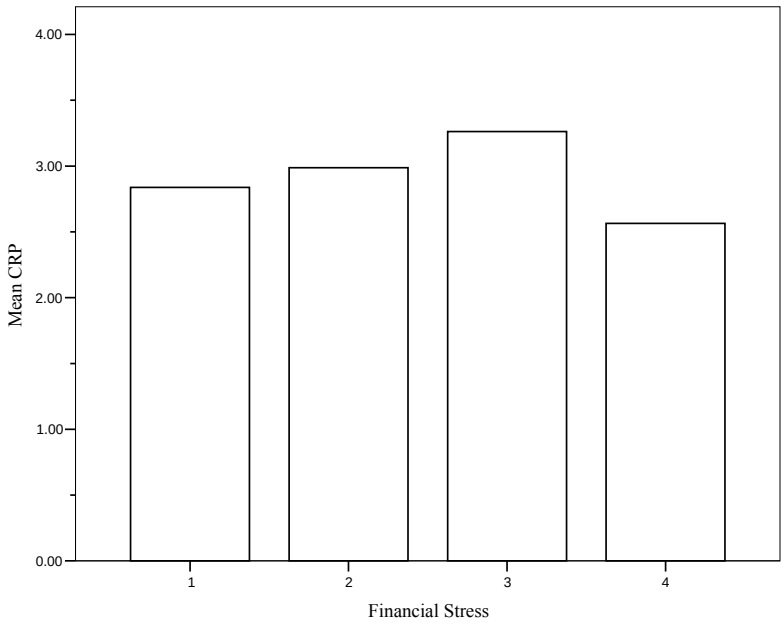

Figure 44: CRP concentration by quartile of Financial Stress

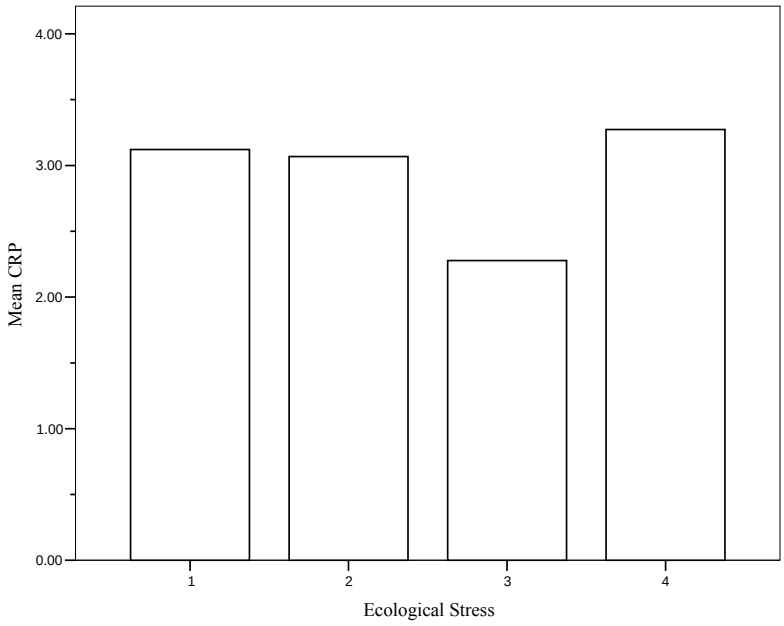

Figure 45: CRP concentration by quartile of Ecological Stress

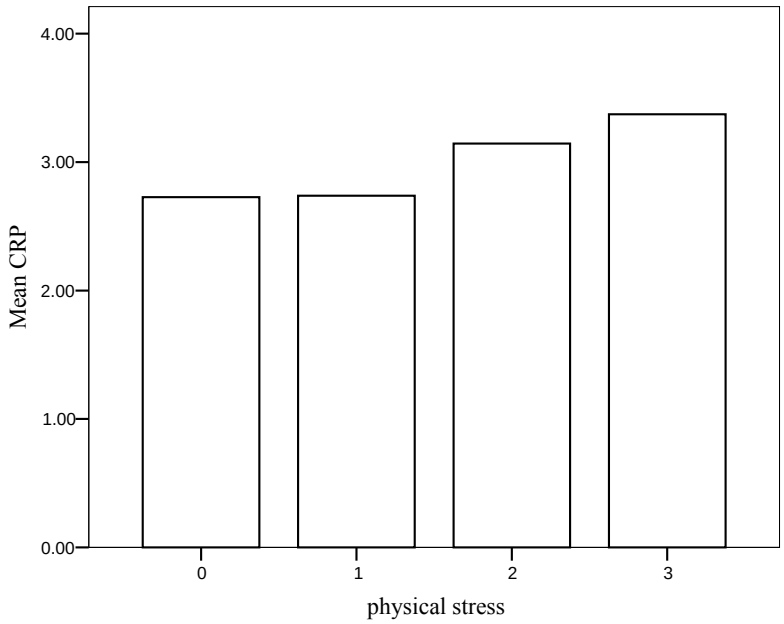

Figure 46: CRP concentration by zero, low, middle, and high Physical Stress groups

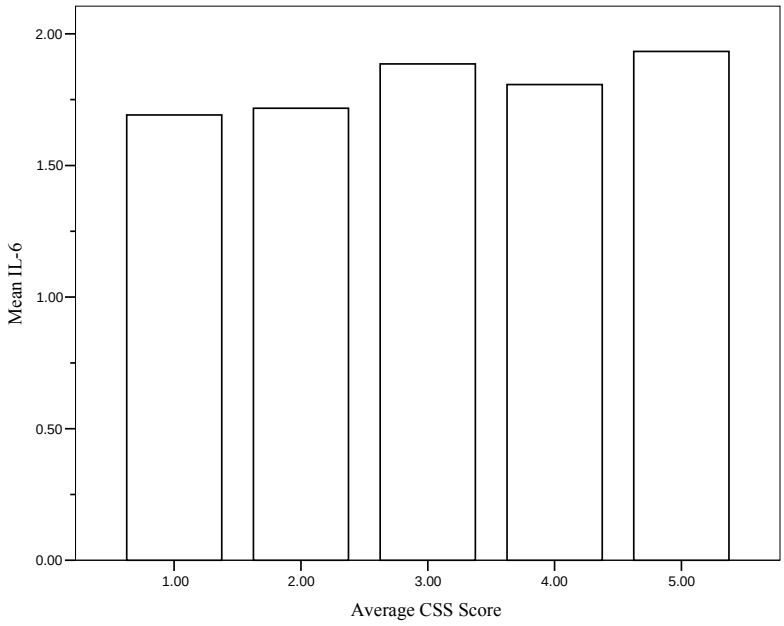

Figure 47: IL-6 concentration by quintile of average CSS score

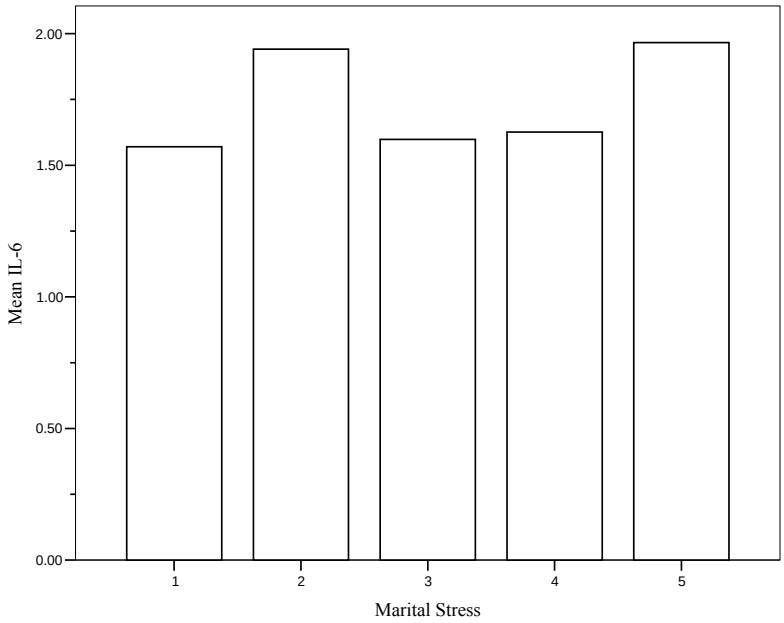

Figure 48: IL-6 concentration by quintile of Marital Stress

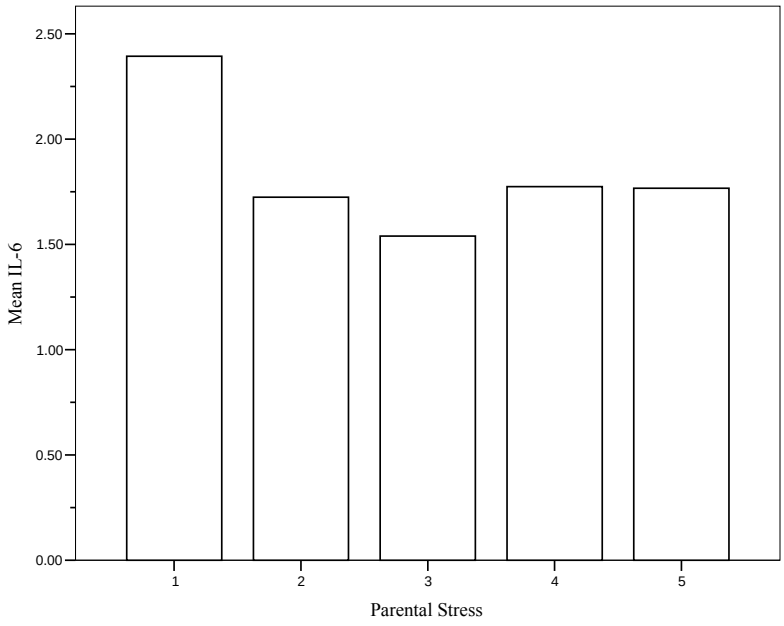

Figure 49: IL-6 concentration by quintile of Parental Stress

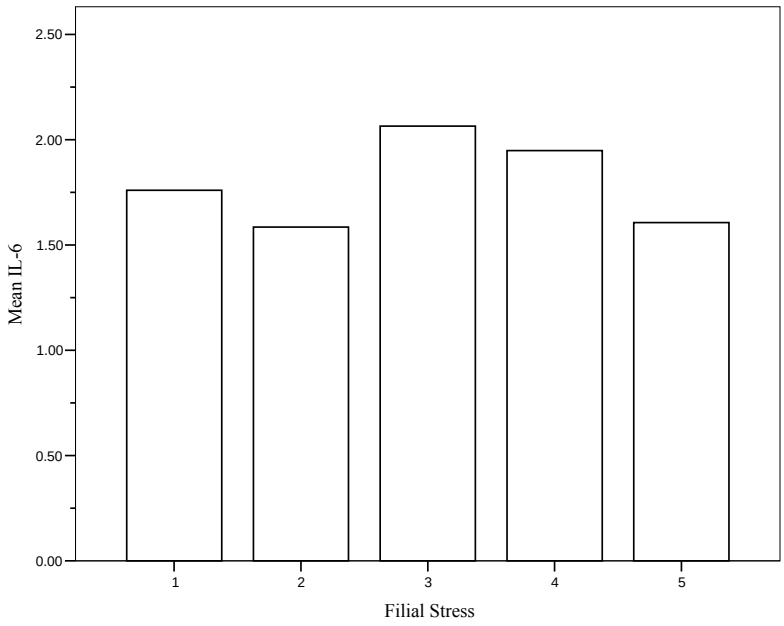

Figure 50: IL-6 concentration by quintile of Filial Stress

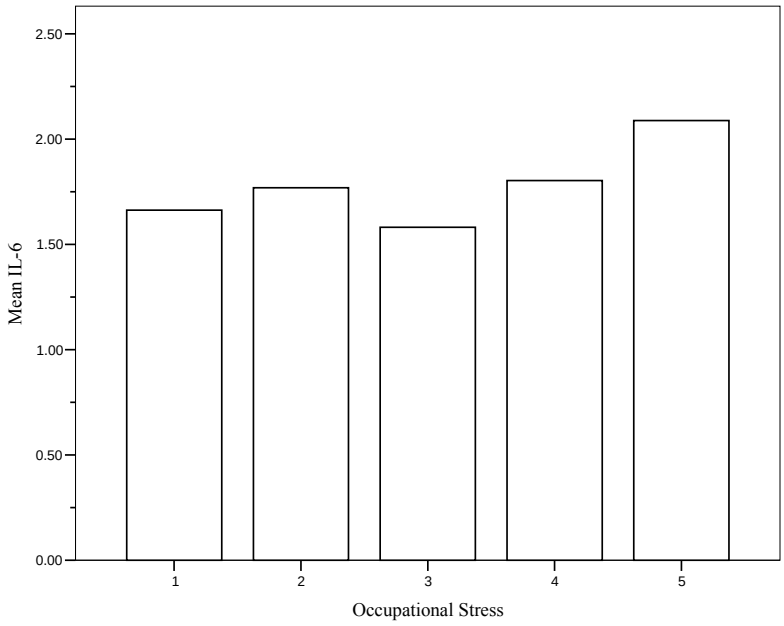

Figure 51: IL-6 concentration by quintile of Occupational Stress

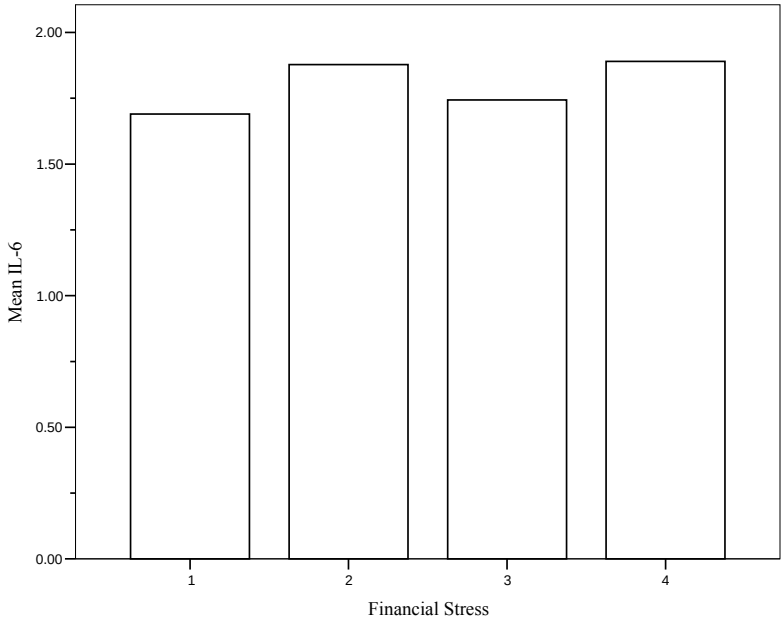

Figure 52: IL-6 concentration by quartile of Financial Stress

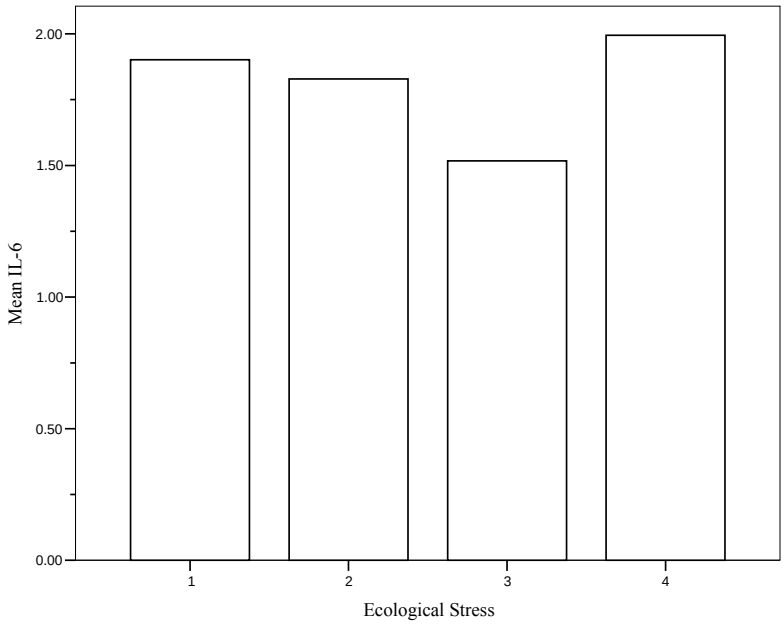

Figure 53: IL-6 concentration by quartile of Ecological Stress

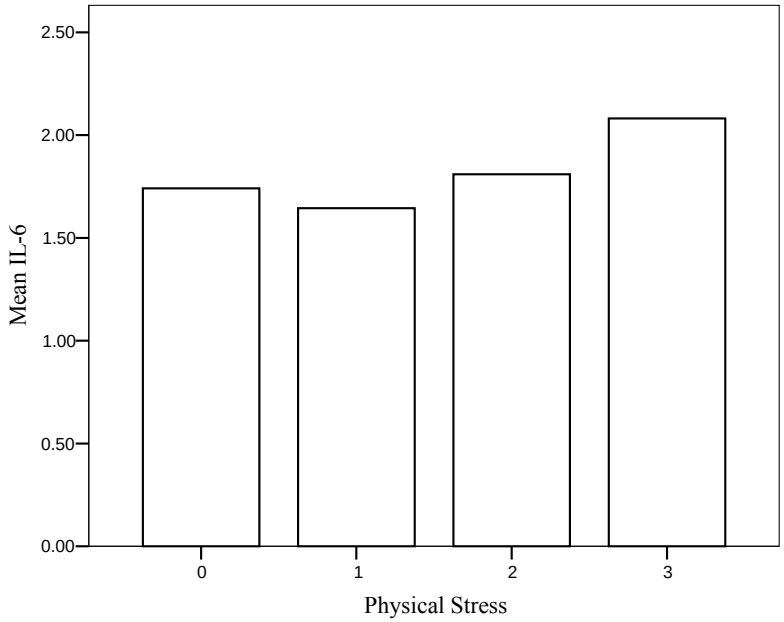

Figure 54: IL-6 concentration by zero, low, middle, and high Physical Stress groups

APPENDIX E: MAIN ANALYSES STRATIFIED BY SEX

Table 38: Pearson correlations between CSS scores and carotid artery measures—Men

	Baseline IMT	IMT Change	Baseline Plaque	Plaque Change
Average CSS	.11	.07	-.07	-.14
Marital	-.04	-.11	-.07	-.09
Parental	-.12	-.01	-.07	-.09
Filial	-.09	.04	-.13	-.06
Occupational	-.02	.07	-.10	-.11
Financial	-.07	-.04	-.08	-.16†
Ecological	-.12	.09	.02	-.12
Physical	.05	.21*	.12	.001

* < .05. † < .10.

Table 39: Pearson correlations between CSS scores and carotid artery measures—Women

	Baseline IMT	IMT Change	Baseline Plaque	Plaque Change
Average CSS	.003	.01	.04	.03
Marital	-.08	-.18†	-.05	.09
Parental	-.09	-.09	-.04	-.02
Filial	.01	-.07	-.02	-.01
Occupational	-.002	.06	.02	-.10
Financial	.03	.17*	.01	.09
Ecological	.08	-.01	.06	-.02
Physical	-.02	.13	.08	.06

Table 40: CSS as predictor of 3-year IMT change—Men*

Stress	b	t	p
Average CSS	.03	1.24	.22
Marital	-.01	-.96	.34
Parental	.001	.15	.88
Filial	.003	.88	.38
Occupational	.01	1.00	.32
Financial	.00	-.04	.97
Ecological	.01	1.41	.16
Physical	.02	2.35	.02

*Includes controls for baseline IMT, age

Table 41: CSS as predictor of 3-year IMT change—Women*

Stress	b	t	p
Average CSS	-.02	-.97	.34
Marital	-.01	-1.79	.08
Parental	-.003	-.98	.33
Filial	-.003	-1.47	.14
Occupational	-.001	-.20	.84
Financial	.005	1.29	.20
Ecological	-.002	-.75	.46
Physical	.004	.60	.55

*Includes controls for baseline IMT, age, use of prescription pain medication

Table 42: CSS as predictor of 3-year plaque change—Men

Stress	Odds Ratio	Confidence Interval	p
Average CSS	.71	.36, 1.39	.31
Marital	.90	.75, 1.08	.28
Parental	.95	.84, 1.08	.42
Filial	1.00	.92, 1.08	.91
Occupational	.98	.82, 1.17	.81
Financial	.92	.82, 1.03	.14
Ecological	.95	.85, 1.06	.35
Physical	1.02	.82, 1.27	.85

*Includes controls for baseline plaque, BMI, pulse pressure

Table 43: CSS as predictor of 3-year plaque change—Women

Stress	Odds Ratio	Confidence Interval	p
Average CSS	.94	.46, 1.90	.86
Marital	1.08	.91, 1.28	.39
Parental	.98	.87, 1.10	.68
Filial	1.00	.92, 1.07	.91
Occupational	.89	.73, 1.08	.23
Financial	1.06	.92, 1.23	.41
Ecological	.98	.87, 1.09	.67
Physical	.99	.78, 1.26	.94

*Includes controls for baseline plaque, LDL cholesterol, use of prescription pain medication

Table 44: Correlations between inflammatory markers and carotid artery measures—Men

	Baseline IMT	IMT Change	Baseline Plaque	Plaque Change
CRP	.04	-.08	.13	.06
IL-6	-.05	-.01	.10	-.08

Table 45: Correlations between inflammatory markers and carotid artery measures—Women

	Baseline IMT	IMT Change	Baseline Plaque	Plaque Change
CRP	.10	.03	-.05	-.05
IL-6	.07	.03	.06	-.11

Table 46: Correlations between CSS scores and inflammatory markers—Men

Stress	CRP	IL-6
Average CSS	.11	.04
Marital	.11	-.03
Parental	-.06	-.18*
Filial	-.12	-.04
Occupational	-.12	.07
Financial	.22*	.02
Ecological	.09	.13
Physical	.22*	.19*

* < .05. † < .10.

Table 47: Correlations between CSS scores and inflammatory markers—Women

Stress	CRP	IL-6
Average CSS	.16†	.07
Marital	.04	.18†
Parental	.09	.03
Filial	.13	.02
Occupational	.10	-.08
Financial	-.05	-.03
Ecological	.12	.14†
Physical	.12	.06

* < .05. † < .10.

Table 48: CSS as predictor of baseline CRP—Men*

Stress	b	t	p
Average CSS	.01	.31	.76
Marital	.01	.73	.47
Parental	-.01	-1.49	.14
Filial	-.001	-.30	.77
Occupational	-.01	-1.13	.26
Financial	.01	1.87	.06
Ecological	.003	.53	.60
Physical	.003	.21	.83

* Includes controls for BMI, smoking status, use of prescription pain medication

Table 49: CSS as predictor of baseline CRP—Women*

Stress	b	t	p
Average CSS	.04	1.17	.24
Marital	.003	.28	.78
Parental	.01	.86	.39
Filial	.01	1.82	.07
Occupational	.001	.13	.90
Financial	-.01	-.68	.50
Ecological	.01	.91	.37
Physical	-.001	-.12	.90

* Includes controls for BMI, triglycerides

Table 50: CSS as predictor of baseline IL-6—Men*

Stress	b	t	p
Average CSS	-.01	-.39	.70
Marital	-.01	-1.03	.31
Parental	-.01	-2.09	.04
Filial	-.001	.54	.59
Occupational	.001	.25	.81
Financial	.003	.75	.46
Ecological	-.001	-.26	.80
Physical	-.001	-.18	.86

* Includes controls for BMI, smoking status, rheumatologic condition

Table 51: CSS as predictor of baseline IL-6—Women*

Stress	b	t	p
Average CSS	-.004	-.20	.84
Marital	.01	1.16	.25
Parental	-.001	-.22	.83
Filial	.00	.10	.92
Occupational	-.01	-1.36	.18
Financial	-.002	-.68	.50
Ecological	.004	1.25	.21
Physical	-.005	-.80	.43

* Includes controls for BMI, triglycerides

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