SPECIFIC FEATURES OF STRESSFUL EXPERIENCES, DISPOSITIONAL
INTERPERSONAL SENSITIVITY, AND BIOLOGICAL STRESS MEDIATORS IN
CHILDREN AND ADOLESCENTS

by

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Abstract

This thesis examines the associations between stressful experiences and biological mediators in young people, with an emphasis on specific features of the stressor (such as its duration and emotion-eliciting qualities) and the moderating role of dispositional interpersonal sensitivity. Study 1 examined the effects of major life events, chronic interpersonal stress, and the interaction between the two among 104 adolescent women. The biological outcomes were cortisol output, the expression of glucocorticoid receptor (GR) mRNA, C-reactive protein (CRP), insulin, and glucose. Results indicated that neither life events nor chronic interpersonal stress was significantly associated with biological outcomes. However, adolescents who experienced the combination of a major life event and higher levels of chronic interpersonal stress showed increased cortisol output and decreased expression of GR mRNA. This indicates that the simultaneous exposure to both acute and chronic stress may be particularly detrimental. Study 2 aimed to replicate these findings among 71 youth with asthma. This study followed participants over 2 years. Life stress, asthma-relevant immune activity, and daily asthma symptoms were measured every 6 months. Findings were analyzed using hierarchical linear modeling (HLM). Similar to the study 1 findings, youth who were double-exposed to a major life event and chronic family stress showed increased production of asthma-relevant cytokines and increased symptom expression. Finally, study 3 examined the interaction between interpersonal sensitivity and life event dimensions of loss, danger, and humiliation among 144 adolescent women. Study outcomes included markers of early cardiovascular risk, such as blood pressure and indicators of systemic inflammation (serum CRP, serum interleukin-6, and stimulated IL-6). Life stress dimensions and biological outcomes were measured every 6 months over 2.5 years. HLM analyses indicated that interpersonal sensitivity moderated the relations of danger events to CRP
and diastolic blood pressure, such that the biological response was amplified among interpersonally sensitive adolescents. Interpersonal sensitivity also interacted with loss events, such that adolescents high on interpersonal sensitivity showed increased production of the pro-inflammatory cytokine IL-6 in the aftermath of major loss. Overall, findings indicate that stressor impact is a complex phenomenon that depends on specific properties of the stressor, as well as dispositional factors like interpersonal sensitivity.
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List of Abbreviations

ANS = autonomic nervous system
CRP = C-reactive protein
CVD = cardiovascular disease
BP = blood pressure
DBP = diastolic blood pressure
GR = glucocorticoid receptor
HLM = hierarchical linear modeling
HPA = hypothalamic-pituitary-adrenocortical
IFN = interferon
IL = interleukin
SNS = sympathetic nervous system
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Co-Authorship Statement

Chapters 2 and 3 are manuscripts that have already been published in peer-reviewed journals. Chapter 2 was published in *Health Psychology* in 2007. I am the first author, and the other authors are Tara Martin, Ekin Blackwell, Cinnamon Stetler, and Gregory Miller (in that order). Gregory Miller designed the research project, while I analyzed the data and drafted the manuscript. Feedback from Gregory Miller was incorporated into the final versions. Chapter 3 was published in *Psychosomatic Medicine* in 2009. I am the first author, and Edith Chen and Gregory Miller are the second and third authors. Edith Chen designed the research project. I performed all of the data analyses and wrote the first draft of the manuscript. Feedback from both Edith Chen and Gregory Miller was incorporated into the final versions.

Chapter 4 is a manuscript that will soon be submitted for publication. I am the first author, and Edith Chen and Gregory Miller are the second and third authors. Gregory Miller designed the research project. I analyzed the data and wrote the paper under the guidance of Gregory Miller.
Chapter 1: General Introduction

Stressful life experiences increase vulnerability to adverse medical conditions. For instance, prolonged exposure to stress can increase susceptibility to upper respiratory illness, accelerate progression of cardiovascular and infectious diseases, and trigger exacerbations of inflammatory conditions like asthma and rheumatoid arthritis (Cohen, Janicki-Deverts, & Miller, 2007; Miller, Chen, & Cole, 2009). As a result of the robust associations between stressful experiences and adverse health outcomes, current research aims to understand the biological mechanisms by which stressors “get under the skin.”

Stress occurs when environmental demands exceed an individual’s adaptive capacity or ability to cope (Cohen, Kessler, & Gordon, 1997). Some conceptions of stress focus on the environmental event (also referred to as the stressor), while others emphasize the individual’s perception and evaluation of the potential harm posed by the environment. According to general models of stress and health, stressors or life events cause negative affective states like fear and anxiety, which in turn, influence health-relevant behaviors and biological processes (Cohen et al., 1997; Cohen et al., 2007). The assumption underlying the behavioral pathway is that people who are experiencing stress are more likely to engage in poor health practices, such as cigarette smoking and alcohol use, and are less likely to engage in beneficial behaviors such as adherence with medical regimens. In regard to biological pathways, the emphasis has been on 2 main stress systems that are activated by stress, the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympathetic nervous system (SNS). HPA activation triggers the release of cortisol, a glucocorticoid hormone with wide-ranging effects on other body systems, including the immune, cardiovascular, and metabolic systems. SNS activation triggers the release of catecholamines, including epinephrine and norepinephrine, which exert effects on a number of systems, including
the skeletal, pulmonary, and cardiovascular systems (Cohen et al., 2007). The physiological changes elicited by the SNS, which include elevated heart rate and blood pressure (BP), and increased blood flow to active muscles, are the types of responses that are thought of as “fight or flight” reactions (Cannon, 1932). Although the biological stress response can help people to deal effectively with acute threat, prolonged or repeated exposure to stress can lead to dysregulation in these systems, which fosters pathogenic processes like high BP, insulin resistance, and low-grade chronic inflammation (McEwen & Stellar, 1993; Sapolsky, 2004).

This thesis focuses on neuroendocrine changes following life stress and their implications for pathogenic processes involved in major diseases, including asthma and cardiovascular disease (CVD). These processes were studied in children and adolescents. This is important because stress exposures in the early decades of life have the potential to permanently alter HPA and SNS regulation. For example, evidence from animal models suggests that pubertal animals are more sensitive to stress-induced alterations in brain and behavior compared to adult animals, which may have lasting implications for stress reactivity (Romeo, 2010). Moreover, there is mounting evidence that chronic diseases, particularly CVD, begin in the first and second decades of life. For example, autopsy studies reveal that many young individuals have developed atherosclerotic plaque by the time they reach adolescence (Berenson et al., 1992; Berenson et al., 1998), and CVD precursors such as insulin resistance and endothelial dysfunction are identifiable in some youth (Berenson & Srnivasan, 2005). Moreover, risk factors for CVD, including obesity, high BP, and elevated lipids track across childhood and adolescence and into adulthood (Lauer, Burns, Clarke, & Mahoney, 1991; Mahoney et al., 1996). Thus, stressful experiences may promote biological alterations that foster the earliest subclinical manifestations CVD (Matthews, 2005). Finally, asthma is one of the most common chronic illnesses among
North American youth, and it has important consequences for daily life functioning and health care costs. For example, in the United States in 2003, there were 12.8 million missed school days among youth with asthma, and in 2004, there were 198 000 hospitalizations for severe exacerbations (Akinbami, 2006). Psychological stress has been implicated in the onset of asthma, as well as symptom exacerbation among patients with asthma (Wright, Cohen, & Cohen, 2005; Chen & Miller, 2007; Miller et al., 2009).

This general introduction provides a background for the studies presented later in the thesis. The literature linking life stress to biological outcomes in young people is reviewed, with an emphasis on the conceptual and methodological limitations of past work. This is followed by a discussion of how stress models have progressed from more general to more specific, and how this progression is reflected in the measurement of life stress. Next, examples from the literature are used to demonstrate that the relationship between life stress and biological outcomes can be influenced by specific features of the stressor, including its duration and emotion-eliciting properties. This is followed by an introduction of a personality construct referred to as interpersonal sensitivity (Marin & Miller, under review), which is likely to intersect with the stress process. Finally, the major aims of the dissertation are described. These involve identifying features of life stress that elicit the most profound biological changes, as well as delineating the moderating role of dispositional interpersonal sensitivity. Following the general introduction, Chapter 2 reports on an initial study of life stress and HPA regulation in female adolescents, with an emphasis on the effects of acute versus chronic stressors. Chapter 3 expands on this work by testing similar questions using a repeated measures design and looking at biological outcomes relevant to asthma. In the 4th chapter, the focus changes to the emotion-eliciting properties of life stress and their interaction with dispositional interpersonal sensitivity.
This study looks at biological outcomes associated with pre-clinical cardiovascular risk. Finally, Chapter 5 concludes the dissertation with a general discussion of study findings.

Background

The Role of Family Difficulties

Most of what is known about the role of life stress in shaping disease-relevant biological processes in young people comes from a growing body of literature on risky family environments (Repetti, Taylor, & Seeman, 2002; Troxel & Matthews, 2004). Risky families are characterized by conflict and aggression, as well as by relationships that are cold, neglectful, and unsupportive (Repetti et al., 2002). Thus, the following paragraphs review the literature documenting associations between aspects of the family environment and markers of SNS and HPA function, pre-clinical CVD, and inflammatory processes relevant to asthma.

SNS activity. Youth from family environments characterized as conflictual and unsupportive show altered sympathetic nervous system (SNS) responses to laboratory stressors. In one study, college students who reported poor childhood family relationships showed elevated BP, both at resting and in response to two laboratory tasks (an impromptu speech and viewing a sad movie) (Luecken, 1998). In a similar study, poor family relations were associated with delayed SPB recovery following the stress manipulation (Luecken, Rodriguez, & Appelhans, 2005). In yet another study, Salomon, Matthews, and Allen (2000) measured family difficulties by asking parents and adolescents to report the extent of openly expressed anger, aggression, and conflict among family members. Results indicated that youth who had experienced increased family difficulties were more likely to show heightened sympathetic activation in response to a mirror tracing task and a social competence interview. Finally, research evidence indicates that children from high-conflict homes show elevated cardiovascular reactivity to audiotaped
arguments compared to children from low-conflict homes (El-Sheikh & Harger, 2001). Moreover, this association was amplified among children who responded to their parents’ conflicts with higher levels of self-blame and threat appraisals.

Importantly, the biological alterations associated with these types of family variables extend beyond measures of laboratory reactivity and recovery. For example, one study found that African American adolescents with higher perceived family conflict showed increases in resting diastolic blood pressure (DBP) over 2 clinic-based assessments, spaced 6 months apart (Clark & Armstead, 2000). Another study showed that adolescents who reported a higher degree of chronic family stressors over the previous year showed increased ambulatory systolic blood pressure (SBP) over 2 consecutive school days (Brady & Matthews, 2006). These findings suggest that some youngsters are unable to adapt or habituate to repeated or ongoing family stressors. These studies also show that chronic exposure to family difficulties can have a long-term impact on resting BP, which may take a toll on the cardiovascular system over time.

**HPA activity.** Growing evidence indicates that the family environment affects cortisol profiles in children and adolescents. In one study, aspects of the family environment were assessed among 264 children and adolescents residing in a rural Caribbean village. Results indicated that family environments characterized by high levels of conflict and irrational punishment were associated with abnormal cortisol profiles, diminished immunity, and frequent illness (Flinn & England, 1997). More recently, Essex and colleagues (2002) showed that maternal stress beginning in infancy was associated with elevated cortisol levels at 4.5 years compared to children who had never been exposed to stress. Further analyses demonstrated that maternal depression beginning in the child’s infancy was the strongest predictor of children’s
cortisol. However, another study found evidence of lower basal cortisol levels among children who experienced long-term separation from a parent (Carlson & Earls, 1997).

Family variables have also been linked to cortisol reactivity. For example, a recent study showed that young adults from divorced families showed significantly lower cortisol both before and after a laboratory speech task compared to young adults from intact families, even after controlling for family conflict and current depression and anxiety (Kraft & Luecken, 2009). In another study, Luecken and Appelhans (2006) demonstrated that early parental loss interacts with the quality of the family environment in the prediction of cortisol responses in adolescence. In particular, among adolescents who experienced early parental loss, a poor relationship with the surviving parent was associated with increased cortisol reactivity to a speech task. Taken together, these studies indicate that family variables are associated with indices of HPA activity. However, there are inconsistencies regarding the direction of the effect, with some studies showing increased cortisol output and others showing lower basal levels and blunted responses to psychosocial triggers.

Preclinical CVD. Family stressors have also been linked to biological alterations more proximately related to CVD, although the evidence in this area is quite limited. In one study, parent ratings of family conflict were associated with lipid profiles among boys (but not girls) between the ages of 6 and 18 (Weidner, Hutt, Connor, & Mendell, 1992). Specifically, boys from families characterized by higher levels of family conflict were more likely to show unfavorable lipid profiles (i.e., higher ratios of plasma cholesterol to high-density lipoprotein). In addition, a recent study looked at trajectories of family stress over 3.3 years among 158 healthy adolescents (Low, Salomon, & Matthews, 2009). The biological outcomes included cardiovascular reactivity to a mental arithmetic task and a mirror image tracing task, as well as
intima-media thickness, a measure of the degree of atherosclerosis in the carotid artery. Results indicated that adolescents exposed to family stress that worsened over time showed increasing trajectories of cardiovascular reactivity. Although family stress trajectories were not directly associated with intima-media thickness, increasing trajectories of DBP reactivity over time were associated with increased thickness. Another study examined the roles of cumulative risk exposure and maternal responsiveness in physiological processes among 207 adolescents living in rural areas (Evans, Kim, Ting, Tesher, & Shannis, 2007). Cumulative risk scores were based on aspects of the family environment (e.g., turmoil, violence), aspects of the physical home environment (e.g., crowding, noise), and personal characteristics such as poverty and single parenthood. Maternal responsiveness was a combination of the youth’s perceptions (e.g., willing to talk to me when needed) and observations of a mother-child interaction. Results indicated a significant longitudinal association between cumulative risk and allostatic load (based on the upper quartiles of 6 physiological indices, including cardiovascular and neuroendocrine parameters and body mass index). However, this association was qualified by an interaction with maternal responsiveness. In particular, higher cumulative risk was associated with elevated allostatic load only among adolescents with mothers low on responsiveness. Finally, a study of children and adolescents with insulin-dependent diabetes showed that aspects of the family environment influenced glycemic control over the first 4 years after diagnosis (Jacobson et al., 1994). In particular, low family cohesiveness, high family conflict, and the inability to openly discuss and express feelings within the family were associated with deterioration in glycemic control over the course of the follow-up.

**Asthma outcomes.** Family adversity is an important predictor of asthma outcomes (Kaugars, Klinnert, & Bender, 2004). For instance, one study focused on maternal risk,
including emotional availability for the child, commitment to child care, and perinatal depression (Klinnert et al., 2001). Results indicated that parenting assessments taken when the infants were 3 weeks old were prospectively associated with infant respiratory or wheezing illness, as well as the persistence of asthma between ages 6 and 8. Similarly, Wright and colleagues (Wright, Cohen, Carey, Weiss, & Gold, 2002) showed that parental stress prospectively predicted wheezing in infancy, independent of caregiver smoking and breast-feeding behaviors. Aspects of the family environment also appear to influence asthma symptoms and asthma-relevant immune processes in children and adolescents with asthma. A recent study showed that family support (i.e., “the degree to which youths lack a parent who understands, values, or cares about them”) was associated with biological processes relevant to asthma, as well as clinically relevant outcomes (Chen, Chim, Strunk, & Miller, 2007). In particular, to the extent that youth perceived lower family support, they showed increased symptom expression, poorer pulmonary function, and heightened allergic inflammation. Family support has also been linked to glucocorticoid resistance and higher circulating levels of eosinophil cationic protein in youth with asthma (Miller, Gaudin, Zysk, & Chen, 2009). This suggests that difficult parent-child relationships can diminish cortisol’s ability to regulate cytokine activity and subsequent airway inflammation. These studies indicate that family difficulties are particularly detrimental to youth with asthma.

The Role of the Broader Social Environment

Although the majority of studies have focused on the family environment, there’s growing evidence that stressors in the broader social environment can contribute to health risks as well. This may be especially true in adolescence, when there is increasing emphasis on the peer group. For instance, a recent study found that adolescents who reported interpersonal mistreatment (e.g., threats and harassment) showed elevated ambulatory BP over the course of 2 school days.
(Matthews, Salomon, Kenyon, & Zhou, 2005). In addition, a study from the mental health literature showed that young teenagers who experienced recurrent peer victimization were more likely to report symptoms of depression and anxiety one year later (Bond, Carlin, Thomas, Rubin, & Patton, 2001). Thus, interpersonal tension and conflict with friends and peer groups appears to be an important source of stress in adolescence. Furthermore, most adolescents have had a romantic relationship by middle adolescence, and concerns over the presence, absence, and quality of romance are particularly salient at this age (Graber, Brooks-Gunn, & Petersen, 1996; Steinberg & Morris, 2001). Although little is known about the association between romantic relationships and biological outcomes, research evidence indicates that a recent romantic breakup is a significant risk factor for the onset of major depressive disorder in adolescence (Monroe, Rohde, JR, & Lewinsohn, 1999). Moreover, a recent study showed that chronic stress across multiple interpersonal domains (including romantic relationships, close friendships, family relationships, and the larger social network) is associated with increasing 6-month trajectories of an indicator of pro-inflammatory activity (Miller, Rohleder, & Cole, 2009). Over the long-term, this tendency could favor the development of a pro-inflammatory environment in the body that sets the stage for chronic diseases like atherosclerosis.

Limitations of Previous Work

Taken together, these findings suggest that stressors in children and adolescents’ social world have biological consequences. However, our knowledge of this relationship is limited in several important ways. First, the majority of these studies measured the social environment using self-report questionnaires. These measures often suffer from what has been referred to as intracategory variability (Dohrenwend, 2006), which means that people interpret life event descriptors in highly personal ways. For example, the investigator may intend to measure the
occurrence of a major family conflict, but a subject may endorse the item for a minor argument or tension between family members (Monroe, 2008). Thus, event checklists that were designed to measure major life events often have the problem of over-inclusiveness, in that minor events often lead participants to make a positive response to a given checklist category. The problem of intracategory variability can also occur when measuring other important characteristics of events, such as their valence and source, and its consequences in traditional checklists include unreliability of recall and susceptibility to recall bias (Dohrenwend, 2006). In a similar vein, the ability to cope with life events may affect retrospective reports as well as the biological processes of interest. As an example, an individual who is unable to harness social support following a stressful event may recall that event as being more stressful compared to someone with ample social resources. This can be problematic because social support is an important predictor of biological stress mediators, and thus can act as a third variable. Therefore, more objective measures are needed to help rule out individual difference factors as potential confounders. It should also be noted that stress checklists fail to capture stressor severity and duration, which are likely to influence the magnitude and/or duration of the biological response.

Second, many of the studies have used cross-sectional designs. These designs present interpretational difficulties because they cannot elucidate the temporal relations between stressful experiences and biological outcomes. They also may provide somewhat misleading conclusions about how biological systems are modified by stress. Indeed, there is growing evidence of dynamic associations between stress exposures and outcomes, which cannot be captured in a single snapshot (Miller, Chen, & Zhou, 2007). For example, there is evidence of temporal variability in biological outcomes in a recent study of caregiving stress (Rohleder, Marin, Ma, & Miller, 2009). In particular, results indicated that there was no difference between caregivers
and controls on a marker of systemic inflammation at study entry, but trajectories diverged over time as caregivers’ concentrations increased markedly. Thus, a cross-sectional study near the onset of caregiving stress would have failed to capture this effect. Moreover, the trajectories for 3 other biological outcomes followed a u-shaped function, such that the direction of the trajectories changed at about 18 to 20 weeks after study entry. This means that single time-point assessments can generate misleading conclusions about which direction a process changes, depending on when the assessments are conducted.

Finally, there are some inconsistencies in the current literature regarding the specific biological processes that are affected by family and interpersonal difficulties. For instance, the literature on family difficulties and children’s cortisol profiles reveals discrepant findings across studies. For example, maternal stress and parental loss have been linked to increases in both basal cortisol levels and cortisol reactivity, whereas marital dissolution and parental separation have been linked to lower basal levels and blunted responses to acute stress (Carlson & Earls, 1997; Essex et al., 2002; Luecken & Appelhans, 2006; Kraft & Luecken, 2009). This may be a result of studies overlooking distinctions between stressors, such as the duration of exposure and the particular type of threat, which determine whether cortisol increases or decreases (Kemeny, 2003; Miller et al., 2007). Another possibility is that dispositional factors influence the direction and/or magnitude of individuals’ biological responses to stress. Thus, it may be possible to gain a more complete understanding of stress, biology, and disease by taking a more nuanced approach to the study of stressful life experiences.

Specific Properties of Life Stress

Early theories postulated that biological responses to life stress were nonspecific and did not differ depending on the nature of the situation (the generality model) (e.g., Selye, 1978).
This is reflected in the instruments used to measure life stress, which started off as very general, and then gradually became more refined. Specifically, the first measures consisted of a checklist of common events, with each event counting equally toward an overall stress score (Hawkins, Davies, & Holmes, 1957). The standard checklist was then adapted to account for stressor severity. For example, the events listed in The Social Readjustment Rating Scale were weighted based on judges’ ratings of the degree of difficulty required to adjust to the event (Holmes & Masuda, 1973). These weights were called life change units. The assumption here was that the effects of stressors operate largely through the creation of excessive adaptive demands. Moreover, the magnitude of life change was considered more important than if the event was positive or negative (Cohen, Kessler, & Gordon, 1995).

Over time, models of stress began to incorporate the notion of appraisal to deal with the observation that there are profound individual differences in responsivity to most situations. Appraisals represent the process of categorizing a situation in terms of its significance for wellbeing (Lazarus & Folkman, 1984). According to the transactional model, primary appraisal relates to perceptions of goal threat, whereas secondary appraisal relates to perceptions of coping resources available to meet the demands of the circumstance (e.g., financial resources, social support). Thus, psychological stress results from an imbalance between demands and resources. This model drove changes in stress measures, which started accounting for the appraisal process. This was done in two ways. One was to measure perceived stress by adding a subjective element to the traditional stress checklist (e.g., Sarason & Johnson, 1978). The other approach used an investigator-based rating (e.g., Brown & Harris, 1978). These ratings are often referred to as contextual measures because they estimate the impact of an event in a specific context for the average person, while avoiding individual subjective reactions. To make these ratings of
contextual threat, the raters consider the key dimensions identified by Lazarus and other appraisal theorists (e.g., goal threat, controllability) (e.g., Lazarus & Folkman, 1984; Scherer, 1984). Thus, the appraisal is made for the participant in a way that is consistent with models of the appraisal process, but which may or may not correspond with the way people themselves report stressor severity.

In recent years, specificity models have been receiving increasing attention, although they have yet to be tested in any depth. The idea here is that the behavioral and biological response is suited to coping with the specific demands presented by the environment. These models suggest that while there is some generic stress response, it is probably only evident in the most severe of circumstances where the whole system goes into overdrive (e.g., physical threat) (Cacioppo et al., 1992). In current times, humans tend to encounter situations that are more subtle and nuanced, and the brain is able to tailor the response to the context. Specifically, environmental demands and cognitive appraisals of those demands elicit specific emotional, motivational, and biological responses that promote adaptive responses to the specific threat (Kemeny, 2003). For example, threats that are appraised as controllable are thought to elicit active motivational states and physiological changes that support active coping processes. In contrast, threats that are deemed uncontrollable are thought to elicit a pattern of responses that support disengagement from the goal that is threatened by the stressor. These responses may include physiological changes that bring about withdrawal and inactivity, as well as affective changes like depressed mood.

To test these specificity models, it is necessary to measure and account for specific characteristics of the stressor (e.g., its duration, frequency), and to understand the reasons why it is likely to be threatening to the individual (e.g., because s/he has lost something important or
has the potential do so). The studies presented in this thesis use an in-depth interview of life stress that explicitly captures these features. It also considers a dispositional factor, interpersonal sensitivity, that is likely to influence the types of stressors that people find most threatening, and their biological responses to them.

*Acute Versus Chronic Stress*

A growing body of research suggests that it is important to differentiate between acute stressors, such as an isolated conflict or a move to a new city, and more enduring difficulties, like being part of a family that lacks trust, intimacy, and mutual respect. There are compelling reasons to believe that these situations elicit different biological responses (see Kop, 1997; Mohr & Pelletier, 2006; Miller et al., 2007). For example, the association between stress and HPA activity depends on the duration of the threat. In particular, the onset of stress is associated with HPA activation, however, if the stressor persists over time, the body mounts a counter-regulatory response such that cortisol rebounds below normal (Miller et al., 2007). Thus, stressors that are short-lived (acute stressors) are associated with elevated concentrations of cortisol, whereas those that have been playing out over months (chronic stressors) are associated with lower cortisol levels. Components of the immune system also appear to be sensitive to the duration of the stressor. In particular, a recent meta-analysis showed that short-term stressors are associated with upregulated parameters of natural immunity and a decrease in proliferative response (Segerstrom & Miller, 2004). Given that these immune changes require minimal time and energy investment, they may represent an efficient redistribution of cells in the context of a “fight or flight” reaction. The authors suggest that this immune response may have evolved to prepare the organism for infections resulting from bites, scrapes, or other injuries. In contrast,
stressors that are more chronic in nature (e.g., caring for an ill spouse) are associated with more global immunosuppression, as evidenced by decreases in functional immune measures.

In general, exposures to chronic stress are thought to be most toxic because they are most likely to bring about long-term biological changes (McEwen, 2004). In contrast, the effects of acute stressors are thought to be relatively short-lived. For example, a meta-analysis of acute laboratory stressors showed that cortisol levels returned to baseline between 21 and 40 minutes following social-evaluative performance tasks (Dickerson & Kemeny, 2004). Moreover, in a recent study of daily experiences and HPA activity, feelings of loneliness, sadness, threat, and lack of control on one day were associated with a higher cortisol awakening response on the following day (Adam, Hawkley, Kudielka, & Cacioppo, 2006). Thus, the effects of daily psychosocial experiences act on a relatively rapid time scale.

However, very little is known about the duration of the biological response in the aftermath of major life events in the real world. This is in part a methodological issue because it is difficult to identify individuals who have experienced a recent major life event, enroll them in a study, and then begin tracking their biology within days or even weeks of the event. In comparison, chronic stressors are still ongoing at the time of biological assessment. Indeed, studies on life stress in children and adolescents suggest that it is the chronic stressors rather than the major life events that are most strongly related to biological outcomes. This is true when accounting for major life events over the past year (Brady & Matthews, 2006) and over the past 3 months (Miller & Chen, 2006).

It should be noted that arbitrary language is used to explain the temporal aspects of stressors. Acute life events (also referred to as major life events or episodic stressors) are short in duration compared to chronic stressors, but they should not be confused with acute laboratory
stressors, which unfold over the course of minutes. For example, an acute life event like a conflict between best friends may play out over several days, whereas an acute laboratory stressor like the Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993) may last for only 15 minutes (i.e., 10 minutes to prepare and 5 minutes to deliver a speech). Thus, acute and chronic are relative terms only, and they do not refer to specific timeframes.

The Combination of Acute and Chronic Stress

Apart from differentiating between stressors that are acute and chronic, research has indicated that there is value in considering their co-occurrence. In particular, study results indicate that chronic background stress can accentuate and/or prolong the effects of acute exposures. Although the mechanisms remain unclear, it may be that people do not have the adaptive capacity (e.g., coping resources, emotional energy) to deal with multiple demands simultaneously. Examples of this effect have been shown both inside and outside the laboratory. Specifically, Matthews and colleagues (1997) found that children and adolescents with ongoing interpersonal stress showed heightened DBP and total peripheral resistance responses to acute laboratory stressors compared to their lower stress counterparts. Moreover, a recent study examined children’s biological reactivity both before and after the 9/11 attacks (Gump, Reihman, Stewart, Lonky, & Darvill, 2005). It was hypothesized that the attacks would act as a potent background stressor for children living near New York City, and that this would influence their biological responses to laboratory computer tasks. Results indicated that children tested after the attacks showed elevated cardiovascular reactivity compared to children tested prior to the attacks. Moreover, the responses of children tested following 9/11 showed significantly lower reactivity after one year. This suggests that background stressors like terrorism can temporarily elevate children’s cardiovascular responses to acute stress tasks.
The combination of acute and chronic stress also has implications for asthma outcomes. For example, Sandberg and colleagues (2000) examined the association between stressful experiences and asthma exacerbations among children with asthma. Findings indicated that severe life events significantly increased risk for an asthma attack in the 4 weeks that followed. However, when these events took place in the context of chronic stress, the effect was stronger and came almost immediately (within 2 weeks). Miller and Chen (2006) have since provided insights into the biological mechanisms mediating this effect. In particular, they showed that simultaneous exposure to major life events and chronic stress is accompanied by reduced expression of receptors that bind cortisol, epinephrine, and norepinephrine among youth with asthma. This process is thought to decrease sensitivity to the anti-inflammatory effects of hormones like cortisol and epinephrine (Miller, Cohen, & Ritchey, 2002), and therefore foster inflammation in the airways that is characteristic of asthma.

**Emotion-Eliciting Qualities of Stressful Experiences**

As alluded to earlier in this chapter, contemporary theorists have been proposing specificity in the biological response to stressful situations (Kemeny, 2003). According to Weiner’s integrated specificity model (Weiner, 1992), both behavior and physiology are part of an integrated response to address a specific environmental condition. In other words, specific signals in the environment elicit a distinct pattern of hormonal and neural changes that help the organism to deal with the specific nature of the threat. Importantly, cognitive appraisals and emotion are thought to function as the intermediaries through which the demands get transduced into behavioral and biological responses. For instance, threat versus challenge appraisals of a given situation are associated with distinctive autonomic nervous system (ANS) alterations (Tomaka, Blascovich, Kelsey, & Leitten, 1993). Specifically, motivated performance situations
in the lab (e.g., mathematical tasks like serial subtractions and verbal tasks like speech anticipation and delivery) are associated with activation of some physiological systems, which reflects energy mobilized to cope with the situation. However, the specific nature of the response depends on challenge versus threat appraisals of the situation. In particular, threat appraisals occur when environmental demands are perceived as exceeding resources to cope, and they are associated with high negative affect and disorganized mobilization of physiological resources (i.e., moderate cardiac reactivity coupled with an increase in overall systemic vascular resistance). This pattern of arousal is thought to reflect anxiety and uncertainty over options for coping (Blascovich & Tomaka, 1996). In contrast, challenge appraisals occur when environmental demands are appraised to be within the person’s resources or ability to cope, and they are associated with organized mobilization of physiological resources (maximal cardiac reactivity coupled with a decline in systemic vascular resistance). This pattern of arousal may represent an adaptive response that allows for the speedy and efficient distribution of metabolic resources. Thus, cognitive appraisals can explain why two people might show a different pattern of biological responses to the same task. However, a focus on the specific emotions elicited by an event may reveal even more about the nature of the response because emotions are thought to provide a co-ordinated, adaptive response to specific environmental demands (e.g., Keltner & Gross, 1999; Levenson, 1994; Tooby & Cosmides, 1990).

Research on discrete emotions is consistent with this hypothesis. In particular, emotion induction studies have shown distinctive patterns of activation in the ANS. Ekman and colleagues (1983) presented some of the first convincing evidence of emotion-specific peripheral responses. Among other findings, they showed that fear and anger were both associated with increases in heart rate (HR), which distinguished them from happy, disgust, and surprise.
Moreover, fear and anger could be discriminated based on skin temperature, which was higher in the case of anger. Other studies testing this hypothesis have also shown evidence of differentiation, but these findings suggest that anger acts more on the vasculature and less on the heart than fear (Cacioppo, Berntson, Larsen, Poehlmann, & Ito, 1993). According to a functionalist perspective, the physiological changes associated with fear facilitate inhibition and flight, whereas the physiological changes associated with anger make it easier to attack the antagonist. Indeed, animal research shows that specific neural and peripheral changes occur in concert with behaviors such as fighting, fleeing, defending, and submitting (Weiner, 1992).

Taken together, laboratory studies provide some convincing evidence in support of specificity models. However, little is known about the impact of different types of real-world stressors on biological processes. In this regard, stressful life events can be conceptualized as having core emotional themes involving loss, humiliation, and danger (Brown, 2002). The loss dimension captures whether an event results in the individual having a diminished sense of connectedness or well-being. This could happen in any aspect of a person’s life. Some examples would include the loss of a person through death, separation, or decreased interaction, the loss of a significant material possession, or an event that resulted in the loss of respect in a social group. The danger dimension captures whether the event might pose a threat to the person in the future. This could happen if the event itself recurred, or if it’s full threat unfolded at a later time. Some examples of an event that would be high in danger would include a family member receiving a cancer diagnosis, or a friend or partner threatening to end a relationship. Finally, the humiliation dimension indicates the likelihood of an event leaving a person feeling devalued in relation to others or to a core sense of self. Some examples of humiliating events might be getting fired from a job, or having a partner commit an act of infidelity that becomes public.
Using this dimensional approach, Kendler and colleagues (2003) have demonstrated a degree of specificity in the association between life event dimensions and the onset of mood disorders. In particular, they showed that life stress that involved a major loss increased the risk for onset of major depression. However, it was the events that had an element of loss and humiliation that posed the greatest risk. In contrast, events characterized by danger predicted the onset of generalized anxiety syndrome, but not major depression. This pattern of findings points to specificity in the biological pathways linking life events to anxiety and depression. However, it is only recently that studies have begun to examine the impact of specific types of events on biological processes that might be relevant to physical illnesses.

*Humiliation and shame.* In recent years there has been a particular emphasis on stressful situations that are likely to elicit the emotion shame. Specifically, Dickerson and colleagues (2004) have argued that social threats (much like threats to the physical self) elicit a coordinated psychobiological response, including shame. Specifically, situations that involve a threat to one’s social status or social self-esteem (e.g., rejection, loss of status) trigger feelings of shame, which in turn, activate the HPA axis and pro-inflammatory pathways. The system that coordinates this set of responses is referred to as the social self-preservation system because it is thought to serve the basic needs of belonging and positive self-regard (Taylor & Brown, 1988; Baumeister & Leary, 1995). In particular, shame is associated with a set of motives, non-verbal displays, and behaviors characterized by submission and withdrawal that serve as an appeasement strategy to reduce the chances of social conflict (Keltner, Young, & Buswell, 1997). Importantly, increases in pro-inflammatory activity may extend and prolong disengagement-related behavioral, cognitive, and motivational effects. Given that these psychological and behavioral changes overlap with the symptoms of depression, some
researchers have argued that biological alterations following shame-eliciting stressors have the capacity to bring about depressive episodes (Dickerson, Kemeny, Aziz, Kim, & Fahey, 2004; Miller & Blackwell, 2006).

Growing evidence points to the importance of social threat and shame in the prediction of cortisol responses. In a meta-analysis of studies investigating the acute stress response in humans, laboratory stressors that included a social evaluative component produced the greatest elevations in cortisol (Dickerson & Kemeny, 2004). In fact, research evidence has shown that the extent of cortisol reactivity increases in a linear fashion with shame (Gruenewald, Kemeny, Aziz, & Fahey, 2004). In addition, a recent meta-analysis showed that life events that are likely to elicit feelings of shame (e.g., abuse or assault) were associated with higher afternoon/evening cortisol (Miller et al., 2007). However, there is evidence of an opposite pattern for more long-term severe stress. In combat veterans who suffer from post-traumatic stress disorder, shame is associated with hypocortisolism (Mason et al., 2001).

There is also some evidence of an association between shame and pro-inflammatory activity. In one study, Dickerson and colleagues (Dickerson et al., 2004) experimentally induced self-blame by asking participants to write about traumatic situations that made them feel badly about themselves or for which they blamed themselves. Participants in the control group wrote on a neutral topic. Results indicated that the manipulation successfully induced shame and guilt, as well as increases in oral concentrations of the soluble tumour necrosis factor-alpha receptor II as an index of inflammatory activity. In addition, a recent study examined the association between trait shame and a number of biological outcomes including inflammatory activity (Rohleder, Chen, Wolf, & Miller, 2008). Results indicated that women high on trait shame showed greater inflammatory activity and heightened SNS activity, independent of other
negative emotional states like guilt and depression. However, trait shame was unrelated to daily cortisol output. Thus, shame appears to be a robust predictor of physiological responses, yet more work is needed to clarify the impact of acute versus chronic shame on HPA, SNS, and inflammatory responses. Moreover, studies of social threat in the real world are needed to further examine the generalizability of the social self-preservation theory.

*Loss and sadness.* Loss situations are associated with the emotion sadness (Lazarus, 1991; Levenson, 1994). Sadness is a unique emotion in that it is not connected to an urgent action tendency. In fact, the response is characterized by conservation and withdrawal or passive coping, which is thought to involve energy conservation and decreased responsivity to the environment (Engel, 1962; Bohus et al., 1987; Schneiderman & McCabe, 1989). In regard to its biological correlates, HR responses are smaller in sadness compared to fear (Cacioppo et al., 1993), and a recent study revealed HR deceleration in response to a sadness induction (Kreibig, Wilhelm, Roth, & Gross, 2007). Thus, there is some evidence of biological conservation during sadness. However, induced sadness has also been linked to increases in peripheral cortisol levels (Brown, Sirota, Niaura, & Engebretson, 1993). Moreover, it has been argued that the association between loss events and the onset of major depression is mediated by altered HPA activity (Nicolson, 2004; Meinlschmidt & Heim, 2005).

In support of this hypothesis, there is growing evidence of a link between loss experiences and cortisol activity. Research in rodents and non-human primates shows that maternal separation early in development leads to altered neuroendocrine responsiveness (Sanchez, Ladd, & Plotsky, 2001), a finding that has been replicated in humans. For instance, one study found an association between childhood parental loss and increased cortisol reactivity to a speech task in early adulthood, but only in the context of perceived low caring from the
surviving parent (Luecken, 2000). Moreover, Pfeffer and colleagues (2007) showed that children bereaved by sudden, unexpected parent death showed significantly higher cortisol output across 4 days of sampling compared to non-bereaved children.

Loss experiences also appear to influence diurnal patterns of cortisol output. This work has been synthesized in a recent meta-analysis, which coded stressful life events based on the likelihood that they would elicit feelings of loss (Miller et al., 2007). Results indicated that loss events (e.g., death of a spouse, loss of a job) were accompanied by a flattened diurnal cortisol profile, namely blunted morning cortisol responses and more gradual declines in cortisol output throughout the day. Thus, withdrawal and decreased responsivity to the environment following loss events may have the capacity to alter the circadian rhythm of cortisol secretion (or both may be a manifestation of decreased corticotrophin-releasing hormone drive emanating from the hypothalamus).

**Danger and fear.** Danger situations are likely to elicit the emotion fear, as well as cardiovascular and respiratory changes that facilitate flight, fight, freezing, enhanced startle, and avoidance. These biological changes were first referred to as “fight or flight” reactions, and they include increases in HR and BP (Cannon, 1932). Although fear responses can be acute and reflexive, they may also be sustained responses to lingering threat or danger, and cortisol is thought to play a role in sustaining fear (Schulkin, Morgan, & Rosen, 2005).

The experience of fear and anxiety has been linked to changes in cortisol and BP, as well as markers of cardiovascular morbidity. In a study on rumination about painful social interactions (e.g., betrayals of confidence and romantic infidelity), fear of the transgressor was linked to within-person increases in cortisol (McCullough, Orsulak, Brandon, & Akers, 2007). Thus, it may be that HPA activation helps people to maintain a vigilant posture toward threats
from the past that could be threats again. In addition, vigilant behavior in the lab has been linked to increases in BP, but not HR (Smith, Ruiz, & Uchino, 2000). In regard to life situations involving danger, the threat of future job loss has been linked to biological changes like increases in body mass index (BMI) and BP, elevated adrenal hormones, and increased risk of ischemic heart disease (see review by Ferrie (2001)).

There is also evidence of biological change in the context of chronic fear and anxiety. One study showed that chronic fear of terror was associated with elevated systemic inflammation among healthy women. These findings were independent of generalized anxiety and symptoms of depression (Melamed, Shirom, Toker, Berliner, & Shapira, 2004). Symptoms of anxiety have also been linked to higher concentrations of systemic inflammation in healthy men and women (Pitsavos et al., 2006). Finally, in a study on personality, mood, and BP, adults who were high on trait anxiety showed elevated ambulatory BP over the course of 3 days, independent of daily mood (Räikkönen, Matthews, Flory, Owens, & Gump, 1999). Thus, acute fear and fear-related behaviors may be associated with HPA and SNS activation, and chronic fear and anxiety may also influence inflammatory processes.

The Moderating Role of Interpersonal Sensitivity

Even when the duration and emotion-eliciting properties of events are considered, there is still much variability in how people respond to any given stressful experience. This raises the possibility that there are individual difference variables that influence the magnitude and direction of people’s responses. Given that most of the stressors people encounter in life are social in nature, the level of dispositional interpersonal sensitivity has the potential to be a strong moderator.
Interpersonal sensitivity is a disposition that makes people vigilant for and sensitive to criticism, rejection, and interpersonal conflict (Marin & Miller, under review). This sensitivity to the social world has potential biological ramifications. In particular, when interpersonally sensitive individuals experience interpersonal difficulties, they tend to interpret them as having especially damaging consequences for their status within social groups. Thus, social threats activate concerns about belongingness and acceptance among interpersonally sensitive individuals, which may amplify or prolong the biological response. As mentioned earlier in this chapter, concerns about negative social evaluation have been linked to robust neuroendocrine, cardiovascular, and inflammatory responses. In particular, social evaluative threat and shame are strong elicitors of cortisol reactivity (Gruenewald et al., 2004; Dickerson & Kemeny, 2004), and there is some evidence that negative social evaluation activates pro-inflammatory pathways (Dickerson, Gable, Irwin, Aziz, & Kemeny, 2009) and elicits heightened autonomic and cardiovascular responses (Cacioppo, Rourke, Marshall-Goodell, Tassinary, & Baron, 1990; Smith, Nealey, Kircher, & Limon, 1997; Bosch et al., 2009). Thus, knowing who is and isn’t interpersonally sensitive may explain some of the variability in the biological response to acute threat.

In addition to cognitive and affective aspects of interpersonal sensitivity, this disposition has a behavioral component characterized by submission and inhibition. Interpersonally sensitive individuals are thought to adopt these behaviors to avoid negative social evaluation (Marin & Miller, under review). However, doing so may actually have the opposite effect because inappropriate submissive behavior can elicit negative reactions from others. Moreover, interpersonally sensitive individuals have the tendency to perceive rejection or disapproval in response to benign social cues. Thus, in addition to having heightened biological responses to
social threat, interpersonally sensitive individuals may experience these types of threats with increased frequency. However, for the purposes of this thesis, the focus will be on the moderating effects of IS in the association between acute stress and biological reactivity.

*Functional significance.* Concerns about negative social evaluation may serve the basic human need for belongingness and acceptance (Baumeister & Leary, 1995). Belongingness needs are thought to have evolved from our ancestors’ dependence on group membership for survival and reproduction. Specifically, in the environment of evolutionary adaptation, the motive to create and maintain social bonds would have increased the chances of group membership and decreased the chances of social isolation. Groups offer a number of benefits including the opportunity to share food and resources, help caring for offspring, and the ability to maintain defensive vigilance against predators. Therefore, a great deal of human thought, emotion, and behavior is likely to have evolved to serve this fundamental interpersonal motive. In this regard, a focus on negative social evaluation may promote social connectedness by increasing sensitivity to social cues. In particular, if a person is able to detect signs of disapproval from others, s/he is in a better position to repair the social damage.

As discussed earlier in this chapter, social signals like submissiveness and withdrawal can be thought of as appeasement strategies that bring about social reconciliation. In fact, theorists have argued that submissive displays like holding the head down, gaze avoidance, and hiding are part of a primitive social defense system that evolved to control aggression (Gilbert, 2000). Specifically, in a potential or real conflict situation, submissive behaviors signal that an animal is not going to contest resources or escalate conflict, which in turn, reduces the attacker’s aggression. For humans, the greatest social dangers were probably not aggressive fights. Thus, human appeasement (apologetic, submissive, and affiliative behavior) and its emotional
correlates (embarrassment and shame) are thought to serve the important social outcomes of acceptance and being liked.

The ability to perceive others’ disapproval and to appease when necessary is an important part of maintaining social connections. Yet, individuals high on interpersonal sensitivity have lingering concerns about negative evaluation by the social world, and they tend to recruit submissive options across various social interactions (Gilbert & Trower, 1990). Moreover, interpersonally sensitive individuals over-interpret the impact of abrasive encounters, and they probably ruminate on them as well. Thus, it’s likely that these cognitive and behavioral components of interpersonal sensitivity will accentuate biological responses to social world threats.

*Interpersonal sensitivity and stress reactivity.* Most of what is known about the role of interpersonal sensitivity in the biological stress response comes from studies of acute stress in the lab. For example, one study showed that introversion amplifies the effects of lecturing on heart rate reactivity, both in the anticipation period and at the beginning of a lecture (Houtman & Bakker, 1991). Another study showed that introversion moderated the effects of serial subtraction on heart rate reactivity, such that introverts showed heightened responses compared to extraverts (Hinton & Craske, 1977). In another study, social inhibition amplified the effects of serial subtraction on SBP and DBP reactivity (Habra, Linden, Anderson, & Weinberg, 2003). In another study, the association between social inhibition and immune responses were examined, with a focus on a process called delayed type hypersensitivity responses (Cole, Kemeny, Weitzman, Schoen, & Anton, 1999). Results indicated that social inhibition moderated the effects of high social engagement (intensive personal contact with unfamiliar professionals) on immune responses, such that socially inhibited individuals showed increased delayed type
responses (which are interpreted by the authors as being maladaptive because they reflect an allergic-type response). However, social inhibition did not emerge as a significant moderator under low engagement conditions. This increased delayed type hypersensitivity response among socially inhibited individuals persisted across repeated exposures to the high engagement condition (over the course of 3 weeks). Thus, interpersonal sensitivity may interfere with people’s ability to habituate to psychosocial triggers. Finally, a recent study used a non-verbal behavior analysis to measure women’s use of submissive strategies during a stress interview (i.e., patterns used to appease the interviewer and prevent or inhibit hostile responses). Results indicated that submissive behavior amplified the effects of the interview on heart rate and vagal withdrawal, both during the interview and the recovery phase (Pico-Alfonso, Mastorci, Ceresini, & Ceda, 2007).

Thus, the effects of acute social threat in the lab are amplified among individuals high on interpersonal sensitivity compared to individuals low on this dimension. However, to our knowledge, there is no evidence regarding the impact of real-world stressors on interpersonally sensitive individuals’ biological profiles. It is likely that interpersonally sensitive individuals are more reactive to real-world stressors compared to individuals low on this dimension, but there may be specific features of stress that interact with this disposition. Specifically, given interpersonally sensitive individuals’ concerns about status and belongingness, it’s expected that they would be particularly reactive to life events with themes of rejection, disapproval, and/or loss of status.

Summary of the Literature

Stressful experiences in childhood and adolescence may promote biological alterations that set the stage for disease. Research evidence indicates that stress in children and adolescents’
lives activates the HPA axis and SNS, and there is some evidence that it also has downstream consequences for BP, inflammation, and metabolic outcomes. In particular, these hormones bind to receptors in cells of the immune, metabolic, and vascular systems, altering their functions so that over the long term these pathogenic processes arise. These pathogenic changes have the potential to influence health outcomes in children and adolescents. In youth who are sick with asthma, for example, the changes would likely affect the magnitude of the inflammatory response to allergens, irritants, and infections. If these responses were accentuated, it would have consequences for inflammation and obstruction of the airways, and in turn, for symptom expression (Chen & Miller, 2007). For healthy youngsters, these changes could also be important for health by setting the stage for CVD. In particular, a pro-inflammatory environment in the body could contribute to the early stages of atherosclerosis, the underlying pathogenic condition that drives CVD. Atherosclerosis is a chronic inflammatory response to injuries of the endothelium (the lining of the arteries) caused by a number of factors, including infections and high BP. To the extent that the immune system responds aggressively to these injuries and infections, there is increased risk for the formation and growth of atherosclerotic plaque.

Studies have shown that children and adolescents with family and interpersonal difficulties are more likely to show biological alterations that can be detrimental to health. However, few studies have differentiated the biological impact of acute versus chronic stress. Moreover, there is evidence to suggest that stressful events characterized by loss, danger, and humiliation are differentially associated with the onset of depression and anxiety, yet it is unclear whether there is specificity in the biological responses they elicit or whether they have consequences for health. Laboratory studies on specific types of threat and specific emotions (e.g., social evaluative threat and shame) suggest that the emotional tone of stressors has implications for biology, but this has
rarely been tested in the context of real world stressors. Finally, recent evidence suggests that dispositional interpersonal sensitivity plays a role in shaping the magnitude of individuals’ biological responses to life events. Individuals high on this dimension may show heightened biological reactivity to acute stressors in the lab, especially if those stressors occur in the interpersonal domain. However, little is known about the interaction between interpersonal sensitivity and real-world stressors.

The Current Studies

The goal of this thesis is to identify specific features of life stress that give rise to the most profound biological alterations in young people. There were two main objectives. The first was to examine the differential impact of various features of life stress with a focus on the duration of the stressor and its emotion-eliciting properties. The second was to examine whether interpersonal sensitivity shapes the magnitude of the biological response to various kinds of real world stressors. Thus, each of these studies uses an in-depth interview to measure stress across multiple domains in the lives of children and adolescents.

The first article (or Chapter 2) examines cross-sectional associations between life stress and markers of HPA regulation in a sample of female adolescents. The question of interest here is whether HPA activity differs as a function of acute stress, chronic stress, or a combination of the two. The major outcomes of interest are cortisol output and glucocorticoid receptor (GR) expression. GRs are the binding sites for cortisol and other glucocorticoids, and it is through these receptors that cortisol acts on target tissues. This article also looks at downstream consequences of HPA activation. Cortisol acts on immune and metabolic systems, and excess levels of the hormone are thought to have a dysregulating effect (Bjorntorp & Rosmond, 1999). Thus, this article also looks at one indicator of systemic inflammation (CRP) and two indicators
of metabolic control (insulin and glucose). The second article (or Chapter 3) also examines the differential effects of acute versus chronic stress, but in this case, an actual disease model is used to see if these differences have clinical relevance, in terms of affecting the degree of inflammatory response and symptom expression in asthma. Moreover, a repeated measures design is used to examine within-person changes in immune markers as a function of major life events and chronic family stress. By examining associations at the within-person level of analysis, most between-person confounders can be ruled out, and the temporal ordering of events can be better established. Finally, the third article (or Chapter 4) examines the interaction between dispositional interpersonal sensitivity and life event dimensions of loss, danger, and humiliation. Study outcomes include markers of early cardiovascular risk, such as BP and indicators of systemic inflammation. Inflammation is a key pathogenic mechanism in many diseases, including CVD. This study also uses a repeated measures design. This means that within-person associations between life event components and biological outcomes can be examined as a function of dispositional interpersonal sensitivity. Again, this design helps to establish the temporal ordering of events because the within-person nature of this analysis precludes most of the alternative explanations for linkages between stressors and biological outcomes.

General Hypotheses

The first hypothesis was that the association between life stress and biological stress mediators would depend on the duration of the stressor. In particular, children and adolescents undergoing chronic interpersonal stress were expected to show elevated biological outcomes (including increased cortisol output and higher BP) compared to children and adolescents without these ongoing interpersonal difficulties. Although past work suggests that acute
stressors in the laboratory are robust predictors of biological change, relatively few studies have examined the biological impact of acute events in the real world. However, the available evidence suggests that the effects will be weaker for acute stressors compared to chronic stressors.

The second hypothesis was that the combination of acute and chronic stress would emerge as a robust predictor of biological outcomes, such that chronic interpersonal stress would prolong the effects of major life events. For example, the expectation was that healthy participants who were exposed to a major life event in the context of chronic interpersonal stress would show increased cortisol output, elevated BP, and heightened pro-inflammatory activity. Among youth with asthma, the combination of acute and chronic stress was expected to be associated with immune changes relevant to asthma, as well as increased symptom expression.

The third hypothesis was that life event dimensions of loss, danger, and humiliation would shape the nature of the biological response. For example, consistent with predictions made by the social self-preservation theory, it was anticipated that life events with a major humiliation component (e.g., peer rejection, being cheated on by a romantic partner) would be accompanied by increased pro-inflammatory activity. In addition, acute danger events can evoke the “fight or flight” reaction, thus the expectation was that these events would be accompanied by increases in BP.

The final hypothesis was that the association between major life events and biological stress mediators would be moderated by dispositional interpersonal sensitivity. In particular, it was hypothesized that interpersonally sensitive people would show amplified biological responses to major life events compared to their less sensitive counterparts. However, it was expected that there would be some specificity in terms of the types of events that would be most
salient to interpersonally sensitive individuals. In particular, it was thought that interpersonal sensitivity would interact with themes of rejection, disapproval, and/or loss of status, such that these events would be particularly impactful among interpersonally sensitive individuals.
References


Chapter 2: Differentiating the Impact of Episodic and Chronic Stressors on HPA Axis Regulation in Young Women

Psychological stress is associated with morbidity and mortality across a variety of medical conditions. Prolonged exposure to stress can increase susceptibility to upper respiratory illness, accelerate progression of cardiovascular and infectious diseases, and foster exacerbations of autoimmune conditions like multiple sclerosis and rheumatoid arthritis (Miller & Cohen, 2005; Mohr et al., 2004; Pereira & Penedo, 2005; Rozanski, Blumenthal, & Kaplan, 1999). Given the widespread evidence that stressful experiences are related to adverse health outcomes, current research aims to understand the biological mechanisms through which stressors exert their influence. One candidate mechanism that has received a great deal of attention is the hypothalamic-pituitary-adrenocortical (HPA) axis. Activation of this system initiates a hormonal cascade that results in the secretion of cortisol, a glucocorticoid that has wide-ranging effects on the metabolic, immune, and nervous systems. For this reason, cortisol is often viewed as a primary mechanism through which stressors “get inside the body” to bring about disease (Dickerson & Kemeny, 2004; Heim et al., 2000; Miller, Chen, & Zhou, 2007).

Although the impact of stressful experience on HPA regulation is of considerable theoretical interest, our knowledge of this phenomenon is limited in several important respects. One of the most salient problems is that little is known about how real-world stressors modify functions of the HPA axis. The vast majority of studies in this area have been conducted with animals, or have examined people’s hormonal responses to acute stressors in the laboratory (Dickerson & Kemeny, 2004). There have been studies of longer-term stressors in the real-world,

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but they have yielded conflicting and ambiguous findings, perhaps because they overlooked distinctions between stressors, such as the duration or frequency of exposure (Miller, Chen, & Zhou, 2007). In this regard, it is important to differentiate between episodic stressors, such as an isolated social conflict or a move to a new city, and more enduring difficulties like being part of a marriage that lacks trust, intimacy, and mutual respect. There are compelling reasons to believe that these situations will elicit different biological responses (see Baum, Cohen, & Hall, 1993; Kop, 1999; Mohr & Pelletier, 2006). Although we have a limited understanding of why HPA responses differ under conditions of acute versus chronic stress, there appears to be a re-regulation of the system that occurs with increased duration of the stressor. This re-regulation involves a transition from excess to diminished cortisol production (Miller, Chen, & Zhou, 2007).

Apart from differentiating between stressors that are episodic and chronic, research indicates that there is value in considering their co-occurrence. When people facing chronic difficulties are exposed to an episodic stressor, that event’s biological consequences can be markedly accentuated (Matthews, Gump, Block, & Allen, 1997; Miller & Chen, 2006). By contrast, the impact of episodic stressors on biological systems is attenuated (and sometimes eliminated altogether) among people who are not facing chronic difficulties (Matthews, Gump, Block, & Allen, 1997; Miller & Chen, 2006). Finally, some research indicates that in the absence of chronic stress, episodic stressors may even confer physiological benefits such as a reduced risk for infectious disease (Boyce et al., 1995).

Knowledge regarding stressful experience and HPA regulation is also limited by the fact that many studies have focused solely on hormone dynamics. For cortisol to act on target tissues in the body, it must bind to a glucocorticoid receptor (GR) inside cells. This receptor-hormone
complex can then initiate a molecular cascade, which eventually results in the cell’s program of genetic expression being modified. The extent of GR expression can therefore provide an indication of how sensitive a biological system will be to cortisol’s influence (Rohleder, Wolf, & Kirschbaum, 2003). It can also provide an index of recent exposure to cortisol, since cells often downregulate GR when exposed to increased hormone concentrations. In fact, a number of theories suggest that by triggering persistent cortisol secretion, stressful experiences downregulate the expression of GR in various bodily tissues. This downregulation is thought to facilitate a low-grade inflammatory response in the body and give rise to metabolic dysfunctions like impaired glucose control (Bjorntrop & Rosmond, 1999; Miller, Cohen, & Ritchey, 2002).

A final limit of existing research in this area is that it has focused mainly on middle-aged and older adults. While much can be learned from people at this phase of life, adolescence and early adulthood may represent other important stages to consider stressful experience and HPA regulation. These are times of life that can be particularly stressful, with young people struggling to develop a sense of personal identity, maintain close relationships with friends and peers, and attain an increasing amount of independence from their parents (Laursen & Collins, 1994). Indeed, young people with family and personal difficulties exhibit a number of health risks including higher ambulatory blood pressure, enhanced autonomic reactivity to stress, poorer glycemic control, and abnormal cortisol responses to laboratory stress (Jacobson et al., 1994; Repetti, Taylor, & Seeman, 2002; Salomon, Matthews, & Allen, 2000; Troxel & Matthews, 2004; Taylor, Lerner, Sage, Lehman, & Seeman, 2005). There is also mounting evidence that disease processes, especially those related to diabetes mellitus and cardiac disease, begin to develop in the early decades of life (Berenson & Srinivasan, 2005; Berenson, Srinivasan, Bao, Newman, Tracy, & Wattigney, 1998; Berenson et al., 1992). Researchers have thus called for
increasing attention to stressful experience, and its biological consequences, in populations of children, adolescents, and young adults (Matthews, 2005).

The current article examines episodic and chronic stressors in a cohort of young women ages 15-19 and how they relate to cortisol output and GR expression. To explore the potential downstream consequences of altered HPA dynamics, we also assess indicators of metabolic control (glucose, insulin) and systemic inflammation (C-reactive protein), which are more proximally related to the development and progression of disease (Willerson & Ridker, 2004; Adult Treatment Panel III Report, 2002). We hypothesized that episodic stressors would be unrelated to biological outcomes because of their time-limited nature. However, to the extent that they had chronic interpersonal stressors in their lives, we expected that adolescents would exhibit increased basal cortisol output, reduced expression of GR, and higher levels of CRP, glucose, and insulin. Finally, we expected that the most pronounced alterations in biological outcomes would occur in those participants exposed simultaneously to episodic and chronic stressors (Gump & Matthews, 1999; Miller & Chen, 2006). Conversely, the biological consequences of episodic stressors were expected to be attenuated among those without chronic difficulties.

Methods

Participants

Data for the present study were collected as part of a larger research project involving young women at high-risk for depression. Adolescent females were recruited from the Vancouver, British Columbia community through advertisements in newspapers and magazines. Young women were eligible for the study if they were (1) between the ages of 15 and 19, (2) fluent in the English language, (3) free of acute and chronic medical conditions, (4) without a
lifetime history of major psychiatric disorders, and (5) at high risk for developing an initial episode of major depression. High-risk was defined as having a first-degree relative with a history of depression, or as scoring in the top quartile of the sample distribution on one of two indices of cognitive vulnerability, the Dysfunctional Attitudes Scale (Weissman & Beck, 1978) or the Adolescent Cognitive Style Questionnaire (Hankin & Abramson, 2002). The study received approval of the institutional review board at the University of British Columbia, and participants were paid $70 for completion of this portion of the research. The final sample consisted of 104 young women whose characteristics are described in Table 2.1.

Procedures

All participants attended an initial laboratory session. Upon arriving at our laboratory, a research assistant described the study procedures in detail. Written consent was obtained from the participant, or if she was younger than 18 years, written assent was obtained and formal consent from her parent. The Structured Clinical Interview for DSM-IV was then administered to determine eligibility in terms of lifetime history of psychiatric disorders. Next, research assistants administered an in-depth interview regarding life stress (see below). Following the interview, the participant was seated in a comfortable chair, and 30-ml of blood was collected through antecubital venipuncture.

Over the course of the next two days, participants gathered salivary cortisol samples as they went about their normal daily activities. To facilitate the collection process, we lent participants a handheld computer (Palm Zire 21) which signaled them to collect saliva at waking, and at 1/2, 1, 4, 9, and 14 hours after waking. Specifically, when participants woke up, they took their first saliva sample and activated a customized software application on the Palm. This application “programmed” the computer so that it would sound alarms at the appropriate times
for the rest of the day’s samples. To collect the saliva samples, participants chewed lightly on a cotton dental roll for 1 minute so that it became saturated in saliva (Salivette; Sartstedt Corp., Numbrecht, Germany). Participants were instructed to avoid taking saliva samples immediately following tooth brushing and food intake. The dental roll was then placed in a plastic container and stored in the refrigerator until the end of the ambulatory monitoring period. To ensure compliance with the saliva sample protocol, the computer flashed a three digit code each time the alarm sounded. Participants recorded the code on the plastic container. When the Salivettes were returned to the lab, a research assistant matched the computer codes with those recorded by the participant, and samples without proper codes were excluded from analyses.

Life-Stress Interview

To assess participants’ exposure to stressful experiences, we administered the UCLA Life Stress Interview- Adolescent Version, which was developed from earlier versions for adults and children (e.g., Hammen, 1991). This semi-structured interview covers episodic and chronic forms of stress over the past six months. It focuses on stress in multiple domains, including romantic relationships, friendships, and family relationships. In each domain a trained interviewer asks a series of open-ended questions, and uses the information gathered to rate the level of chronic, ongoing stress. Ratings range from 1-5, with 1’s reflecting superior functioning and higher numbers reflecting more severe and persistent difficulties. Separate ratings are made in each domain. This interview also yields information regarding the occurrence of episodic stressors, which in this context are defined as specific events with a discrete onset and offset. To judge the objective impact of an episodic event, our research team made a consensus impact rating, after being briefed on event details by the primary interviewer. Impact ratings can range from 1 (no long-term impact) to 5 (severe long-term impact) and they explicitly consider the
context in which an event has occurred. Thus, higher ratings represent greater contextual threat. For example, if a participant was expelled from school, we would make a rating based on a number of factors, such as reactions from parents and friends, and the extent to which the expulsion interfered with academic progress. The goal would be to capture how the average person in similar biographical circumstances would respond. The rating process is also meant to eliminate the influence of reporting biases; thus, a participant’s subjective experience is not discussed by the team or factored into its rating. This interview has been used successfully in adolescent populations (e.g., Hammen, Brennan, & Shih, 2004), and there is evidence to support its reliability and validity. In the current project our raters agree with each other on chronic stress ratings 91% percent of the time. Agreement in this case is defined as being within ½ point of each other. In terms of validity, high stress ratings predict the onset of a depressive episode among children and adolescents (Adrian & Hammen, 1993; Hammen, Adrian, & Hiroto, 1988; Rudolph & Hammen, 1999), as well as biological outcomes among children with asthma (Miller & Chen, 2006).

Chronic stress ratings were averaged across four domains (i.e., family life, social life, romantic, and closest friend) to create an interpersonal chronic stress score for each participant. We focused on interpersonal chronic stress because peer and family relationships are an important focus in adolescence and adulthood and past research has shown that interpersonal stress is a strong predictor of disease outcomes (Smith & Ruiz, 2002). Examples of chronic interpersonal stressors in this sample include having a sibling with a mental illness, living in a conflictual family environment, feeling rejected by a peer group, and the absence of a confidant. The mean interpersonal chronic stress score was 2.40 (SD=0.49). Each participant’s maximum episodic event rating in the past six months was used to create an episodic stress score.
Participants with no episodic event were given a score of 1 (no long-term impact). The average episodic rating across the sample was 1.92 \((SD=0.86)\), which corresponds to an event with mild impact.

*Cortisol Secretion*

Cortisol was measured utilizing a commercially available chemiluminescent technique (IBL-Hamburg; Hamburg, Germany) at the Technical University of Dresden. This assay has a sensitivity of 0.16 ng/ml and intra- and inter-assay coefficients of variation less than 12%. After cortisol values had been log-transformed, each day’s data were used to create two area-under-the-curve (AUC) indices of secretion for later analysis. The first index was the morning response measure reflecting the volume of cortisol secretion over the first hour after waking.\(^2\) Cortisol values at waking, ½ hour after waking, and 1 hour after waking were used for these calculations. The second index was the total volume of cortisol secretion over the day. For these calculations, we used all cortisol values across the day excluding the ½ hour sample which is an indicator of morning response and has a disproportionate influence on daily output calculations. Both of these measures were computed using a trapezoidal method, such that higher values reflect greater cortisol release. To obtain more reliable indices of cortisol secretion, we averaged the AUC values calculated for each day of ambulatory data collection. The correlation between morning response values from the 2 days was \(r=0.45\), \(p<.001\), and the correlation between daily output values was \(r=0.58\), \(p<.001\). AUC values are in arbitrary units that reflect nmol/L over time.

*Data Cleaning.* Because the sampling schedule we used was specially designed to capture diurnal fluctuations in cortisol secretion, we felt that it was important to monitor subjects’ compliance with ambulatory monitoring carefully, and to exclude any samples that did
not conform to the protocol’s requirements. The handheld computers’ capacity to time-state and date-stamp each diary entry facilitated this process greatly. On an a priori basis, we chose to define compliance as taking a sample within 20 minutes of target in either direction for the waking, ½ hour, and 1 hour samples, and within 60 minutes of target for the remainder of the samples. When this definition was applied, a total of 1037 of the 1248 samples (83%) met our criteria for compliance. Only these values were used to compute morning cortisol response and daily cortisol output AUC scores. In the case of a missing sample at waking, ½ hour after waking, or 1 hour after waking, we did not compute a morning cortisol response score for that day. We computed daily output scores when we had at least four samples across the day.

**C-Reactive Protein, Glucose, and Insulin**

Blood draw was collected in the morning following a 12 hour fast. 10ml was drawn into a serum separator tube and then centrifuged at 1000 x g for 25 minutes. The serum was then aspirated, divided into 1-ml aliquots, and frozen at -20 degrees C until analysis. CRP was analyzed using a high-sensitivity, chemiluminescent technique on an IMMULITE 2000 (Diagnostic Products Corporation, Los Angeles, California). This assay has a an inter-assay coefficient of variation of 2.2% and a detection threshold of .20 mg/L. Glucose analyses were carried out on an ADVIA 1650 Chemistry system (Bayer Diagnostics, Tarrytown, New York). This analysis is an enzymatic technique that utilizes hexokinase and glucose-6-phosphate dehydrogenase enzymes. The assay has an inter-assay coefficient of variation of 1.2%. Insulin analyses were carried out on the IMMULITE 2000 using a solid-phase, two site chemiluminescent immunometric assay with an inter-assay coefficient of 3.1%.

**Glucocorticoid Receptors**
The expression of GR was quantified by measuring mRNA in leukocytes through real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) using a commercially available one-step assay purchased from Applied Biosystems (see Miller & Chen, 2006). Results are expressed as relative quantities of GR mRNA, such that higher relative quantities indicate greater expression of the GR.

Potential Confounders

We measured a number of processes that could provide alternative explanations for relations between stressors and biological outcomes. We collected demographic information, including participant age and ethnicity. Because the majority of the sample (90%) was of Caucasian or Asian descent, we created a dichotomous ethnicity variable coded as 1 for Caucasian and 0 for Other. Participants reported on tobacco use and alcohol consumption. There were no cigarette smokers in the sample, defined as smoking daily. The mean number of alcoholic beverages consumed per week was 1.28 (SD=4.93). Body Mass Index (BMI) was calculated based on height and weight measures obtained in the lab using a medical-grade scale balance-beam score. The average BMI was 21.63 (SD=2.72).

Statistical Analyses

In the first wave of analyses, we examined the distribution of study variables. The distribution of CRP scores was substantially positively skewed; so, this variable was analyzed following a log-10 transformation. We also screened for outliers and found a score in the daily cortisol distribution greater than 3 SD’s from the sample mean. We performed a log-10 transformation which brought the outlier within 3 SD’s of the mean, and the data were analyzed using these transformed scores. In the second wave, we conducted bivariate analyses to assess the relationship between study variables and potential confounds. In the third wave of analyses,
we tested our major hypotheses. We analyzed the main effects of episodic and chronic stressors using first-order and partial correlations. Finally, we tested the interaction between episodic and chronic stressors using multiple regression. The interaction term was created by taking the cross-product of centered episodic stress and chronic stress scores. When testing the interaction, main effects and the interaction term were entered together into the same regression equation. When a statistically significant interaction emerged, it was interpreted according to guidelines by Aiken and West (1991). We plotted predicted scores at low, medium, and high levels of chronic and episodic stress, which corresponded to -1 SD, the mean, and +1 SD.

Results

Preliminary Analyses

To identify potential confounders, correlations were computed between young womens’ demographic and behavioral characteristics and study variables. Episodic and chronic interpersonal stress, cortisol indices, and glucocorticoid receptors were not significantly associated with age, ethnicity, alcohol consumption, or BMI ($p > .05$). Glucose was significantly associated with age, such that older participants had lower glucose levels ($r = -.20, p < .05$). Both higher CRP and insulin were significantly associated with higher BMI, $r = .311, p < .001$ and $r = .26, p < .05$, respectively. Based on the results of these analyses, we included age as a covariate in analyses of glucose, and we included BMI as a covariate in analyses of CRP and insulin.

Exposure to Chronic and Episodic Stressors

Pearson correlations indicated that the extent of chronic interpersonal stress was unrelated to biological outcomes. This was true for daily cortisol output, morning cortisol response, GR mRNA, CRP, glucose, and insulin ($p > .10$).
Pearson correlations indicated that episodic stressors were inversely related to CRP. To the extent that they had experienced a more contextually threatening event in the past six months, participants exhibited lower circulating concentrations of the inflammatory molecule \( r = -0.23, p < .05 \). The extent of contextual threat in the last 6 months was unrelated to daily cortisol output, morning cortisol response, GR mRNA, glucose, and insulin \( p > .10 \). Due to the fact that our interview captured episodic stressors as far back as 6 months, we also examined whether recent events were more strongly related to outcomes. However, analyses indicated that episodic stressors in the 1- and 3-month periods before enrollment were unrelated to outcomes \( p > .05 \).

**The Interaction of Chronic and Episodic Stressors**

We used multiple regression to test the interaction between chronic and episodic stress in the prediction of cortisol secretion. These analyses indicated that episodic stress and chronic interpersonal stress interacted in the prediction of daily cortisol output, \( \beta = 0.21, t(87) = 1.99, p < .05 \). As Figure 2.1 illustrates, the impact of episodic stressors depended on the amount of chronic interpersonal stress a participant was experiencing. Among young women facing higher levels of chronic interpersonal stress, daily cortisol output increased with the severity of episodic stressors. The opposite pattern emerged among young women with the lowest levels of chronic interpersonal stress; daily output was reduced to the extent they experienced more contextually threatening episodic events. Among young women rated as having relatively moderate levels of chronic interpersonal stress, cortisol output did not vary as a function of episodic stressors.

Analyses indicated an interaction between chronic interpersonal stress and episodic stress in the prediction of the morning cortisol response, \( \beta = 0.37, t(87) = 3.29, p < .01 \). As shown in Figure 2.2, the interaction of episodic and chronic stress predicted the morning cortisol response in a pattern similar to that of daily cortisol output. Specifically, among young women who faced
higher levels of chronic interpersonal stress, the morning cortisol response increased as they experienced more contextually threatening events, whereas among young women who experienced lower levels of interpersonal stress, the morning cortisol response decreased with the extent of contextual threat. Again, episodic stress had minimal impact on cortisol secretion among young women who experienced relatively moderate levels of ongoing interpersonal stress.

A similar pattern emerged in the prediction of GR mRNA, $\beta = -.26$, $t(93) = -2.58$, $p<.05$. Figure 2.3 shows that among young women facing higher levels of chronic interpersonal stress, GR mRNA declined to the extent they experienced more contextually threatening events. Again, the opposite pattern was evident among young women with lower levels of chronic interpersonal stress, and GR mRNA was not affected by episodic stressors in young women with relatively moderate levels of chronic interpersonal stress.

Episodic and chronic stress also interacted significantly to predict CRP, $\beta = -.20$, $t(93) = -2.03$, $p<.05$. However, the pattern was somewhat different, as shown in Figure 2.4. As chronic interpersonal stress increased, so did the impact of episodic events. Specifically, under conditions of low chronic interpersonal stress, ratings of contextual threat had no effect on CRP. However, as young women faced increased chronic interpersonal stress the impact of episodic events increased in magnitude, such that young women who experienced more contextually threatening events had lower levels of circulating CRP.

Episodic and chronic interpersonal stress scores did not interact in the prediction of glucose and insulin, $p>.10$. 
Discussion

This study had three major hypotheses: (1) that by virtue of their time-limited nature, episodic stressors would be unrelated to biological outcomes; (2) to the extent that they had chronic interpersonal stress, young women would show evidence of hormonal, inflammatory, and metabolic dysregulation; and (3) that episodic and chronic stressors would interact to predict these outcomes, such that the impact of acute events would be accentuated by chronic interpersonal stress. Our first two hypotheses proved to be incorrect. There was no consistent pattern of associations between episodic or chronic interpersonal stressors and any of the biological outcomes we measured. However, there was strikingly consistent evidence in support of our last hypothesis, as chronic and episodic stressors interacted to predict four separate outcomes: daily cortisol output, morning cortisol response, GR mRNA, and CRP. By exploring the pattern of these interactions, it became evident why neither episodic nor chronic interpersonal stress related directly to outcomes; the influence of episodic events depended entirely on the extent of chronic interpersonal stress, and vice versa.

Among young women facing higher levels of chronic interpersonal stress, more severe episodic stressors (during the previous six months) were associated with amplified cortisol output, both in the morning and across the day, reduced expression of GR mRNA, and lower concentrations of CRP. These findings are in line with research showing that the impact of acute events is accentuated in people who are in the midst of chronic stressors (Gump & Matthews, 1999; Miller & Chen, 2006). This may be the case because people generally do not have the coping resources, emotional energy, or social support to manage acute and chronic demands simultaneously. Our findings suggest that when people face these situations, the magnitude of their cortisol output is increased, both in the morning hours and through the daytime and
evening. Persistently increased output of this nature might then foster a compensatory downregulation of GR mRNA in leukocytes.

Among young women with relatively moderate levels of chronic interpersonal stress, episodic events were unrelated to morning cortisol response, daily cortisol output, or GR mRNA. Why might the impact of episodic stressors be attenuated in these young women? We know that the transition to adulthood is a time filled with interpersonal successes and failures, as teenagers try to negotiate close relationships with friends and peers and become more independent from their parents (Laursen & Collins, 1994). Some degree of tension seems to be inherent in the life of women at this stage; those with moderate difficulties are likely to be having developmentally normative experiences. Furthermore, young women who fall into this group have a chronic interpersonal stress score around 2.4, suggesting that they have basically stable and positive relationships, with some occasional but not serious problems in them. When a stressor arises, these young women are likely to have at least adequate social resources to call on. These resources may be what help them to minimize the biological consequences of exposure to an episodic stressor.

Among young women with the lowest levels of chronic interpersonal stress, episodic stressors were related to reductions in the morning cortisol response and daily cortisol output, as well as increased expression of GR mRNA by leukocytes. How do we explain these effects? It is clear that these young women have strong relations with friends and family, marked by high levels of trust and intimacy. So when they are exposed to episodic stressors, these teenagers have many resources to call on, and they may emerge from these experiences looking healthier than if they had not been exposed. These findings add to a growing literature suggesting that children in supportive environments can develop physiological resilience in response to stressful
experiences (Boyce et al., 1995; Bugental, 2005). In other words, the combination of supportive parenting history and confrontations with stressors and challenges may provide inoculation against subsequent stressors (physiological toughening; Dienstbier, 1989). To test this hypothesis more completely, relations between the social environment, stressful events, and health trajectories would need to be examined prospectively over the course of development.

To examine any downstream consequences of hormonal alterations, we assessed indicators of metabolic functioning and systemic inflammation. Episodic and chronic stressors were unrelated to levels of glucose and insulin. We had expected that stressful life experiences would be associated with altered metabolism, as indicated by higher levels of glucose and insulin. It is possible that the impact of stress on metabolic functioning accumulates slowly over time, requiring prolonged elevations in the amount of cortisol before it emerges. Our focus on relatively recent stressors (those that had occurred within the past 6 months), rather than ongoing stressors over the course of childhood and adolescence, may account for these null findings.

Episodic stress and chronic interpersonal stress did however interact to predict CRP concentrations, but in a fashion that was partially inconsistent with our initial hypothesis. For young women with the lowest amount of chronic interpersonal stress, episodic stressors were unrelated to the expression of CRP. This pattern of findings makes sense conceptually, given the high-quality of social relations in this subgroup of young women. However, as chronic interpersonal stress increased in magnitude, so did the impact of episodic stressors on CRP. The direction of this association was negative – with more severe episodic stressors, subjects exhibited smaller amounts of CRP. This was unexpected. We predicted that in this cohort of double-exposed young women, systemic inflammation would be the most pronounced. It is not clear what accounts for these findings. Output of cortisol in these young women was
significantly elevated, and according to our model, this should result in greater systemic inflammation, secondary to the downregulation of GR numbers or function (Miller, Cohen, & Ritchey, 2002). We are not sure why this pattern failed to emerge, but it may be due to the cross-sectional design of the study. Our findings suggest that, at high levels of chronic interpersonal stress, cortisol concentrations and GR numbers are adjusted depending on the presence or absence of episodic stressors. Thus, HPA responses to these types of stressors may not persist long enough to impact systemic inflammation. A multi-wave study that tracks changes in these parameters over time is needed to clarify these points.

To the extent that this scenario is accurate, it has interesting theoretical and practical implications for research. Double-exposed young women showed a variety of biological alterations, and they were not uniformly negative or positive. Though increased morning cortisol and reduced expression of GR in leukocytes could heighten risk for later metabolic and cardiac disease, reduced concentrations of inflammatory molecules like CRP would offset this to a large extent. This pattern of findings suggests that simple theories linking stressful experience, increased cortisol output, and metabolic/inflammatory dysregulation are likely to be incorrect (Miller, Chen, & Zhou, 2007). Collectively, these findings highlight the need for more complex accounts of the pathways linking stressors and disease. Such accounts will need to acknowledge that stressful experiences can activate multiple interacting biological systems, which have differing and sometimes opposing consequences for later disease (McEwen, 1998; Weiner, 1992).

There are a number of limitations to this study that should be noted. First, young women in this sample were at high risk of developing an initial episode of depression. It is likely that these women are generally more susceptible to the effects of stress. So, our findings may
exaggerate the relationship between stress and hormonal and inflammatory responses in the general population of female adolescents. It is therefore necessary to replicate these findings among more diverse samples. Second, our inability to detect main effects of episodic events may reflect the fact that HPA indices were not always assessed shortly after the stressor occurred, when they would be most likely to emerge. Future research that examines HPA activity within weeks of the stressful event may be more likely to detect such findings. Third, this study employed a cross-sectional design. To clarify the temporal relations between stressful experiences and biological processes, we will need to test these associations longitudinally. Fourth, our GR findings were in leukocytes, and it will be important for future research to determine whether they extend to other tissues. Finally, our findings may be better explained by an underlying personality characteristic. For instance, if hostile or neurotic young women are prone to experiencing (or simply reporting) episodic stressors and chronic interpersonal stressors, their biological outcomes might be better explained by personality features than life events. However, we used objective contextual interviews that minimize the influence of self-report biases and as a result are unlikely to reflect the influence of personality.

Despite these limitations, our findings provide several interesting contributions to the literature. First, they suggest that stressor impact is a complex phenomenon, and depends on both episodic and chronic exposure. The simple presence of an episodic or chronic stressor did not reliably impact biological outcomes. Thus, future research must assess both types of stress to clarify the biological consequences of either. Only then will we be able to identify people who may be susceptible to HPA dysregulation and its subsequent impact on other systems. Second, among young women in this study, episodic events and chronic interpersonal stressors were most strongly related to hormonal and inflammatory outcomes, suggesting that these biological
processes are particularly sensitive to social stressors in adolescent life. Metabolic indicators may be less sensitive to these types of stressors, or they may be better examined in longitudinal designs that capture the impact of cumulative stress over time. Finally, we found that adolescents with low to moderate chronic interpersonal stress showed relatively adaptive biological responses to episodic stressors. These results contribute to existing literature that challenges the assumption that stress is uniformly bad for one’s health. In the context of a supportive environment, stressful experiences may toughen the body, rendering it more resilient to subsequent stressors. With future studies in this area, we will be able to clarify the conditions under which hormonal and inflammatory responses to stressful experiences are risk factors for versus protective factors against future health problems.
Table 2.1.
Demographic and Health-Related Characteristics of Participants

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<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>17.2 ± 1.34</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>42%</td>
</tr>
<tr>
<td>East Asian</td>
<td>48%</td>
</tr>
<tr>
<td>Other</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Parents’ education</strong></td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>25.1%</td>
</tr>
<tr>
<td>Some college</td>
<td>15.9%</td>
</tr>
<tr>
<td>College graduate</td>
<td>59.2%</td>
</tr>
<tr>
<td><strong>Parents married</strong></td>
<td>82%</td>
</tr>
<tr>
<td><strong>Risk for depression</strong></td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>11%</td>
</tr>
<tr>
<td>Cognitive vulnerability</td>
<td>64%</td>
</tr>
<tr>
<td>Both</td>
<td>21%</td>
</tr>
<tr>
<td><strong>Cortisol 2-day means (nmol/L)</strong></td>
<td></td>
</tr>
<tr>
<td>Waking</td>
<td>9.10 ± 8.34</td>
</tr>
<tr>
<td>½ hr after waking</td>
<td>12.57 ± 8.50</td>
</tr>
<tr>
<td>1 hr after waking</td>
<td>11.96 ± 11.75</td>
</tr>
<tr>
<td>4 hrs after waking</td>
<td>4.40 ± 4.89</td>
</tr>
<tr>
<td>9 hrs after waking</td>
<td>2.76 ± 4.18</td>
</tr>
<tr>
<td>14 hrs after waking</td>
<td>1.76 ± 2.62</td>
</tr>
<tr>
<td><strong>Cortisol morning response</strong></td>
<td>0.89 ± 0.29</td>
</tr>
<tr>
<td><strong>Cortisol daily output</strong></td>
<td>6.66 ± 2.76</td>
</tr>
<tr>
<td><strong>CRP (mg/L)</strong></td>
<td>0.61 ± 0.74</td>
</tr>
<tr>
<td><strong>Glucose (mmol/L)</strong></td>
<td>4.48 ± 0.36</td>
</tr>
<tr>
<td><strong>Insulin (pmol/L)</strong></td>
<td>53.63 ± 23.83</td>
</tr>
<tr>
<td><strong>GR mRNA (RQ)</strong></td>
<td>4.15 ± 1.93</td>
</tr>
</tbody>
</table>
Note. CRP = C-reactive protein; GR = glucocorticoid receptor; RQ = relative quantity.

a Four percent of young women in our sample were not considered to be at high risk for depression. Excluding these women from our analyses did not affect our findings.
Figure 2.1.

*Chronic interpersonal stress interacts with episodic stress in the prediction of daily cortisol output.*

Daily cortisol output as a function of episodic stressor exposure and chronic interpersonal stress. Episodic stressors are specific events with a discrete onset and offset, and higher scores indicate events with greater impact. Chronic interpersonal stress represents ongoing family, social, and/or romantic difficulties with higher scores indicating more severe and persistent difficulties. Predicted scores are plotted at low, medium, and high levels of chronic and episodic stress, which corresponds to -1 SD, the mean, and +1 SD.
Figure 2.2.

Chronic interpersonal stress interacts with episodic stress in the prediction of the morning cortisol response.
Chronic interpersonal stress interacts with episodic stress in the prediction of glucocorticoid receptor mRNA.
Figure 2.4.

*Chronic interpersonal stress interacts with episodic stress in the prediction of C-reactive protein.*
References


Chapter 3: Double-Exposure to Acute Stress and Chronic Family Stress is Associated with Immune Changes in Children with Asthma

While it has long been known that stressful experiences can exacerbate symptoms of asthma (Wright et al., 1998), it has only recently become clear which kinds of stressors pose the greatest risk for patients. In this regard studies indicate that simultaneous exposure to acute life events and ongoing chronic stressors is particularly detrimental. For instance, in a prospective study of children with asthma, those exposed to high levels of acute and chronic stress showed a 3-fold increase in risk for an attack in the 2 weeks that followed (Sandberg et al., 2000, 2004). Apart from the issue of duration, research indicates that the type of stressor is an important determinant of its influence, with family difficulties emerging as particularly detrimental. For example, prospective studies have shown that parental stress at 2 to 3 months of life predicts subsequent wheezing in infancy (Wright et al., 2002), and that parenting difficulties during the first years of life are associated with the onset and persistence of school-age asthma (Gustafsson et al., 2002; Klinnert et al., 2001).

Given the growing evidence of the role of psychosocial factors on clinical asthma outcomes, researchers are beginning to investigate the mechanisms through which stressful experiences contribute to the development and/or persistence of symptoms (Rattanjeet et al., 2006; Wright et al., 1998). Previous research has found that when asthmatic patients are exposed to stressors like writing an important exam or living in a low SES environment, they show higher eosinophil counts, greater lymphocyte proliferative responses to allergic triggers, and heightened in vitro production of inflammatory cytokines implicated in asthma such as interleukins-5 and –

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Stressors also accentuate the airway inflammatory response to allergen challenges (Liu et al., 2002). However, the bulk of the research on mechanisms has been cross-sectional, meaning that the temporal ordering and causal direction of these associations remains unclear. To begin resolving some of these issues, the current study followed children with asthma over 2 years, taking repeated measures of life stress and asthma-related immune markers. Immune markers included the \textit{in vitro} production of asthma-related cytokines including IL-4, IL-5, IL-13, and IFN-\(\gamma\). This design enabled us to identify within-person changes in cytokine dynamics following exposure to stressful experiences. We expected that children who were simultaneously exposed to acute life events and chronic family stress would show alterations in cytokine production in a direction detrimental to asthma.

\textbf{Methods}

\textit{Patients}

The sample consisted of 71 children with asthma and 76 medically healthy children. They were recruited from the Vancouver, BC community through advertisements in physicians’ offices, newspapers and magazines, and community settings. Children were eligible for the study if (1) they were between the ages of 9 and 18, (2) they were fluent in English, and (3) had been free of upper-respiratory illness for the past 4 weeks. To be included in the asthma group, children were required to have a physician diagnosis of asthma and be free of other chronic medical illness. Healthy children were required to have a history without chronic medical and psychiatric illness. Information regarding children’s medical history was gathered from parents. See Table 3.1 for a description of the sample.

\textit{Procedures}
Children visited the research center accompanied by a parent every six months over the course of two years. Eighty-nine percent of the children had completed 3 or 4 study visits at the time of data analysis; they as well as the other 11 percent are included in the statistical analyses presented below. Written consent was obtained from the parent, and assent was obtained from the child. A local anesthetic cream (EMLA; AstraZeneca, Wilmington, DE) was applied to the child’s arm. Children were interviewed regarding life stress (see below), and then blood was collected using antecubital venipuncture. Children with asthma reported on their asthma symptoms twice daily for 14 days following each laboratory visit. Data collection for this portion of the study began in April of 2004 and ended in September of 2007. The study was approved by the Research Ethics Board of the University of British Columbia.

Life-Stress Interview

To assess children’s exposure to stressful experiences, we administered the University of California Los Angeles Life Stress Interview, Child Version (Hammen and Rudolph). This semistructured interview covers acute and chronic forms of stress over the past 6 months. It has been used successfully in children as young as 8, and has demonstrated reliability and validity (Adrian & Hammen, 1993; Hammen et al., 1988; Rudolph & Hammen, 1999). The interview focuses on stress in multiple life domains, including family relationships, friendships, school, and home life. Interviewers rate the level of chronic, ongoing stress on a 5 point scale, with higher numbers reflecting more severe and persistent difficulties. Here we focus on chronic family stress, which is concerned with the quality of interpersonal relationships among family members. Chronic family stress was quite stable over time (Cronbach’s alpha=0.90; intercorrelations range from .61 to .84), thus we could not justify modeling it within-person. More specifically, the average change across time per subject was about a half-point, which is actually
within our margin of error across different raters. Thus, chronic family stress ratings were averaged across the 4 visits and modeled as a between-subjects factor (see below for more information on our data analytic strategy).

The interview also probed for acute stressors, defined as specific events with a discrete onset and offset. To judge the objective impact of an acute event, a consensus rating is made by a team of interviewers after it has been briefed on event details by the primary interviewer. Impact ratings are made on a 5-point scale ranging from 1 (no impact) to 5 (severe impact). Consensus ratings take into account the context in which an event has occurred. For example, if a participant had failed a test at school, we would make a rating based on a number of factors, such as his or her previous academic record, and reactions from parents and teachers. In the present study we consider acute events rated 2.5 or higher (mild-moderate impact) to be significant life events. This follows the convention in the Life Events and Difficulties Schedule area of using a threshold for defining major life events (e.g., Hammen et al., 2000; Miller & Chen, 2006; Monroe et al., 1992). Examples of acute life events from the current study include a mother being fired from her job, a father being admitted to the hospital for depression, and a child’s best friend moving to another city.

*Cytokine Production*

Cytokine release by peripheral blood mononuclear cells (PBMC) in response to mitogenic stimulation was measured in an *in vitro* model. Peripheral blood was collected into BD Vacutainer Cell Preparation Tubes containing sodium heparin, and PBMCs were separated and stimulated with phorbol myristate acetate (PMA; final concentration 25 ng/ml) and ionomycin (final concentration 1 µg/ml) for 48 hours at 37°C in 5% CO₂. This PMA/ionomycin combination has been successful in stimulating the cytokines of interest in other asthma studies.
(Magnan et al., 2000; Schuerwegh et al., 1999). After centrifugation, supernatants were collected and frozen at -80 °C. Supernatants were then assayed to determine levels of IL-4, IL-5, IL-13, and IFN-γ using enzyme-linked immunosorbent assays (ELISA) (R&D System, Minneapolis, MN). Inter- and intra-assay variations were below 10%.

**Asthma Symptoms**

Children rated each of 4 asthma symptoms (cough, wheeze, chest tightness, and shortness of breath) on a scale from 0 (none) to 4 (really bad). Symptoms experienced over the course of the night were rated at waking and symptoms experienced during the day were rated before bed on each day of sampling. Compliance to the diary schedule was monitored using electronic time and date stamps that the participants would “punch-in” before making their ratings. To create symptom scores for each visit, we computed average daily symptoms scores, and then averaged the daily scores across the 14 days.

**Potential Confounders**

To account for the impact of medication, parents were asked to bring all of their child’s medications to the research center. Names and dosages were recorded directly from the label, and the number of days each medication was taken in the last 2 weeks was ascertained. Using this information, we created variables reflecting the number of days in which inhaled corticosteroids and β agonists were used in the past 2 weeks.

Asthma severity was determined from the National Asthma Education and Prevention Program, Expert Panel Report 2 (NAEPP/EPR2) guidelines based on the higher of symptom frequency and medication use (Bacharier et al., 2004). Symptom frequency was based on parent reports of the child’s daytime, nighttime, and exertional symptoms over the past month. Medication use and asthma severity were included as covariates in statistical analyses.
Finally, socioeconomic status as a possible covariate was measured using current family income. Current family income was defined as the family’s total gross income for the past 12 months before taxes.

Statistical Analyses

Data were analysed using hierarchical linear modeling (HLM 6.03 (Raudenbush et al., 2006)), a multi-level modeling technique that allowed us to test both within-person and between-person contributions to changes in cytokine production and asthma symptoms over time. Acute stress was modeled as a within-person factor because it varies over time, whereas chronic family stress was modeled as a between-subjects factor because it varies from child to child, but remains quite stable over time. In the within-person (or level-1) model, we estimated cytokine production as a function of factors that vary over time including exposure to a significant life event and the covariates of days of medication use in the last 2 weeks. These models yielded a series of person-specific slopes reflecting the difference in cytokine production between visits when patients were versus were not exposed to a significant life event ($\beta_{1i}$). In the between-person (or level-2) models, we estimated the above slopes for each subject as a function of factors that vary across people, including chronic family stress and the covariate asthma severity. The critical parameter was the coefficient $\gamma_{11}$ which reflects the cross-level interaction between acute stress and chronic stress in the prediction of cytokine levels. In other words, the coefficient $\gamma_{11}$ indicates whether the relationship between acute stress and cytokine production depended on the extent of chronic family stress. When significant interactions emerged, cytokine production was plotted at the 25th and 75th percentiles of chronic family stress in both the absence and presence of an acute event. In addition, the magnitude of the interactions was examined by
calculating the proportion of between-person variability in the relationship between acute stress and cytokine production that was explained by chronic family stress.

Results

*Relations of life stress to cytokine production among patients with asthma.* First we tested the main effects of acute stress at level 1. Results indicated no significant effects for any of the outcome measures, p’s>.10, meaning that patients had similar patterns of cytokine production at times when they had experienced an acute event compared to times when they had not. We then tested the main effects of chronic stress at level 2, and again, results indicated no statistically significant effects, p’s>.05. However, there was a trend toward a positive relationship between chronic family stress and IL-4 production, \( \gamma_0=1.84, \ SE=.96, \ p=.06 \). Specifically, children with higher family chronic stress showed marginally higher IL-4 levels.

Next, we tested for a level 1 by level 2 interaction, in terms of whether the relationship between acute stress and IL-4 production depended on the extent of chronic family stress. Results indicated a significant interaction between acute and chronic stress, \( \gamma_{11}=8.16, \ SE=3.15, \ p=.01 \). Consistent with expectations about the detrimental nature of double-exposure, children with higher levels of chronic family stress showed increased IL-4 at times when they had experienced an acute event compared to times when they had not (see Figure 3.1). In contrast, children with lower levels of chronic family stress showed no changes in IL-4 at times when they had experienced an acute event compared to times when they had not. The moderating effect of chronic stress was large, as it accounted for approximately 77% of the between-person variability in the relationship between IL-4 and acute events.

We found similar cross-level interactions for IL-5, \( \gamma_{11}=43.74, \ SE=15.46, \ p=.007 \) and IFN-\( \gamma \), \( \gamma_{11}=11334.98, \ SE=3803.80, \ p=.004 \) (see Table 3.2 and Figures 3.2 and 3.3). Children with
higher levels of chronic family stress showed increased IL-5 and IFN-γ production at times when they had experienced an acute event compared to times when they had not. Conversely, children with lower levels of chronic family stress showed no difference in production of these cytokines at times when they had experienced an acute event compared to times when they had not. Chronic stress accounted for approximately 73% of the between-person variability in the relationship between acute events and each of these outcomes.

In contrast to our other findings, acute and chronic stress did not interact in the prediction of IL-13 production (p>.20).

To test whether these findings could be explained by family socioeconomic status, we re-ran analyses including family income as a control variable at level 2. Acute stress and chronic family stress continued to interact in predicting IL4, IL5, and IFN-γ production, independent of family income (p’s<.05).

Analyses were also repeated using a 6-month (rather than 3-month) window for major life events. However, these analyses indicated no significant main effects of acute stress (p’s>0.10) and no significant acute by chronic interactions (p’s >0.20). Thus, only more recent events (within the 3 months leading up to each visit) appeared to interact with chronic stress in the prediction of IL-4, IL-5, and IFN-γ.

Relations of life stress to asthma symptoms. We next asked whether chronic and acute stress would predict asthma symptoms. These analyses were carried out in a subgroup of participants with moderate to severe asthma (N=32) because they were the only ones to report symptoms consistently. The mean symptom score for this subgroup was 1.04±1.01 on a scale of 0 to 4; in the rest of the sample it was .71±.81.
Analyses indicated that while there were no main effects of acute or chronic stress on symptoms, p’> .20, there was a significant cross-level interaction, $\gamma_{11} = 0.41$, $SE = 0.17$, $p = .024$ (see Table 3.2). As shown in Figure 3.4, the pattern of findings for asthma symptoms partially mirrored the pattern for cytokine production. Specifically, as was the case with cytokines, the impact of acute events was amplified among children with high chronic family stress, such that patients with simultaneous exposure to these stressors reported the most symptoms. But unlike the cytokine outcomes, there was evidence that chronic family stress increased symptoms even at times when no acute event had occurred. (Though this amplifying effect of chronic stress was smaller than at times when an acute event had occurred.) The cross-level interaction explained approximately 88% of the between person variability in the relationship between acute stress and asthma symptoms.

Relations of cytokine production to asthma symptoms. Given that life stress was associated with IL-4, IL-5, and IFN-γ production, we tested whether within-person changes in these cytokines were associated with changes in asthma symptoms. This set of analyses was also carried out among the subgroup of asthma participants with moderate to severe symptoms. Results indicated that IL-5 production was significantly associated with asthma symptoms, $\gamma_{01} = .0024$, $SE = .00067$, $p = .002$, meaning that participants reported increased asthma symptoms at times when IL-5 production was higher than their individual averages. In addition, there was a trend for a positive association between IL-4 production and asthma symptoms, $\gamma_{01} = .011$, $SE = .0055$, $p = .06$. However, IFN-γ production was unrelated to asthma symptoms, p>.30.

Relations of life stress to cytokine production among healthy controls. We conducted a parallel set of analyses in the healthy comparison group (see Table 3.2). They showed that exposure to an acute event (within 3 months) was associated with increased production of IL-5,
\( \gamma_{01} = 58.01, SE = 24.80, p = .02 \), meaning that participants showed increased production of IL-5 at times when they had experienced an acute event compared to times when they had not. However, there were no main effects of acute events on IL-4, IL-13, or IFN-\( \gamma \), \( p's > .30 \). The analyses also revealed a significant main effect of chronic family stress on IL-13, such that children with higher levels of chronic family show increased production of IL-13, \( \gamma_{01} = -.73.45, SE = 29.38, p = .02 \). There were no main effects of chronic family stress on production of IL-4, IL-5, or IFN-\( \gamma \), \( p's > .30 \). Finally, we tested the interaction between acute and chronic stress. Unlike the asthma sample, results indicated that acute and chronic stress did not interact to predict IL-4, IL-5, or IL-13, \( p's > .10 \). However, there was some trend toward an interaction between acute and chronic stress in the prediction of IFN-\( \gamma \), \( \gamma_{01} = -.7502.52, SE = 4261.56, p = .08 \).

Discussion

This study examined the relationship between chronic stress, acute events and within-person changes in cytokine production over two years in children with asthma and a healthy comparison group. Within the asthma group, we found that the association between acute events and asthma-relevant cytokines was accentuated among children with chronic family stress. Specifically, children with high chronic family stress showed increased production of IL-4, IL-5, and IFN-\( \gamma \) at times when they had experienced an acute event compared to times when they had not. In contrast, when chronic family stress was low, there was no relationship between acute events and cytokine production. Acute and chronic stress did not interact to predict cytokine changes among medically healthy children, suggesting that the cytokine changes are specific to asthma and not simply part of a general response to stress. Furthermore, in a subgroup of children with moderate to severe asthma, acute and chronic stress interacted to predict asthma symptoms, such that children who were exposed to both acute and chronic stress reported the
most severe symptoms. This pattern of results mirrors previous clinical findings in which double-exposure to acute and chronic stressors was associated with risk for asthma exacerbations (Sandberg et al., 2000). The present study takes this research one step further by providing a plausible biological explanation for how a psychological factor like stress can influence clinical outcomes in asthma.

One reason why double-exposure may be so detrimental is that it may be that while people can manage one key life stressor, they do not have the resources (e.g., time, energy, social support) to deal with multiple stressors effectively. This might prolong the impact or heighten the severity of stressors that could normally be managed. Having to juggle multiple stressors also seems likely to interfere with people’s ability to engage in healthy behaviors like exercising and spending time with friends, that can protect against the detrimental influence of problems at home, work, and school. Double-exposure may also overwhelm a person’s social-support network or, if one of the stressors involves tensions with close friends or family, interfere with his or her ability to mobilize it. Erosion of family support could be especially detrimental for children, who often rely on their parents and siblings for assistance with key life events.

What are the mechanisms by which double-exposure influences cytokine production? One possibility is that stress-related activation of the hypothalamic-pituitary-adrenocortical axis and the sympathetic nervous system among double-exposed children leads over time to a compensatory down-regulation of glucocorticoid and β-adrenergic receptors on target tissues. This process is thought to decrease sensitivity to the anti-inflammatory effects of hormones like cortisol and epinephrine (Miller & Chen, 2006; Miller et al., 2002). Consistent with this theory, double-exposure has been linked to elevated cortisol output through the day and decreased expression of glucocorticoid receptor mRNA among healthy young women (Marin et al., 2007),
as well as decreased expression of both glucocorticoid and β-adrenergic receptor mRNA among youth with asthma (Miller & Chen, 2006). Thus, the present findings of heightened cytokine production may in part result from diminished control over inflammation due to dysregulation at the level of hormones and receptors. Future work would benefit from prospective studies that examine the causal relations between stress exposures, hormone dynamics, and asthma-related immune markers.

This study examined T-helper (T_H) 2 (IL-4, IL-5, and IL-13) and T_H1 (IFNγ) cytokines, which serve different functions during an immune response. In regard to asthma, it is widely believed that the inflammatory response involves a T_H2 mechanism (Barnes, 1994; Chung & Barnes, 1999; Marshall & Agarwal, 2000). In addition, there is some evidence that acute stress involves a shift toward a T_H2 response (increased production of IL-10) and away from a T_H1 response (decreased production of IFN-γ) among healthy young adults (Marshall, 1998). Consistent with these findings, double-exposed children with asthma in our sample showed increased T_H2 cytokine (IL-4 and IL-5) production which could be indicative of an enhanced humoral response to allergens. However, they also showed increased production of IFN-γ, a T_H1 cytokine that is thought to have inhibitory effects on T_H2 pathways. These results are consistent with some previous research— for example, the finding that another type of chronic stress, low socioeconomic status, is associated with elevations in both T_H2 (IL-5) and T_H1 (IFN-γ) responses among children with asthma (Chen et al., 2003). Thus, it may be that in children with asthma, stress leads to a general upregulation of T_H cell activity and signaling (rather than an imbalance between T_H1 and T_H2). What this means for asthma is unclear. Though Th1 activity generally inhibits the Th2 signals that drive asthma, some data suggest that cytokines like IFNg contribute to asthma pathogenesis, possibly by orchestrating anti-viral immune responses in the lungs.
(Holtzman et al., 2002). If this is the case, stress-related priming of IFN-g responses could have detrimental influences on asthma symptoms, especially when they have been triggered by viral infection (Chen & Miller, 2007; Wright et al., 1998).

This study showed that, among children with moderate to severe asthma, acute stress and chronic family stress interacted to predict asthma symptoms. The pattern of the interaction was similar to the interaction of acute and chronic stress predicting IL-4, IL-5, and IFN-γ production. However, in addition to the pattern found with cytokines, chronic family stress also appears to influence asthma symptoms in the absence of an acute event. Specifically, when no event had occurred, chronic family stress was positively associated with asthma symptoms, but to a lesser degree than after an acute event. The difference in patterns for cytokine production versus symptoms suggests that there are pathways in addition to immune changes by which chronic family stress can influence symptoms (Jarvis et al., 2002).

But, are the observed increases in IL-4, IL-5 and IFN-γ production really relevant to asthma? To test this question, we also examined the relationship between cytokine production and asthma symptoms among subjects with higher severity asthma. Results indicated that increased production of IL-5 was associated with within-person increases in asthma symptoms, and there was a trend toward a similar effect for IL-4 production. This provides evidence that changes in IL-5 and IL-4, but not IFN-γ, production are relevant to asthma symptoms.

This study has a number of limitations. First, it is possible that unmeasured variables account for our effects. For instance, increased risk for infection following life events could explain the relations of acute events to cytokine production. However, if this were the case, one would expect to see effects of infection on both the main effect of stressful events as well as the interaction between acute and chronic stressors. Moreover, the within-person nature of our
analyses and findings precludes most of the alternative explanations for linkages between stressors and immunity (e.g., genetics, lifestyle factors). Second, it will be important to see how factors like gender and puberty moderate the effects of double-exposure on cytokine production; however, in the present study, we did not have enough power to test these 3-way interactions. Third, our inability to detect main effects of acute events may reflect the fact that cytokine production was not always assessed shortly after the stressor occurred, when they would be most likely to emerge. Future research that examines inflammatory processes within weeks of the stressful event may be more likely to detect such findings. Fourth, immune processes were measured from peripheral blood cells. Future studies that are able to obtain cells directly from the airways would provide important information about more proximal processes. Fifth, the cytokine production assay used a substance that stimulates cells nonspecifically, rather than through the antigen-specific pathways that are engaged by most allergens in the body. However, given that our sample was not selected for a positive response to any specific allergen, the use of a specific stimulus would not have elicited responses across all children. Finally, it should be noted that youth were recruited into this study on the basis of parent reported physician diagnosis of asthma and that physician diagnosis is not always accurate. However, if we inadvertently included some children who did not truly have asthma, this in theory would add noise to our measurements, making it more difficult to detect associations.

Despite these limitations, our findings provide several important contributions to the literature. First, they suggest that stressor impact is a complex phenomenon- the combination of acute and chronic stress may be necessary to produce a change in cytokine production. Second, the immune changes observed among children who had been exposed to acute and chronic stress are relevant to asthma symptomatology. Third, this type of research contributes to
biopsychosocial models that seek to explain how larger social factors can get “under the skin” to affect an individual child’s health. The fact that experiencing an acute life event on top of high chronic stress predicts detrimental inflammatory responses suggests that psychosocial interventions aimed at helping children manage stress could have implications biologically for children with asthma.
Table 3.1.

Demographic and health-related characteristics of participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Statistic</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asthma (n=71)</td>
<td>Healthy (n=76)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.77±2.68</td>
<td>12.89±2.34</td>
</tr>
<tr>
<td>Sex (males)*</td>
<td>72%</td>
<td>52.6%</td>
</tr>
<tr>
<td>Ethnicity (white)</td>
<td>63%</td>
<td>63%</td>
</tr>
<tr>
<td>Annual family income (more than $50 000)</td>
<td>81%</td>
<td>69.5%</td>
</tr>
<tr>
<td>Asthma severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild intermittent</td>
<td>16.9%</td>
<td></td>
</tr>
<tr>
<td>Mild persistent</td>
<td>38.0%</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>32.4%</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>12.7%</td>
<td></td>
</tr>
<tr>
<td>Diary symptoms(^a)</td>
<td>0.71±0.74</td>
<td></td>
</tr>
<tr>
<td>Atopic asthma</td>
<td>84%</td>
<td></td>
</tr>
<tr>
<td>B-Agonist use(^\d)</td>
<td>83%</td>
<td></td>
</tr>
<tr>
<td>Inhaled corticosteroid use(^\d)</td>
<td>65%</td>
<td></td>
</tr>
<tr>
<td>Chronic family stress(^c)</td>
<td>2.23±0.66</td>
<td>2.38±0.84</td>
</tr>
<tr>
<td>Acute events(^c)</td>
<td>16-19%</td>
<td>18.9-31.1%</td>
</tr>
<tr>
<td>IL-4 production (pg/mL)</td>
<td>6.79±5.53</td>
<td>9.02±9.77</td>
</tr>
<tr>
<td>IL-5 production (pg/mL)</td>
<td>81.90±82.69</td>
<td>91.13±142.46</td>
</tr>
<tr>
<td>IL-13 production (pg/mL)</td>
<td>285.36±194.84</td>
<td>317.64±259.09</td>
</tr>
<tr>
<td>IFN-(\gamma) production (pg/mL)</td>
<td>12373.45±16211.94</td>
<td>13930.88±12797.94</td>
</tr>
</tbody>
</table>

Note. Values represent means ±SDs. Means for cytokine production and diary symptoms were averaged across study visits. We compared the 11% of participants who had only completed 2 study visits to the larger sample and found no significant differences in cytokine production, but
significantly lower acute and chronic stress. Nevertheless, the group with more complete data (the 89% who had completed 3 or 4 study visits) did still represent the full range of scores on chronic family stress.

*Groups were statistically different ($p<.05$) according to $X^2$-test.

*aRatings of asthma symptoms were made on a scale from 0 (none) to 4 (really bad). Morning and evening ratings were summed, and these scores were averaged across the 14 days of sampling.

*bCoded as whether or not the child had been prescribed the medication

*cThe average chronic family stress rating corresponds to a mild to moderate level of family stress. An acute event was defined as an event rate 2.5 or higher. Here we report the range of frequencies of acute events across visits.
Table 3.2

*Hierarchical linear models testing the effects of acute stress, chronic family stress, and the acute by chronic stress interaction on cytokine production and asthma symptoms*

<table>
<thead>
<tr>
<th>Model</th>
<th>Asthma</th>
<th></th>
<th></th>
<th>Healthy</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>SE</td>
<td>p</td>
<td>Effect size (%)</td>
<td>Coefficient</td>
<td>SE</td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>.083</td>
<td>2.15</td>
<td>.97</td>
<td></td>
<td>2.91</td>
<td>2.74</td>
</tr>
<tr>
<td>Chronic</td>
<td>1.84</td>
<td>0.96</td>
<td>.06</td>
<td></td>
<td>-0.55</td>
<td>0.88</td>
</tr>
<tr>
<td>Interaction</td>
<td>8.16</td>
<td>3.15</td>
<td>.01</td>
<td>77</td>
<td>-4.83</td>
<td>3.59</td>
</tr>
<tr>
<td>IL-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>4.98</td>
<td>14.75</td>
<td>.74</td>
<td></td>
<td>58.01</td>
<td>24.80</td>
</tr>
<tr>
<td>Chronic</td>
<td>13.96</td>
<td>12.46</td>
<td>.27</td>
<td></td>
<td>-13.42</td>
<td>18.59</td>
</tr>
<tr>
<td>Interaction</td>
<td>43.74</td>
<td>15.46</td>
<td>.007</td>
<td>73</td>
<td>-22.18</td>
<td>19.26</td>
</tr>
<tr>
<td>IL-13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>-21.87</td>
<td>36.66</td>
<td>.55</td>
<td></td>
<td>21.06</td>
<td>76.25</td>
</tr>
<tr>
<td>Chronic</td>
<td>41.83</td>
<td>29.93</td>
<td>.17</td>
<td></td>
<td>-73.45</td>
<td>29.38</td>
</tr>
<tr>
<td>Interaction</td>
<td>15.94</td>
<td>36.81</td>
<td>.67</td>
<td></td>
<td>20.00</td>
<td>88.51</td>
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*Note.* The effect size measure is the percentage of the between person variability in the relationship between acute stress and the outcome that is accounted for by chronic family stress.
Models testing the main effect of chronic stress and the interaction between acute and chronic stress statistically control for asthma severity and medication use. Models testing the main effect of acute stress statistically control for medication use.

The effect of life stress on symptoms was tested in a subgroup of children with moderate to severe asthma (n=32).
Figure 3.1

Chronic family stress interacts with major life events in the prediction of interleukin 4.

Predicted values of stimulated interleukin-4 at the 25th and 75th percentiles of chronic family stress for asthma patients who had and had not experienced a significant life event (n=71).
Figure 3.2.

Chronic family stress interacts with major life events in the prediction of interleukin 5.

Predicted values of stimulated interleukin-5 at the 25th and 75th percentiles of chronic family stress for asthma patients who had and had not experienced a significant life event (n=71).
Figure 3.3

*Chronic family stress interacts with major life events in the prediction of interferon-gamma.*

Predicted values of stimulated interferon-gamma at the 25\textsuperscript{th} and 75\textsuperscript{th} percentiles of chronic family stress for asthma patients who had and had not experienced a significant life event (n=71).
Figure 3.4

*Chronic family stress interacts with major life events in the prediction of asthma symptoms.*

Predicted symptom scores at the 25th and 75th percentiles of chronic family stress for asthma patients who had and had not experienced a significant life event. These data represent a subgroup of children who have moderate to severe asthma (n=32).
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Chapter 4: The Interpersonally Sensitive Disposition, Stressful Life Events, and Markers of Cardiovascular Health in Young Women

Epidemiologic research has revealed an increased risk of morbidity and mortality among individuals who exhibit features of a disposition we call interpersonal sensitivity (Marin & Miller, under review). For example, one study showed that introverts compared to extraverts were more susceptible to a virus that causes the common cold (Cohen, Doyle, Skoner, Rabin, & Gwaltney, 1997). Another study showed that sensitivity to rejection was associated with accelerated progression of human immunodeficiency virus infection (Cole, Kemeny, & Taylor, 1997). In fact, men at the 75th percentile on rejection sensitivity experienced HIV-related mortality approximately 2 years earlier than their counterparts at the 25th percentile. In another study, socially avoidant men were at increased risk for long-term death from cardiovascular disease (CVD) (Berry, Lloyd-Jones, Garside, Wang, & Greenland, 2007), and, in yet another, introversion was predictive of all-cause mortality among 6158 older adults, independent of baseline medical conditions and health-related behaviors (Wilson et al., 2005). Thus, individuals with characteristics related to interpersonal sensitivity are at increased risk for acquiring major diseases and dying from them more rapidly, and our recent review of the literature suggests that these effects may be strongest for infectious disease and CVD (Marin & Miller, under review).

Little is known about the psychobiological mechanisms through which interpersonal sensitivity might contribute to the progression and/or development of these medical illnesses. One hypothesis is that persons high in interpersonal sensitivity show exaggerated hypothalamic-pituitary-adrenocortical (HPA) axis and sympathetic nervous system (SNS) responses to stressful

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life events, particularly those that involve social threats (Marin & Miller, under review). These responses would expose bodily tissues to high levels of the hormones cortisol, epinephrine, and norepinephrine. Over time this exposure could change the function of these tissues in a manner that fosters pathogenic processes like high blood pressure (BP), resistance to insulin, and systemic inflammation, which ultimately drive the chronic diseases of aging (McEwen, 1998). The goal of this paper is to begin exploring this hypothesis by studying biological responses to real-life stressors in young women who vary in interpersonal sensitivity.

Dispositional Interpersonal Sensitivity

A variety of related social constructs have been studied in relation to health, including introversion, submissiveness, inhibition, social anxiety, and rejection sensitivity (Eysenck & Eysenck, 1984; Eisenberg, Fabes, & Murphy, 1995; Cole et al., 1997; Leary & Kowalski, 1997). As we have argued elsewhere (Marin & Miller, under review), we think that they all capture an underlying interpersonally sensitive disposition. This disposition makes people vigilant for/sensitive to others’ negative evaluations of them. To avoid negative social evaluation, they adopt defensive behaviors like submission and inhibition. However, distressing social situations are impossible to avoid altogether, and this may be especially true for those who are high in interpersonal sensitivity, both because they tend to have a low threshold for interpreting situations as socially threatening, and because they behave in ways that tend to elicit negative reactions from others. Also, when those high in interpersonal sensitivity do experience a frankly aversive social situation, they are inclined to interpret it as having especially damaging consequences for their reputation (Downey & Feldman, 1996; Keltner, Young, & Buswell, 1997; Downey, Lebolt, Rincón, & Freitas, 2008). We believe that these tendencies result in frequent and prolonged activation of the HPA axis and the SNS, which, as noted above, has downstream
consequences for various pathogenic processes that contribute to disease. Consistent with this scenario, interpersonally sensitive personality styles like social inhibition and introversion have been linked to heightened SNS activity, greater epinephrine output, altered immune function, and susceptibility to viral infections (Cohen et al., 1997; Miller, Cohen, Rabin, Skoner, & Doyle, 1999; Cole, Kemeny, Fahey, Zack, & Naliboff, 2003).

**Social-Evaluative Threat and Shame**

The scenario we have outlined is consistent with recent theoretical analyses of the biological consequences of negative social evaluation. Specifically, Dickerson and Kemeny (2004) have argued that situations that pose a threat to one’s social status or self-esteem (e.g., rejection, loss of status) elicit a coordinated psychobiological response consisting of shame, and activation of the HPA axis and pro-inflammatory pathways. The system that coordinates this set of responses is referred to as the social self-preservation system because it is thought to serve the basic needs of social esteem, status, and acceptance (Baumeister & Leary, 1995). In particular, shame is associated with a set of motives, non-verbal displays, and behaviors characterized by submission and withdrawal (Keltner et al., 1997). Importantly, increases in pro-inflammatory activity may extend and prolong disengagement-related motivational states.

The most compelling support for this theory comes from a meta-analysis of acute laboratory stressors and cortisol (Dickerson & Kemeny, 2004). In particular, findings indicated that stressors that included a social evaluative component produced the greatest elevations in cortisol. In fact, research evidence has shown that the extent of cortisol reactivity increases in a linear fashion with shame (Gruenewald et al., 2004). However, there is also evidence to suggest that social evaluative threat elicits SNS responses. In particular, laboratory stressors with a social-evaluative component are associated with larger autonomic and cardiovascular responses.
compared to nonevaluative conditions (Bosch et al., 2009; Cacioppo et al., 1990; Smith et al., 1997). Moreover, there is also growing evidence of a link between negative social evaluation, shame, and inflammatory processes. In particular, a recent study showed that performing laboratory tasks in the presence of an evaluative audience elicited increases in proinflammatory cytokine activity, whereas the same tasks in the absence of an evaluative audience did not (Dickerson, Gable, Irwin, Aziz, & Kemeny, 2009). Moreover, participants in the evaluative condition showed decreased glucocorticoid sensitivity, which means that their immune cells were less responsive to the anti-inflammatory effects of cortisol. Finally, there is some indication that shame and guilt induced in the lab, as well as high levels of trait shame, are associated with increased everyday SNS activity and greater inflammatory activity (Dickerson, Kemeny, Aziz, Kim, & Fahey, 2004; Rohleder, Chen, Wolf, & Miller, 2008).

*Interpersonal Sensitivity and Stress Reactivity*

As we have argued above, individuals who are high in IS should be especially responsive to socially threatening situations because they tend to be concerned about social status and belonging. There is some initial evidence to support this hypothesis. For example, one study showed that introversion amplifies the effects of lecturing on heart rate reactivity, both in the anticipation period and at the beginning of a lecture (Houtman & Bakker, 1991). Another study found that social inhibition was associated with increased systolic blood pressure (SBP) and diastolic blood pressure (DBP) reactivity to a mental arithmetic task with harassment (Habra, Linden, Anderson, & Weinberg, 2003). Another reported that socially inhibited individuals showed significantly increased delayed type hypersensitivity responses (an indicator of cell-mediated immunity) under high engagement conditions (intensive personal contact with unfamiliar professionals), but not under low engagement conditions (Cole, Kemeny, Weitzman,
Schoen, & Anton, 1999). Thus, there is some evidence to suggest that personality styles related to interpersonal sensitivity are associated with greater biological reactivity to experimental stressors. However, it’s unclear whether these laboratory-based findings extend to stressors in the real world.

**Stressful Life Events**

As far as we are aware, there is no evidence regarding the association between interpersonal sensitivity and biological reactivity to real-world stressors. Thus, the goal of the current paper is to explore this association. In general, we would expect interpersonally sensitive individuals to be more reactive to real-world stressors compared to their less sensitive counterparts. However, there may be some specificity depending on the nature of the event. In this regard, stressful life events can be conceptualized as having core emotional themes involving loss, humiliation, and danger (Brown, 2002). The loss dimension captures whether an event results in the individual having diminished sense of connectedness or well-being. Some examples would include the death of a loved one, separation from a loved one, the loss of a significant material possession, or an event that resulted in the loss of respect in a social group. The danger dimension captures whether the event might pose a threat to the person in the future. This could happen if the event itself recurred, or if it’s consequences materialized more fully later. Some examples of an event that would be high in danger would include a family member receiving a cancer diagnosis, or a friend or partner threatening to end a relationship. Finally, the humiliation dimension indicates the likelihood of an event leaving a person feeling devalued in relation to others or to a core sense of self. Some examples of humiliating events might be having a partner break off a relationship, or commit an act of infidelity that becomes public.
Interpersonal sensitivity might accentuate the impact of stressors with each of these core emotional themes. As they typically involve drops in status or rejection by others, events involving loss and humiliation would be expected to be particularly impactful for interpersonally sensitive individuals. Events that are high in danger often pose social threats, too, and when this happens they may be especially impactful for those high in interpersonal sensitivity. (For example, if a loved one was diagnosed with a serious illness, or an argument put a key relationship in jeopardy.) Another reason danger events may be impactful is because their outcome is uncertain. In particular, with events that are high in loss or humiliation, the full extent of the threat being posed is immediately apparent. However, with danger it is unclear how the situation will play out. This uncertainty may be especially stressful.

Life Stress in Adolescence

The influence of interpersonal sensitivity on health-related processes may be especially pronounced in the adolescent years. First, children and adolescents experience a great deal of interpersonal stress (Laursen & Collins, 1994). During this time of life, children struggle to attain increasing independence from their parents, and at the same time, a greater emphasis is placed on the peer network. In fact, close friends become the primary source of social support over the adolescent years, and they play an important role in adolescents’ self-concept and wellbeing (Furman & Buhrmester, 1992). Moreover, most adolescents have had a romantic relationship by middle adolescence, and concerns over the presence, absence, and quality of romance are particularly salient at this age (Graber, Brooks-Gunn, & Petersen, 1996; Steinberg & Morris, 2001). Thus, it’s not surprising that adolescents report interpersonal stressors involving peers as among their most common and most distressing problems (Repetti, McGrath, & Ishikawa, 1999). Taken together, adolescents are exposed to a great deal of interpersonal
stress, whether it is within the family, among close friends, or in the larger social domain, and this impact would be accentuated among interpersonally sensitive adolescents because they are particularly concerned about being accepted and well-liked. Second, it has been argued that the rapid changes in brain structure and function during puberty may represent a critical window during which individuals are particularly sensitive to stress (Dahl, 2004; Romeo & McEwen, 2006). In other words, stress in adolescence has the potential to really disrupt neural development in a way that has long-term effects. Indeed, a recent study showed that neuroendocrine and cardiovascular responses to both interpersonal and performance stressors increases from childhood to adolescence (Stroud et al., 2009). This means that when stressors do occur, adolescents are likely to show significant biological responses to them. These patterns could have important implications for the development of chronic diseases. Indeed, there is mounting evidence that chronic diseases, particularly CVD, begin in the first and second decades of life. In fact, autopsy studies reveal that many young individuals have developed atherosclerotic plaque by the time they reach adolescence (Berenson et al., 1992; Berenson et al., 1998), and CVD precursors such as insulin resistance and endothelial dysfunction are identifiable in some youth (Berenson & Srnivasan, 2005). Researchers have thus called for increased attention to early life stressful experiences and their biological consequences in populations of children and adolescents (Matthews, 2005).

*Inflammation and the Development of Atherosclerosis*

Atherosclerosis is the underlying pathogenic condition that drives CVD. Current thinking maintains that atherosclerosis is a chronic inflammatory response to injuries of the lining of the arteries (called the endothelium) caused by shear stress, infections, irritants, high BP, and other factors (Cai & Harrison, 2000). The injuries trigger an influx of white blood cells
that seek to repair the damage. These cells, particularly macrophages, enter the vessel wall and begin taking up cholesterol in large amounts. When it persists this response causes dysfunction of the endothelium, and causes plaque to grow into the lumen of the blood vessel, which obstructs blood flow to the heart or affected organ. This in turn causes high BP and increased stress on the heart. In some cases these plaques rupture, leading to the formation of a thrombus. This completely obstructs blood flow in the vessel. If this blockage is prolonged and the vessel is a major supplier of blood, the organ it feeds will be deprived of oxygen and nutrients leading to the death of some its tissue (which is the process underlying myocardial infarctions and strokes).

Inflammation is particularly important in the progression of atherosclerosis, as it plays a role in every stage of the process, from the formation and growth of plaques, to their rupture much later in the disease (Pearson et al., 2003; Libby & Theroux, 2005).

The Current Study

In summary, there is evidence that individuals with features of interpersonal sensitivity are at increased health risk. To explain how this happens, we argue that interpersonally sensitive individuals are particularly reactive to the emotion-eliciting features of acute life events. The current article explores this hypothesis.

Adolescent girls were seen every 6 months for 2.5 years. At each study visit, life events were assessed by structured interview and rated by an independent team for the extent of loss, danger, and humiliation. Biological processes involved in CVD pathogenesis were also assessed at each visit. The focus was on 2 key processes in atherogenesis that can be assessed in young people. The first was BP, which is the force on the walls of the arteries as blood circulates. High BP tracks through adolescence and well into adulthood (Lauer, Burns, Clarke, & Mahoney, 1991; Mahoney et al., 1996), and high BP a risk factor for CVD mortality (Lewington, Clarke,
Qizilbash, Peto, & Collins, 2002). The second was inflammation. In this regard, we were interested in both circulating markers of inflammation, which tell us how much is happening in the body at the moment, and responsivity, or how aggressively participants’ cells respond to microbial stimuli that usually trigger inflammation. The latter measure was intended to model how adolescents’ cells would likely respond in vivo to infections and injuries that initiate the inflammatory process that drive atherosclerosis.

We expected that interpersonally sensitive adolescents would be more reactive to the emotion-eliciting features of events compared to adolescents low on this dimension. Given that individuals high in interpersonal sensitivity are particularly sensitive to social threats with themes of rejection, disapproval, and/or loss of status, we expected them to should show elevations in BP and inflammatory markers in the aftermath of major loss or humiliation. In fact, we expected these emotional dimensions to be associated with the most profound changes in biomarker activity. However, we also considered the possibility that interpersonally sensitive adolescents would react to the uncertain nature of danger events, where the full extent of threat has yet to unfold. Thus, a secondary hypothesis was that danger events would elicit heightened reactivity among interpersonally sensitive adolescents, as evidenced by elevated levels of BP and systemic inflammation, and heightened inflammatory responses under microbial challenge.

Method

Subjects

Data for the present study were collected as part of a larger research project involving young women at high-risk for depression. Adolescent females were recruited from the Vancouver, British Columbia community through advertisements in newspapers and magazines. Young women were eligible for the study if they were (1) 15-19 years old, (2) fluent in English,
(3) free of acute and chronic medical conditions, (4) without a lifetime history of major psychiatric disorders, and (5) at high risk for developing an initial episode of major depression. High-risk was defined as having a first-degree relative with a history of depression, or as scoring in the top quartile of the sample distribution on one of two indices of cognitive vulnerability, the Dysfunctional Attitudes Scale (Weissman & Beck, 1978) or the Adolescent Cognitive Style Questionnaire (Hankin & Abramson, 2002).

A total of 157 young women were enrolled in the study. Nearly all of them were recruited to be at high risk for developing an initial episode of depression (N=147). The other 10 young women were enrolled as a low-risk comparison group. Adolescents in this group were without family histories of affective disorder and scored below the 75th percentile on cognitive vulnerability indices. Over the course of the 2.5 year follow-up, 24/157 participants were lost to attrition (15.3%): 6 of them moved away, we lost contact with 4, and 15 withdrew from the study. For the purposes of the analyses in the current paper, we included data from any participant who completed 3 or more visits, which resulted in a sample of 144 young, healthy females, or 91.7 of the original cohort.

**Procedures**

All participants attended an initial laboratory session. Upon arriving at the session, a research assistant described the study procedures in detail. Written consent was obtained from the participant, or if she was younger than 18 years, written assent was obtained and formal consent from her parent. The Structured Clinical Interview for DSM-IV was then administered to determine eligibility in terms of lifetime history of psychiatric disorders. Next, research assistants administered an in-depth interview regarding life stress (see below). Following the interview, the participant completed a battery of personality questionnaires, including measures
of introversion, submissive behavior, and subjective social status. Next, she was seated in a comfortable chair. Measures of resting BP were taken, and then 30-ml of blood was collected through antecubital venipuncture. Participants returned to the lab every 6 months for 2.5 years. Eleven percent of the participants included in this paper completed 3 or 4 study visits, 17 percent completed 5 study visits, and 72 percent completed all 6 visits. Measures of life stress, submissive behavior, subjective social status, and biomarkers were taken at each visit, whereas the introversion scale was administered at every other visit. The project received approval of the institutional review board at the University of British Columbia.

**Composite Measure of Interpersonal Sensitivity**

We define interpersonal sensitivity as a stable trait characterized by ongoing concerns about negative social evaluation. This disposition has a cognitive-affective component that includes vigilance for and sensitivity to others’ evaluations, as well as a behavioral component that includes submission, inhibition and social withdrawal (Marin & Miller, under review). Many different concepts in the literature have elements of interpersonal sensitivity, including introversion, submissiveness, social inhibition, rejection sensitivity, social avoidance, social anxiety, and Type D personality (the combination of social inhibition and negative affect). While these constructs differ in some respects, we believe they share a common element that may be detrimental to health (see Marin & Miller, under review). For the purposes of the current study, we assessed specific features of interpersonal sensitivity using established scales and combined scores on individual measures to produce a composite measure of interpersonal sensitivity.

First, introversion-extraversion was measured by scores on the Big Five Inventory (Pervin & John, 1999). Extraversion captures how a person approaches the social and material world, and includes traits such as sociability, activity, assertiveness, and positive emotionality.
(John, Naumann, & Soto, 2008). Chronbach’s alpha was used to evaluate the scale’s internal consistency. The average alpha across the visits was 0.86 (SD=.015). Second, submissive behaviour was measured using the Submissive Behaviour Scale (Allan & Gilbert, 1997). This is a 16-item questionnaire in which respondents rate a series of statements on a 5-point scale (ranging from 0 to 4). Statements refer to behaviours such as avoiding eye contact with others or apologizing repeatedly for minor mistakes. Higher scores indicate more submissive behaviour. The average alpha across the visits was 0.83 (SD=.022).

The interpersonally sensitive disposition is likely to manifest in how subjects see themselves vis à vis the social groups they belong to. This may be especially true for the submissiveness dimension, which can be construed in terms of relative social ranking. For example, within a given social domain, those with a higher level of assets (e.g., income, savings) or prestige (e.g., educational attainment) are considered higher in status, or more dominant, while individuals with a lower level of assets or prestige are considered lower in status, or more submissive (Newton, 2009). Therefore, as a third indicator of interpersonal sensitivity, we measured perceptions of social standing using the youth version of the MacArthur Scale of Subjective Social Status (Goodman et al., 2001). Subjective social status is an individual difference variable that captures people’s perceptions of their status in social groups. Research indicates that individuals low on this dimension report more shame-related emotions and cognitions, and they are more likely to behave submissively in social situations (Gruenewald, Kemeny, & Adler, 2001). This scale presents participants with a series of 10-rung ladders. On each they indicate where they stand relative to others in a specified community. Higher scores indicate higher perceived rank. The first scale pertained to family status, and adolescents were asked to place their family in comparison with others in society on the ladder, with a focus on
money, amount of schooling, and the jobs that bring the most respect. The other ladder pertained to school status, and adolescents were asked to place their standing in comparison with other students on the ladder, with a focus on respect, grades, and academic standing. Moreover, we created an additional school ladder to capture perceived status in terms of popularity and attractiveness. Thus, the final scale included 3 ladders, one of which focused on family status and two of which focused on school status.

Next, we examined the stability of each scale over the course of the study visits. The intraclass correlations were 0.78 for introversion-extraversion, 0.55 for the MacArthur family scale, 0.53 for the MacArthur school scale focusing on respect and academic standing, 0.59 for the MacArthur school scale focusing on attractiveness and popularity, and 0.74 for submissive behavior. Given that scores on these scales were stable over time, and seemed to reflect genuine between-person differences in the relevant constructs, we then averaged them across the study visits.

Next, scores on each of the scales were standardized. We then ran a factor analysis on the Z-scores to evaluate the relationships among indicators of interpersonal sensitivity. The number of factors to be extracted was determined by factor loading structure and Eigenvalues greater than 1. A principal component analysis showed that each of the scales loaded on a single factor, with loadings ranging from .63 to .83 (see Table 4.1). The first factor that was extracted explained 50 percent of the total variance (with an Eigenvalue of 2.5), whereas the second factor that was extracted explained only 19.4 percent of the total variance and had an Eigenvalue of .97. Based on these results, we accepted the single-factor solution, and Z-scores on the scales were averaged across the measures to create a single composite index of interpersonal sensitivity. Chronbach’s alpha for the final scale was 0.75.
Stressful Life Events

To assess participants’ exposure to stressful experiences, we administered the UCLA Life Stress Interview- Adolescent Version, which was developed from earlier versions for adults and children (e.g., Hammen, 1991). This semi-structured interview assesses acute and chronic forms of stress over the past six months. It focuses on stress in multiple domains, including romantic relationships, friendships, social life, family relationships, academic life, work life, finances, and health. Each domain is probed with open-ended questions, and when a subject notes that an event has occurred, the interviewer gathers details of the event and later presents them to a team of independent and experienced raters. This article focuses on acute stressors, with an emphasis on their emotion-eliciting properties. However, before making ratings along emotional dimensions, the team identified whether a significant life event had occurred or not.

Acute stressors are defined as specific events with a discrete onset and offset. To judge the objective impact of an acute event, our team makes a consensus impact rating, after being briefed on event details by the primary interviewer. The rating process is meant to eliminate the influence of reporting biases; thus, a participant’s subjective experience is not discussed by the team or factored into its rating. The goal of the rating is to capture how the average person in similar biographical circumstances would respond. Impact ratings can range from 1 (no long-term impact) to 5 (severe long-term impact) and they explicitly consider the context in which an event has occurred. For example, if a participant had failed a test at school, the rating would be based on a number of factors, such as her previous academic record, and reactions from parents and teachers. Participants with no acute event were given a score of 1 (no long-term impact). In the present study, we consider events rated 2.5 (mild-moderate impact) or higher to be significant life events. This follows the convention in the Life Events and Difficulties Schedule area of
using a threshold for defining major life events (Hammen, Henry, & Daley, 2000; Miller & Chen, 2006). This interview has been used successfully in adolescent populations (e.g., Hammen, Brennan, & Shih, 2004), and there is evidence to support its reliability and validity. In terms of validity, high stress ratings predict the onset of a depressive episode among children and adolescents (Hammen, Adrian, & Hiroto, 1988; Adrian & Hammen, 1993; Rudolph & Hammen, 1999), as well as biological outcomes among children with asthma (Miller & Chen, 2006).

Acute events rated 2.5 or higher were rated along dimensions of loss, danger, and humiliation using methods developed by Brown, Harris, and Hepworth (1995) and then adapted by Kendler, Hettema, Butera, Gardner, & Prescott (2003) (F. Butera, C. Prescott, and K. Kendler, unpublished manual, *Guidelines for: Contextual Threat and Dependency Rating Humiliation, Entrapment, Loss, and Danger Rating*). Contextual ratings of loss, danger, and humiliation can range from 1 (none) to 5 (severe). The loss dimension is used to indicate a diminished sense of connectedness or well-being. Events that might be rated as high in loss would include the death of a loved one, separation from a loved one, the loss of a significant material possession, or an event that resulted in the loss of respect in a social group. The danger dimension is used to indicate the degree of threat an event is likely to pose in the future. For this dimension, raters consider the potential long-term consequences of an event, particularly those that have not yet materialized, as well as the likelihood of the event repeating itself. Events that might be rated as high in danger include a grandmother’s cancer diagnosis and a fight between parents with threats to divorce. Finally, the humiliation dimension is used to indicate the likelihood of an event leaving a person feeling devalued in relation to others or to a core sense of self, usually with an element of rejection or failure of role. Examples of humiliation include having an abortion and finding out that a boyfriend is seeing another girl. Life event dimensions
of loss, humiliation, and danger have been used successfully to predict the onset of psychological disorders (Kendler et al., 2003). We consider events rated 2.5 (mild-moderate) or higher as major loss, humiliation, and/or danger events.

**Inflammation**

Inflammation was measured in 2 ways. First, we measured circulating markers of inflammation, which indicate extent of ongoing immune activation. Second, we measured stimulated production of inflammatory molecules, which indicates how aggressively participants’ cells respond to microbial stimuli that usually trigger inflammation. We used 2 indicators of systemic inflammation, IL-6 and CRP. IL6 is released by white blood cells and other bodily tissues in response to infection and injuries, and it helps orchestrate the inflammatory response. CRP is a peptide made by the liver in response to IL-6. It does not play a role in inflammation per se, though it is a useful proxy for it. Because they reflect the extent of ongoing inflammation, CRP and IL-6 are prognostic of CVD, with higher levels of both molecules forecasting an increased likelihood of Type 2 diabetes, coronary heart disease, and sudden cardiac death among apparently healthy people (Albert, Ma, Rifai, Stampfer, & Ridker, 2002; Willerson & Ridker, 2004; Libby & Theroux, 2005).

To measure circulating inflammatory markers, blood was collected through antecubital venipuncture following an overnight fast. It was centrifuged at 1000 x g for 25 minutes; the serum was then aspirated, divided into 1-ml aliquots, and frozen at -20 degrees C until analysis. CRP was analyzed using a high-sensitivity, chemiluminescent technique on an IMMULITE 2000 (Diagnostic Products Corporation, Los Angeles, California). This assay has an inter-assay coefficient of variation of 2.2% and a detection threshold of .20 mg/L. IL-6 was analyzed using
high sensitivity assay kits purchased from R&D Systems Minneapolis, MN. This assay has a sensitivity of 0.039 pg/mL and intra- inter-assay coefficients of variation less than 10%.

To measure how aggressively participants’ cells respond to microbial stimuli, we cultured them with lipopolysaccharide (LPS) and measured subsequent production of IL-6. Whole blood was drawn into Lithium-Heparin Vacutainers and diluted 10:1 with saline, and then co-incubated with LPS at a concentration of 50 ng/ml (Sigma, Saint Louis, Missouri) for 6 hours at 37ºC with 5% CO₂. The supernatants were then harvested and frozen at -30ºC until assayed for IL-6 by ELISA (DuoSet ELISA Development Systems; R&D Systems). These kits have a minimum detectable threshold of 0.7 pg/ml and inter- and intra-assay variability of <5%.

Resting BP

SBP and DBP were monitored using a BP TRU automated BP monitor (VSM MedTech Devices Inc., Coquitlam, BC) with a standard occluding cuff on the participant’s arm. Measurements were taken 4 times in 2-minute cycles, and the initial measure was tossed. The averages of the 3 measures were used in statistical analyses. Coefficient alphas for baseline SBP and DBP were .93 and .91, respectively.

Potential Covariates

To determine whether behavioral and biomedical characteristics might be acting as confounders, we collected information regarding age, ethnicity, oral contraceptive use, smoking history, central adiposity, and strenuous physical activity. We chose these covariates because they have been linked to interpersonal sensitivity, reactivity to stress, and risk for CVD, and as such could provide alternative explanations for any associations observed (Tangney, 1990; Paffenbarger Jr, Wing, & Hyde, 1995; Kikuchi et al., 1999; Matthews et al., 2004; Landen et al., 2004; Hamer & Steptoe, 2007; Hampson, Goldberg, Vogt, & Dubanoski, 2007). Because the
majority of the sample (92%) was of Caucasian or Asian descent, we created a dichotomous ethnicity variable coded as 1 for Caucasian and 0 for Other. Smoking history was a dichotomous variable indicating whether or not participants smoked cigarettes daily. Only a small percentage of adolescents endorsed this item (0.7-6.1% across the study), and among those who did, the range was from 1 to 12 cigarettes per day. Central adiposity was indexed as the ratio of waist to hip circumference. Strenuous physical activity was measured using items from the Paffenbarger Activity Scale (Paffenbarger, Blair, & Lee, 1993). This scale provides estimates of the number of minutes each week the respondent engages in “regular activity akin to brisk walking, jogging, bicycling, etc, long enough to work up a sweat.”

Statistical Analyses

Data were analyzed using hierarchical linear modeling (HLM 6.0) (Raudenbush, Bryk, & Congdon, 2006), a multilevel modeling technique that allowed us to test both within-person and between person contributions to changes in biomarkers activity over time. Life event dimensions (i.e., loss, danger, and humiliation) were modeled as within-person factors because they vary over time, whereas interpersonal sensitivity was modeled as a between-subjects factor because it varies from adolescent to adolescent but remains stable over time. In the within-person (or level-1) model, we estimated CRP, IL-6, stimulated IL-6, and BP as a function of factors that vary over time including exposure to a major life event and the covariates of WHR, physical activity, oral contraceptive use, cigarette smoking, time since baseline, and a residual term. All life event variables were person-centered. These models yielded person-specific intercepts reflecting the biological parameter at each person’s typical life event load ($\beta_{0i}$), and person-specific trajectories reflecting the rate of change over the study in that biological parameter ($\beta_{1i}$). Separate models were run for each of the life event dimensions (loss, danger, and humiliation).
In the between-person (or level-2) models, we estimated the above coefficients for each subject as a function of factors that vary across people, including interpersonal sensitivity, age, and ethnicity. The critical parameter was the coefficient $\gamma_{11}$, which reflects the cross-level interaction between life event dimensions and interpersonal sensitivity in the prediction of biological outcomes. In other words, the coefficient $\gamma_{11}$ indicates whether the relationship between the occurrence of a major life event and the study’s various biological outcomes depended on the degree of interpersonal sensitivity. When significant interactions emerged, biological outcomes were plotted at the 25th and 75th percentiles of interpersonal sensitivity in both the absence and presence of major life events (significant overall impact, loss, danger, or humiliation). It should be noted that HLM can account for missing data at level 1, thus a participant’s data could be included in study models, even if she missed a visit.

Results

Preliminary Analyses

Table 4.2 describes the sample characteristics and provides descriptive statistics for major predictors, covariates, and outcomes. The study had an ethnically diverse sample of teen-age females, and the majority of participants (75%) came from families where at least one parent had a college degree. Analyses of psychometric characteristics revealed that all of the study’s predictors and outcomes were distributed normally. The only exceptions to this were serum IL-6 and serum CRP, both of which were substantially positively skewed. First, we discarded IL-6 and CRP values that were greater than 10 mg/L because they are suggestive of an acute inflammatory infection (Pearson et al., 2003). Next, both variables were transformed using log-10 transformations.
Next we ran bivariate correlations among study predictors and covariates (see Table 4.3). Pearson correlations indicated that interpersonal sensitivity was significantly associated with ethnicity, such that Caucasian participants were lower on interpersonal sensitivity compared to non-Caucasian participants. In addition, interpersonal sensitivity was significantly and negatively associated with physical activity. This means that to the extent that adolescents were higher on interpersonal sensitivity, they engaged in less physical activity. Interpersonal sensitivity was unrelated to the likelihood of experiencing life events at baseline, regardless of whether they included elements of loss, danger, or humiliation. The life event dimensions were inter-correlated (r’s ranged from .26 to .84) suggesting (a) that participants who experienced one kind of event were more likely to experience another, and (b) that some events were rated as having multiple core emotional themes at the same time. For example, one major life event involved parents separating due to the mother’s drinking problem. This event was rated as having elements of loss, danger, and humiliation. In particular, the event involves a loss of stability in the family, as well as decreased contact between the daughter and mother. However, there’s also an element of danger, as this event may lead to eventual divorce, as well as further declines in the mother’s health. Finally, there is an aspect of humiliation because of others’ potential judgment of the situation. Now that the parents are separating, close friends and family may become more aware of the family’s difficulties.

*Main Effects of Stressor Loss, Danger, and Humiliation*

The first wave of analyses examined whether life event dimensions of loss, danger, and humiliation triggered changes in the biological processes of interest across the sample. A series of level-1 models were estimated where outcomes at each visit were predicted by loss, danger, or humiliation in the six months prior. The error terms were modeled as random for the intercepts
and life event components, but fixed for the level 1 covariates. These models tested for within-person effects of life event components. The question of interest here was whether participants’ biological profiles differed significantly at study visits that had versus had not been preceded by major loss, danger, or humiliation.

Results indicated that the humiliation component was significantly and positively associated with IL-6 production, $\gamma_{10} = 3099.60$, $SE = 1596.19$, $p = .05$. This means that at times when young women had experienced a major humiliation event they showed higher IL-6 production compared to times when they had not experienced this kind of event. However, humiliation was unrelated to serum IL-6, serum CRP, and BP, and there were no significant associations of loss and danger with the outcome measures ($p$’s > .10).

**Main Effects of Interpersonal Sensitivity**

The second wave of analyses examined whether IS was associated with biomarker activity. These models tested for between-person effects of IS at level 2, controlling for level 1 covariates, as well as age and ethnicity. The question of interest here was whether participants’ biological profiles varied as a function of their interpersonal sensitivity. Results indicated no statistically significant associations between interpersonal sensitivity and biological outcomes, either at study entry or over the course of the project ($p$’s > .05). However, there was a trend towards an effect of interpersonal sensitivity on SBP at study entry. In particular, higher interpersonal sensitivity was marginally related to lower SBP, $\gamma_{01} = -10.83$, $SE = 6.28$, $p = .086$.

**Interactions Between Acute Stress Components and Interpersonal Sensitivity**

In the third wave of analyses, we tested the cross-level interactions between life event components and interpersonal sensitivity in the prediction of biomarkers. The question of interest here was whether interpersonal sensitivity would accentuate the biological consequences
of major loss, danger, and/or humiliation. Again, the error terms were modeled as random for
the intercepts and acute stress components, but fixed for the covariates.

First, we tested for level 1 by level 2 interactions, in terms of whether the relationship
between danger and biological outcomes depended on the level of interpersonal sensitivity.
Results indicated significant interactions for CRP and DBP, $\gamma_{11} = 0.044, SE=0.022, p = 0.044,$
and $\gamma_{11} = 1.90, SE = 0.76, p = 0.014,$ respectively. As shown in Figure 4.1, adolescents higher in
interpersonal sensitivity showed increased CRP and DBP at times when they experienced a
danger event compared to times when they had not. In contrast, adolescents characterized by
lower interpersonal sensitivity showed no changes in DBP and a decrease in CRP at times when
they experienced danger compared to time when they had not. There were no significant
interactions between danger events and interpersonal sensitivity in the prediction of serum IL6,
IL-6 production, and SBP (p’s>.10).

Next we tested for interactions between interpersonal sensitivity and biological responses
to major loss events. Results indicated a significant interaction for IL-6 production, $\gamma_{11} =
4230.58, SE = 1582.13, p = .009.$ As shown in Figure 4.2, adolescents high on interpersonal
sensitivity showed increases in IL-6 production at times when they experienced loss compared to
times when they had not. In contrast, low IS individuals show no changes in inflammatory
activity in the presence or absence of a loss event. Results indicated no significant interactions
between loss and interpersonal sensitivity in the prediction of serum CRP, serum IL-6, or BP (p’s
>.50).

Finally, we tested for interactions between interpersonal sensitivity and humiliating events.
Analyses revealed a significant interaction for SBP, $\gamma_{11} =2.38, SE = 0.87, p = .007.$ As shown in
Figure 4.3, the pattern of findings was in the unexpected direction, with adolescents high on
interpersonal sensitivity showing decreases in SBP at times when they had experienced humiliation. In contrast, individuals low on interpersonal sensitivity showed increases in SBP at times when they experienced a humiliating event compared to times when they had not. There were no significant interactions between IS and humiliation in the prediction of DBP or inflammatory markers (p’s>.20). See Tables 4.4 and 4.5 for a summary of findings.

Discussion

Recent evidence indicates that interpersonally sensitive individuals are at increased risk for morbidity and mortality from chronic diseases. To explain the mechanisms underlying this phenomenon, we have argued that individuals high on this dimension are biologically reactive to specific features of acute life stress. In the current article we began testing this hypothesis in a sample of adolescent females. Every six months we measured the occurrence of major life events that involved loss, danger, and humiliation, and tracked biological processes that are involved in the development of CVD.

We expected that interpersonal sensitivity would be associated with pronounced biological responsivity to major life events involving loss and humiliation. These kinds of events should pose a high degree of threat to interpersonally sensitive individuals, in that they activate concerns about rejection, criticism, belongingness, and acceptance. We found modest support for this hypothesis in the form of a significant interaction between interpersonal sensitivity and loss in the prediction of IL-6 production. The pattern of this interaction indicated that at times when adolescents high in interpersonal sensitivity had experienced significant loss, they showed increases in IL-6 production compared to times when they had not. In contrast, adolescents low on interpersonal sensitivity showed no changes in IL-6 production in the aftermath of loss. This finding suggests that when a major loss event occurs, the immune cells of those high in
interpersonal sensitivity begin to respond more aggressively to microbial stimuli. Over the long-term, this tendency could favor the development of a pro-inflammatory environment in the body that sets the stage for chronic diseases like atherosclerosis.

We also expected interpersonal sensitivity to be associated with more pronounced responses to major events that involved danger, as they often involve social threats, albeit ones that have not yet fully materialized. There was stronger evidence in favor of this hypothesis. In particular, analyses revealed significant interactions between interpersonal sensitivity and danger events in the prediction of CRP and DBP. This means that the within-person associations between danger and both CRP and DBP depended on the extent of interpersonal sensitivity. Specifically, adolescents high on interpersonal sensitivity showed higher than average CRP and DBP at times when they had experienced a danger event compared to times when they had not. In contrast, adolescents low on interpersonal sensitivity showed no changes in DBP and decreases in CRP at times when they had versus had not been exposed to danger. Thus, among adolescents high on interpersonal sensitivity, danger events bring about elevated resting levels, both in terms of diastolic pressure and systemic inflammation. Although biological changes of this magnitude (i.e., 2-point increases in DBP and CRP concentrations that remain within the normal range) are unlikely to have immediate medical consequences, repeated exposures may take an allostatic toll on the cardiovascular system over time.

Why might we see more consistent effects of danger compared to loss and humiliation? First of all, the bulk of events reported in this study were interpersonal in nature (e.g., conflict with best friend, break-up with boyfriend, death of a loved one), and other sources of stress, like performance-based stressors (e.g., failing an exam), are likely to have an interpersonal component (e.g., disappointment of teachers or parents). As a result, the emotion-eliciting
themes of loss, humiliation, and danger may not differentiate between events that are likely to bring about perceptions of negative social evaluation versus those that are not. This may be particularly true of individuals high on interpersonal sensitivity, who are likely to perceive negative social evaluation in response to ambiguous cues (Pozo, Carver, Weflens, & Scheier, 1991; Downey & Feldman, 1996; Magnusdottir & Smari, 1999; Leary, 2001). However, these themes do differentiate between events that have already occurred (loss and humiliation) and those that signal the potential for future threat (danger). Thus, our findings suggest that, for adolescents high on interpersonal sensitivity, it’s the increased likelihood of future social threat, or the uncertainty surrounding whether it will materialize, that relates to changes in biological processes related to CVD risk. From a theoretical perspective, these findings suggest that the key psychological mechanism of action here is the possibility of impending social difficulties, rather than rumination about events that have already transpired. If confirmed, this observation may also have clinical implications. For instance, interventions could be developed to help IS adolescents cope with the uncertainties that life throws their way.

We speculate that highly interpersonally sensitive adolescents respond to danger events with heightened vigilance for the potential future threat. Vigilance is part of a self-protective, or defensive, stance toward the social environment, and as we have argued elsewhere (Marin & Miller, under review), it is a defining feature of the interpersonally sensitive disposition. Vigilant behavior in anticipation of future social threat may prolong the biological response to stress. Although relatively few studies have examined this proposition, there is some evidence consistent with it. For example, studies have shown that vigilant behavior in the lab is accompanied by BP elevations (Smith, Ruiz, & Uchino, 2000) (Ewart, Jorgensen, Schroder, Suchday, & Sherwood, 2004). Moreover, a recent daily diary study showed that anticipatory
fears about potential social threat were associated with within-person increases in cortisol output (McCullough, Orsulak, Brandon, & Akers, 2007). Finally, Melamed and colleagues (2004) reported elevated concentrations of CRP among healthy women experiencing fear of terror (i.e., tension in crowded places, fear of terror strike harming self or family). However, more work is needed to test this vigilance hypothesis directly.

Indeed, the differential effects for danger versus loss and humiliation might be a function of our study design. As mentioned above, danger events involve the potential for future threat, thus their effects may be more prolonged than those of loss and humiliation. Given that the study visits were spaced 6 months apart, and life events could occur at any point in between them, we may have been more likely to capture the biological consequences of danger, compared to loss and humiliation. In particular, danger events were often still unfolding during study visits, whereas other types of events were no longer “hot” because adolescents had been coping with them for weeks or months.

That said, our findings provide some evidence that when events are overtly humiliating, individuals high and low on interpersonal sensitivity respond with increased inflammatory activity. In particular, our findings indicated a main effect of humiliation on IL-6 production, which means that regardless of the level of interpersonal sensitivity, adolescents showed increases in stimulated IL-6 production at times when they had experienced humiliation compared to times when they had not. According to the social self-preservation theory, threats to the social self activate proinflammatory pathways, which induce behavior consistent with a disengaged motivational state (Dickerson, Gruenewald, & Kemeny, 2004; Dickerson & Kemeny, 2004). Thus, the observed changes in stimulated IL-6 following humiliation may represent an adaptive response to overt threats to belongingness, which is why we see it among individuals
both high and low on interpersonal sensitivity. In contrast, the tendency to perceive negative social evaluation in response to ambiguous social situations (as we expect to see in high IS individuals) can be maladaptive (Downey & Feldman, 1996; Pickett, Gardner, & Knowles, 2004).

Findings also indicated a significant interaction between interpersonal sensitivity and humiliation in the prediction of SBP, however the pattern of effects was in the unexpected direction. In particular, adolescents high on interpersonal sensitivity showed relatively stable SBP at times when they had experienced humiliation compared to time when they had not, whereas adolescents low on interpersonal sensitivity showed elevated SBP in the aftermath of these events. Thus, adolescents low on interpersonal sensitivity appear to be more reactive to humiliation events, at least in terms of SBP. Although the majority of past work has linked IS constructs to heightened reactivity, there is some recent evidence consistent with the current data. For example, Gramer and Sprinschnick (2008) showed that socially anxious individuals exhibited lower BP and HR reactivity during an evaluative speaking task compared to low anxious individuals. Moreover, Gruenewald and colleagues (2006) reported that college students with low subjective social status did not mount a significant cortisol response to negative social evaluation, whereas students with high subjective social status showed significant cortisol increases. How do we explain this pattern of findings? One explanation that has been offered is that individuals low on interpersonal sensitivity can be more threatened by negative social evaluation since they have more to lose, at least in terms of relative social status (Gruenewald et al., 2006). However, if this was true, we wouldn’t expect our other study findings to have emerged (e.g., heightened IL-6 production among adolescents high and low on interpersonal sensitivity following humiliation). Clearly, more work is needed to examine the conditions
under which interpersonally sensitive individuals are likely to exhibit an exaggerated versus blunted biological response, as well as to test the potential mediating roles of threat appraisals.

Finally, our findings provide some evidence of biological specificity in response to events that involve danger versus loss and humiliation. In particular, danger was associated with elevated resting levels (systemic inflammation and resting DBP), while loss and humiliation were associated with greater cellular IL-6 responses to microbial stimulation. These patterns are consistent with models that posit biological specificity in the stress response (Weiner, 1992; Kemeny, 2003). The idea here is that specific signals in the environment elicit a distinct pattern of hormonal and neural changes that help the organism to deal with the specific nature of the threat. As these models suggest, specific appraisals and emotions may underlie some of the response specificity we observed, and in future research it will be important to gather data on them to evaluate this proposition. In a similar way, we can speculate on the differential BP findings for danger and humiliation. Specifically, stressors can be appraised as threatening or challenging, with each having somewhat distinct physiological consequences (Tomaka, Blascovich, Kibler, & Ernst, 1997). Threat appraisals are characterized by increases in vascular reactivity, linked to DBP, whereas challenge appraisals are characterized by increases in cardiac reactivity, linked to SBP. Thus, the observed elevations in DBP in response to danger among highly sensitive adolescents may be mediated by threat appraisals. This would be consistent with evidence linking vigilant attention to an impending threat (as in danger) to hemodynamic changes characterized by vascular reactivity (Ewart et al., 2004). In contrast, the observed elevations in SPB in response to humiliation among individuals low on interpersonal sensitivity may be mediated by challenge appraisals. For instance, if an adolescent who is low in interpersonal sensitivity is rejected from a social group, she may be able to disengage from those
friendships and focus her energy on forming new ones. Clearly, more work is needed to examine the cognitive and affective processes that underlie this effect, and more generally, to test the specific biological consequences of various emotion-eliciting situations.

There are a number of limitations to this study that should be considered. First, adolescents in this sample were at high risk of developing an initial episode of depression. These participants may be more susceptible to the effects of stress than the broader population, especially in combination with interpersonal sensitivity. Thus, it will be important to evaluate these hypotheses in a normal community sample of adolescents before any generalizations are made. Second, given the lag of up to 6 months between life event occurrences and biological assessments, our study may have failed to capture important associations between life event dimensions and biological outcomes. In particular, adolescents may have recovered from life events by the time they returned to the lab for a visit. Although this suggests that the effects that did emerge reflect particularly prolonged biological responses, our design may have obscured other pieces of the puzzle. Future research that examines biological processes within weeks of the exposure may be more likely to detect main effects of life events, as well as their interactions with interpersonal sensitivity. Third, it is possible that unmeasured variables account for the observed associations. This is unlikely for the humiliation-inflammation association we observed because of the within-person nature of this analysis precludes most of the alternative explanations for linkages between stressors and biomarker activity (e.g., genetics, lifestyle factors). However, there are other factors that could account for the for the cross-level interactions between interpersonal sensitivity and major events. For example, these effects may be better explained by hostility, an established risk factor for CVD that is placed in a similar region of the Interpersonal Circumplex (Kiesler, 1991; Kiesler, 1996) as constructs related to
interpersonal sensitivity. Thus, more work is needed to differentiate the roles of interpersonal sensitivity and hostility in the early development of CVD. Finally, future work should incorporate indices of HPA function, including cortisol output and glucocorticoid sensitivity, which would shed light on potential pathways from stressful events to proinflammatory activity (Miller, Cohen, & Ritchey, 2002).

Despite these limitations, our findings provide several important contributions to the literature. First, they show that interpersonal sensitivity interacts with features of life stress to produce changes in biomarker activity. Thus, there appears to be specificity in the types of events that are most threatening to interpersonally sensitive adolescents. Second, the interactions between interpersonal sensitivity and danger in the prediction of BP and systemic inflammation suggest that interpersonally sensitive individuals are reactive to events that foreshadow future social difficulties. Thus, a vigilant social stance following danger may prolong the stress response among adolescents who are high on interpersonal sensitivity. Finally, the BP and immune changes observed contribute to our understanding of how psychosocial factors like interpersonal sensitivity and stressful events get “inside the body” to affect long-term cardiovascular risk. Interventions aimed at helping interpersonally sensitive adolescents to manage stressful experiences may have implications for their cardiovascular health later in life.
Table 4.1. Principal Component Factor Analysis.

<table>
<thead>
<tr>
<th>Personality Construct</th>
<th>Factor loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introversion</td>
<td>.69</td>
</tr>
<tr>
<td>SSS Family</td>
<td>.63</td>
</tr>
<tr>
<td>SSS School Respect</td>
<td>.72</td>
</tr>
<tr>
<td>SSS School Popularity</td>
<td>.83</td>
</tr>
<tr>
<td>Submissive Behavior</td>
<td>.65</td>
</tr>
</tbody>
</table>

SSS=Subjective social status
### Table 4.2. Demographic and Health-Related Characteristics of Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ($M \pm SD$)</td>
<td>17.0 ± 1.37</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>49.3</td>
</tr>
<tr>
<td>East or South Asian</td>
<td>42.3</td>
</tr>
<tr>
<td>Other</td>
<td>8.4</td>
</tr>
<tr>
<td>Parents’ education (%)a</td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>14.8</td>
</tr>
<tr>
<td>Some college</td>
<td>10.6</td>
</tr>
<tr>
<td>College graduate</td>
<td>74.6</td>
</tr>
<tr>
<td>Parents married (%)</td>
<td>78</td>
</tr>
<tr>
<td>Risk for depression (%)</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>10.4</td>
</tr>
<tr>
<td>Cognitive vulnerability</td>
<td>63.9</td>
</tr>
<tr>
<td>Both</td>
<td>18.8</td>
</tr>
<tr>
<td>Interpersonal sensitivityb</td>
<td></td>
</tr>
<tr>
<td>Introversion-extraversion</td>
<td>26.8 ± 5.7</td>
</tr>
<tr>
<td>Submissive behavior</td>
<td>22.6 ± 8.2</td>
</tr>
<tr>
<td>SSS family</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>SSS school respect</td>
<td>6.5 ± 1.0</td>
</tr>
<tr>
<td>SSS school popularity</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>Acute events</td>
<td></td>
</tr>
<tr>
<td>Major life eventc (%)</td>
<td>24.2</td>
</tr>
<tr>
<td>Humiliationd (%)</td>
<td>10.4</td>
</tr>
<tr>
<td>Danger (%)</td>
<td>13.9</td>
</tr>
<tr>
<td>Loss (%)</td>
<td>20.1</td>
</tr>
<tr>
<td>Serum CRP (mg/L)</td>
<td>0.68 ± 1.18</td>
</tr>
<tr>
<td>Serum IL6 (pg/mL)</td>
<td>0.68 ± 0.68</td>
</tr>
<tr>
<td>Stimulated IL6 (pg/mL)</td>
<td>43532.9 ± 14861.7</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>104.4 ± 8.7</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>66.3 ± 8.0</td>
</tr>
<tr>
<td>WHR</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>Physical activity (min/week)</td>
<td>156.84 ± 209.21</td>
</tr>
<tr>
<td>Cigarette smoking (%)f</td>
<td>0.7</td>
</tr>
<tr>
<td>Oral contraceptive use (%)</td>
<td>18.8</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation at baseline. SSS=subjective social status.

a Parents’ education is the highest of mother and father

b Scale totals ranged from 8-40 for introversion-extraversion, 0-64 for submissive behavior, and 1-9 for each of the SSS scales.
\(^c\) Indicates percentage of sample with a major life event (an event with an impact rating of 2.5 or higher),
\(^d\) Indicates percentage of sample with significant loss, danger, or humiliation component (an event with a rating of 2.5 or higher on the emotional dimension)
\(^e\) This percentage increased to 6.1 over the course of the study
Table 4.3. Bivariate Correlations Among Interpersonal Sensitivity and Baseline Measures of Acute Stress and Covariates.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Ethnicity</th>
<th>Interpersonal Sensitivity</th>
<th>Loss</th>
<th>Danger</th>
<th>Humiliation</th>
<th>WHR</th>
<th>Smoking</th>
<th>Birth Control</th>
<th>Physical Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.00</td>
<td>.34**</td>
<td>.15</td>
<td>.005</td>
<td>.048</td>
<td>-.063</td>
<td>-.11</td>
<td>.001</td>
<td>.057</td>
<td>-.22</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>1.00</td>
<td>.33**</td>
<td>.010</td>
<td>-.046</td>
<td>-.027</td>
<td>.092</td>
<td>-.086</td>
<td>-.20*</td>
<td>-.19*</td>
<td></td>
</tr>
<tr>
<td>Interpersonal Sensitivity</td>
<td>1.00</td>
<td>-.017</td>
<td>-.14</td>
<td>.066</td>
<td>.071</td>
<td>.00</td>
<td>-.066</td>
<td>-.25**</td>
<td>-.25**</td>
<td></td>
</tr>
<tr>
<td>Loss</td>
<td>1.00</td>
<td>.45**</td>
<td>.62**</td>
<td>-.042</td>
<td>-.042</td>
<td>-.019</td>
<td>-.028</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Danger</td>
<td>1.00</td>
<td>.26**</td>
<td>-.003</td>
<td>-.033</td>
<td>.013</td>
<td>-.067</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humiliation</td>
<td>1.00</td>
<td>.064</td>
<td>-.029</td>
<td>.011</td>
<td>.041</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>1.00</td>
<td>-.11</td>
<td>-.14</td>
<td>.060</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>1.00</td>
<td>.17*</td>
<td>.056</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth Control</td>
<td>1.00</td>
<td>.080</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Activity</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. WHR=waist-to-hip ratio.

*Ethnicity is a dichotomous variable, coded as 1 for Caucasian and 2 for non-Caucasian.
Table 4.4. Hierarchical Linear Models Testing the Effects of Loss, Danger, and Humiliation and their Interaction with Interpersonal Sensitivity in the Prediction of Inflammatory Markers

<table>
<thead>
<tr>
<th></th>
<th>Serum CRP</th>
<th></th>
<th></th>
<th>Serum IL6</th>
<th></th>
<th></th>
<th>Stimulated IL6</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>p</td>
<td>B</td>
<td>SE</td>
<td>p</td>
<td>B</td>
<td>SE</td>
</tr>
<tr>
<td>Acute Stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Danger</td>
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</tr>
<tr>
<td>Main effect</td>
<td>-0.010</td>
<td>0.018</td>
<td>0.57</td>
<td>0.0074</td>
<td>0.018</td>
<td>0.69</td>
<td>3324.79</td>
<td>2063.69</td>
</tr>
<tr>
<td>Interaction</td>
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<td>0.022</td>
<td>0.044</td>
<td>0.0079</td>
<td>0.021</td>
<td>0.71</td>
<td>2986.76</td>
<td>2511.24</td>
</tr>
<tr>
<td>Loss</td>
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<tr>
<td>Main effect</td>
<td>-0.013</td>
<td>0.017</td>
<td>0.45</td>
<td>0.0044</td>
<td>0.013</td>
<td>0.74</td>
<td>1516.05</td>
<td>1323.81</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.0046</td>
<td>0.020</td>
<td>0.82</td>
<td>-0.0045</td>
<td>0.014</td>
<td>0.75</td>
<td>4230.58</td>
<td>1582.13</td>
</tr>
<tr>
<td>Humiliation</td>
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<td></td>
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</tr>
<tr>
<td>Main effect</td>
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<td>0.021</td>
<td>0.21</td>
<td>0.011</td>
<td>0.018</td>
<td>0.56</td>
<td>3099.60</td>
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<td>0.030</td>
<td>0.36</td>
<td>0.0033</td>
<td>0.019</td>
<td>0.87</td>
<td>1720.74</td>
<td>1570.03</td>
</tr>
</tbody>
</table>

Note. Analyses testing the main effect of acute stress components control for time since baseline, WHR, cigarette smoking, physical activity, and oral contraceptive use at level 1. Analyses testing the acute stress by interpersonal sensitivity interaction control for all of the level 1 covariates, as well as age and ethnicity at level 2.
**Table 4.5. Hierarchical Linear Models Testing the Effects of Loss, Danger, and Humiliation, and their Interaction with Interpersonal Sensitivity in the Prediction of BP**

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
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<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>p</td>
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<td>Danger</td>
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<td>0.86</td>
<td>0.74</td>
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<td>Interaction</td>
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<td>0.49</td>
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<td>0.007</td>
<td>-0.68</td>
<td>0.86</td>
<td>0.43</td>
</tr>
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</table>

*Note.* Analyses testing the main effect of acute stress control for time since baseline, WHR, cigarette smoking, physical activity, and oral contraceptive use at level 1. Analyses testing the acute stress by interpersonal sensitivity interaction control for all of the level 1 covariates, as well as age and ethnicity at level 2.
Figure 4.1

*Interpersonal sensitivity interacts with danger in the prediction of C-reactive protein and diastolic blood pressure.*

A

![Graph A: Serum CRP (mg/L log transformed)]

B

![Graph B: Diastolic blood pressure (mm Hg)]

Predicted values of serum C-reactive protein and diastolic blood pressure at the 25th and 75th percentiles of interpersonal sensitivity for adolescents who had and had not experienced a significant danger event.
Figure 4.2

*Interpersonal sensitivity interacts with loss in the prediction of interleukin 6 production.*

Predicted values of interleukin 6 production at the 25th and 75th percentiles of interpersonal sensitivity for adolescents who had and had not experienced a significant loss event.
Figure 4.3

*Interpersonal sensitivity interacts with humiliation in the prediction of systolic blood pressure.*

![Graph showing predicted systolic blood pressure values at 25th and 75th percentiles of interpersonal sensitivity for adolescents with and without a significant humiliation event.]

Predicted values of systolic blood pressure at the 25th and 75th percentiles of interpersonal sensitivity for adolescents who had and had not experienced a significant humiliation event.
References


Chapter 5: General Discussion

Stressful life events are associated with morbidity and mortality across a range of medical conditions (Cohen, Janicki-Deverts, & Miller, 2007; Miller, Chen, & Cole, 2009). As a result, there has been a great deal of interest in the biological pathways linking stressful experiences to biological alterations that set the stage for disease. The emphasis of this work has been on 2 systems that are activated by stress, the HPA axis and the SNS. Prolonged or repeated exposure to stress can lead to dysregulation in these systems, which fosters pathogenic processes like high BP, insulin resistance, and low-grade chronic inflammation (McEwen & Stellar, 1993). These pathogenic changes have the potential for influencing health outcomes in children and adolescents. Among healthy youngsters, these changes foster the earliest subclinical manifestations of CVD (Matthews, 2005), and among youth with asthma, they have consequences for airway inflammation and symptom expression (Chen & Miller, 2007).

However, little is known about the types of stressors that are most detrimental to young people, as well as dispositional factors that make some youth more vulnerable to stressful experiences than others. Thus, the current dissertation examined the effects of stressful life experiences on biological stress mediators in healthy adolescent women and in children and adolescents who had been diagnosed with asthma. There were 2 main objectives. The first was to examine the differential impact of various features of life stress, with a focus on the duration of the stressor and its emotion-eliciting properties. The second was to examine whether dispositional interpersonal sensitivity shapes the magnitude of the biological response to various kinds of real world stressors.
**Chronic Stress Versus Acute Life Events**

The first study hypothesis was that the association between life stress and disease-relevant biological processes would depend on the duration of the stressor. In particular, the biological impact of stressful experiences was expected to be stronger for chronic stressors compared to acute stressors. This is because chronic stressors are thought to bring about long-term biological changes, whereas the effects of acute stressors are thought to be relatively short-lived (Segerstrom & Miller, 2004). However, study results were inconsistent with this hypothesis. Specifically, neither major life events (which are acute in nature) nor chronic difficulties were consistently associated with biological outcomes in Studies 1 and 2. In the first study, there were no associations of major life events or chronic interpersonal stress with cortisol output (both in the morning and throughout the day), the expression of GR mRNA, insulin, or glucose. The only exception to this pattern was that adolescents who had experienced a major life event showed consistently lower concentrations of CRP. In the second study, there were no associations between major life events or chronic family difficulties and asthma-related immune processes or day to day symptoms of disease among children and adolescents with asthma.

These findings suggest that the sole presence of a major life event is not enough to activate biological systems like the HPA axis. Although studies of acute social stressors in the lab are associated with robust cortisol responses and increased heart rate and sympathetic activation (Dickerson & Kemeny, 2004; Bosch et al., 2009), relatively few studies have examined the impact of acute life events in the lives of children and adolescents. Among those that have, the findings have been quite weak. For example, recent studies have reported non-significant relations between major life events and biological processes in healthy adolescents and youth who had been diagnosed with asthma (Brady & Matthews, 2006; Miller & Chen, 2006). Thus, it
may be that the biological response to major life events is relatively short-lived, and therefore
difficult to capture in an observational study. Moreover, when studies examine the effects of life
events that may have taken place months before the biological assessment, as is the case in this
set of studies, the participants have already had time to adapt to the stressor. In order do a
rigorous test of the main effects of major life events, it will be necessary to measure the
biological response within days or weeks of the exposure. However, this is logistically difficult
because there’s no way of predicting when major life events will occur. To keep track of event
occurrences, the research team must keep regular contact with participants, which can be
burdensome for both parties. Moreover, stress interviews and biological assessments need to
take place, all of which may be difficult to coordinate shortly after an event.

The null findings for chronic interpersonal stress among healthy adolescents and chronic
family stress among asthma patients are inconsistent with previous research. For example,
studies have shown that exposure to ongoing difficulties in the family or among peers are
associated with a range of negative outcomes in young people, such as elevated resting BP,
higher cortisol responses to acute laboratory stressors, and increased symptom expression in
asthma (Chen, Chim, Strunk, & Miller, 2007; Clark & Armstead, 2000; Luecken & Appelhans,
2006; Matthews, Salomon, Kenyon, & Zhou, 2005). Thus, it’s surprising that no effects of
chronic stress emerged in Studies 1 and 2. One explanation is the aforementioned studies
focused on specific interpersonal stressors, such as low perceived support from parents,
mistreatment in the peer group, and the combination of early parental loss and a poor relationship
with the surviving parent. Thus, it may be important to hone in on particular aspects of the social
environment to better understand how chronic difficulties impact biological processes. Another
explanation is that children and adolescents can adjust to stable levels of chronic stress, and that
it’s important to assess trajectories of chronic stress over time. For example, Low and colleagues (2009) found that increasing trajectories of life stress were associated with increased cardiovascular reactivity over time, whereas persistent trajectories were unrelated to reactivity parameters. Moreover, increasing trajectories of DBP reactivity over 3.3 years were associated with greater intima media thickness (a measure of the degree of atherosclerosis in the carotid artery). Thus, future work should look at trajectories of life stress and account for its cumulative impact. This may be especially relevant for pathogenic changes, like metabolic dysfunction and chronic low-grade inflammation, which may take time to emerge in healthy young people.

Limitations and future directions. The study findings may have been influenced by methodological issues. First, the study designs did not allow for a rigorous test of major life events on biological change. In particular, there was a time lag of up to 6 months between event occurrence and the next study visit, thus it’s possible that participants had already recovered from the event by the time of biological assessment. One possibility for future work would be to examine the effects of acute stress by focusing on specific naturalistic events, such as school examinations or emergency situations among medical personnel. Given that participants could be enrolled in the study prior to the stressful event, it would be feasible to measure biology within days of the exposure and track subsequent changes thereafter. It’s unclear why there were no significant associations between chronic difficulties and biological mediators, although it may be related to the modest stress levels reported in these samples. On average, these children and adolescents reported fairly good social relationships, and this may explain why biological processes were not influenced by the degree of chronic stress in the current studies but have been in previous work (Chen et al., 2007; Evans, Kim, Ting, Tesher, & Shannis, 2007; Luecken & Appelhans, 2006). Moreover, recent findings point to the importance of examining trajectories
of stress exposures over time in order to gauge whether stressful situations are worsening, staying to same, or improving. These more dynamic stress indices may be stronger predictors of pathogenic processes, such as chronic low-grade inflammation and metabolic dysfunction, which may only emerge in the most challenging social environments. Another potential reason is that the current studies measured chronic stress in somewhat isolated domains (among family and friends in Study 1 and among family in Study 2). It may be that the cumulation of chronic stress across work, social life, finances, and interpersonal relationships matters most. Finally children and adolescents may be generally more resilient, showing lower-magnitude biological responses and faster recovery. Indeed, most of what we know about the biology of chronic stress is from adults and older people (e.g., Kiecolt-Glaser, Glaser, Gravenstein, Malarkey, & Sheridan, 1996; Rohleder, Marin, Ma, & Miller, 2009).

*The Combination of Chronic Stress and Acute Life Events*

The second study hypothesis was that the combination of acute and chronic stress would emerge as a robust predictor of biological outcomes. In particular, it was expected that chronic interpersonal stress would prolong the effects of major life events. Studies 1 and 2 provided strong evidence in support of this hypothesis. Specifically, the simultaneous exposure to major life events and chronic interpersonal stress was associated with elevated morning cortisol response, elevated cortisol throughout the day, and the downregulation of GR mRNA expression among healthy adolescents. Moreover, this effect was replicated in children and adolescents with asthma; children who were double-exposed to a major life event and chronic family stress showed asthma-related immune changes (i.e., increased production of IL-4, IL-5, and IL-13) and increased symptom expression.
What are the potential psychosocial pathways underlying the double-exposure effect? One possibility is that while people can manage one key life stressor, they do not have the resources to deal with multiple stressors effectively. For example, social support is an important resource during times of stress. It has been well documented that individuals with high levels of perceived social support come out of difficult situations looking healthier and happier (stress-buffering model; Cohen & Wills, 1985). It may be that individuals with both chronic and acute difficulties overwhelm their social networks, rendering them vulnerable to the effects of stress. Erosion of family support could be especially detrimental for young people, who often rely on their parents and siblings for assistance with key life events. Another possibility is that double-exposed individuals become emotionally and cognitively taxed, making it difficult to engage in self-regulatory behaviors, including emotion regulation (Vohs & Heatherton, 2000). As a consequence, these individuals may experience increased negative affect and decreased positive affect, both of which have been linked to increased activation of biological stress systems (Shapiro, Jamner, Goldstein, & Delfino, 2001; Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005). Finally, having to juggle multiple stressors also seems likely to interfere with people’s ability to engage in restorative behaviors like spending time unwinding and sharing a meal with friends. Recent evidence indicates that individuals who engage in more frequent leisure activities show lower BP, lower daily cortisol output, and increased perceptions of better physical function (Pressman et al., 2009). Moreover, engaging in these activities plays a role in buffering the negative psychological impact of stress. Thus, for children and adolescents, leisure activities may protect against the detrimental influence of problems at home and school.

In regard to biological pathways, these findings are consistent with a glucocorticoid resistance model, in which psychological stress fosters a pro-inflammatory environment by
causing dysregulation at the level of hormones and the receptors that bind them (Miller, Cohen, & Ritchey, 2002; Miller & Chen, 2006). In particular, Study 1 provides evidence that the combination of a major life event and chronic interpersonal stress activates the HPA axis, which leads to a compensatory down-regulation of GR mRNA expression. This process is thought to decrease sensitivity to the anti-inflammatory effects of cortisol, thereby interfering with the body’s ability to control inflammation. Study 2 provides an indirect suggestion of this. In particular, Study 2 showed increased production of asthma-relevant cytokines among double-exposed youth. Moreover, increases in cytokines were associated with within-person increases in symptom expression.

Thus, Studies 1 and 2 shed light on different parts of a pathway from stress to altered inflammatory activity, and it will be important for future studies to put the pieces together. For example, the results of Study 2 provides compelling evidence for a link between stress exposures, immune changes, and symptom expression in asthma. However, future work should examine the mediating roles or HPA and SNS dynamics. For instance, a prospective study could test the causal relations between stress exposures, hormone dynamics (include cortisol, epinephrine and the receptors that bind them), and asthma-related immune markers. Moreover, similar pathways could be tested in other medical populations, including patients with rheumatoid arthritis and multiple sclerosis.

In healthy adolescents, the link between hormone dynamics and inflammation is less clear. In particular, the results of Study 1 indicate decreased concentrations of CRP among adolescents exposed to both acute and chronic stress, a pattern that was inconsistent with study hypotheses. It may be that the HPA responses to these types of stressors do not persist long enough to affect basal inflammatory activity, or that in otherwise healthy people, cortisol has its normal anti-
inflammatory effects. Thus, glucocorticoid resistance following stress may only happen in youth with diseases like asthma because the endocrine-immune interface is already disrupted by the disease and treatment (Chen & Miller, 2007). Clearly, more work is needed to delineate the impact of stress and stress hormones on inflammatory processes in young people.

In addition to the double-exposure effect, the results of Studies 1 and 2 revealed an interesting pattern of findings among youth with low levels of chronic difficulties. Specifically, exposure to a major life event in the absence of chronic stress actually appeared to be beneficial. Study 1 showed the when adolescents low in chronic interpersonal stress experienced a major life event, they had lower cortisol output and up-regulated GR mRNA expression. Moreover, in Study 2, youth with low levels of chronic family stress showed reductions in asthma-relevant inflammatory products (stimulated production of IL-4 and IL-5) at times when they had been exposed to a major event compared to times when they had not.

These findings are consistent with previous studies showing that children and adolescents in supportive environments can develop physiological resilience in response to stressful experiences. For example, Boyce and colleagues (1995) demonstrated resilience among children in low-stress social environments. In particular, they showed that the combination of low environmental stress and heightened biological reactivity to acute laboratory stressors was associated with decreased rates of respiratory illnesses over 6 months. Thus, a supportive social context fosters resilience among biologically reactive children. A similar pattern of findings has emerged in studies of youth with a history of medical adversity. One study showed that young adults with a history of medical adversity and high perceived emotional closeness with others were better able to habituate to a repeated laboratory stressor compared to young adults with either low perceived emotional closeness or no history of medical adversity (Bugental, Beaulieu,
Fowler, O’Brien, & Cayan, 2010). These findings indicate that feelings of emotional closeness and security are especially beneficial to individuals who have gone through significant life challenges. Not only are these “attachment feelings” beneficial in offsetting the stress, they also help the person grow from it, and turn out looking better than those who didn’t have the experience in the first place. Finally, recent evidence from primate studies indicates that mild early life stress is associated with stress resistance later on (Lyons, Parker, Katz, & Schatzberg, 2009). In particular, monkeys exposed to early life social disruption (i.e., brief intermittent separations from the natal group) show decreased anxiety, increased exploration of novel situations, and decreased cortisol responses to stress compared to monkeys raised in undisturbed social groups. Thus, the early life environment may program arousal regulation, such that individuals who are exposed to manageable stress early on are better able to adapt to subsequent stressors. Although the studies in this dissertation did not account for early life factors, the findings may reflect stress inoculation among children and adolescents in nurturing family environments.

Limitations. Studies 1 and 2 revealed strong evidence in support of the hypothesis that the combination of acute and chronic stress would influence biological processes. However, it’s possible that an unmeasured third variable provides a better explanation for the effects observed in these studies. This may be especially problematic in the first study, which used a cross-sectional design. For example, if hostile or neurotic young women are prone to experiencing (or simply reporting) major life events and chronic interpersonal stressors, their biological outcomes might be better explained by personality features than life events. That said, objective contextual interviews were used to minimize the influence of self-report biases. Moreover, the issue of potential 3rd variables is minimized in the asthma study because effects were observed at the
within-person level of analysis. Thus, most alternative explanations for the association between stress and biological outcomes (i.e., between-person factors like personality and genetics) can be ruled out. Second, there may be other important moderators that we did not examine. For instance, pubertal status (in Studies 1 and 2) and gender (in Study 2) may influence the associations between stress exposures and biological outcomes. For instance, there is some evidence to suggest that the biological response to stress may be amplified among children who have already reached puberty, compared to children who have not (Stroud et al., 2009; Romeo, 2010). Finally, given that the Study 1 findings emerged in a sample of adolescents who were recruited to be at high risk for an initial episode of depression, there was some concern that they would not generalize to other samples. However, the replication of those findings in Study 2 shows that they generalize to at least one other independent sample with very different medical issues.

**Future directions.** Future work should explain why double-exposure to acute and chronic stress is so difficult for children and adolescents. As mentioned above, it will be important to consider both psychosocial and biological pathways. Second, it will be important to clarify which aspects of the social environment have the greatest impact on children versus adolescents. For example, it may be that family stressors are most detrimental in childhood, while stressors in the peer environment are more salient in the teenage years. Third, results from the current studies could be applied to interventions targeting youth. For example, a family-level intervention may provide skills to parents, so they are better equipped to support their children through difficult life situations. Moreover, much can be learned from children and adolescents who show patterns of stress resilience. By identifying what’s working for these children and adolescents in both their personal and interpersonal lives (including coping skills, familial
relationships, and the presence of supportive peers), it may be possible to develop more effective interventions for those who are at higher risk.

*Life Event Dimensions of Loss, Danger, and Humiliation*

The third study hypothesis was that life event dimensions of loss, danger, and humiliation would shape the nature of the biological response. This hypothesis stemmed from models that predict biological specificity in the stress response (Weiner, 1992; Kemeny, 2003). The idea here is that specific signals in the environment elicit a distinct pattern of hormonal and neural changes that help the organism to most effectively deal with the specific nature of the threat. Thus, the third paper in this dissertation examined the differential effects of loss, danger, and humiliation on biological outcomes associated with early cardiovascular risk, including BP and markers of inflammatory activity. Consistent with predictions made by the social self-preservation theory, it was expected that life events with a major humiliation component (e.g., peer rejection, being cheated on by a romantic partner) would be accompanied by increased pro-inflammatory activity. Although previous research on loss and danger events is relatively scarce, it was expected that danger events would be accompanied by increases in BP, given that these events can evoke the “fight or flight” reaction.

In general, the data were inconsistent with these hypotheses. The only significant main effect of an emotional theme on biological outcomes, was the association between humiliation and proinflammatory activity. In particular, adolescent girls showed increased production of IL-6 (a proinflammatory cytokine) at times when they had been exposed to a humiliation event compared to times when they had not. According to the social self-preservation theory, threats to the social self activate proinflammatory pathways, which induce behavior consistent with a disengaged motivational state (Dickerson, Gruenewald, & Kemeny, 2004; Dickerson & Kemeny,
Thus, the observed changes in stimulated IL-6 following humiliation may represent an adaptive response to overt threats to belongingness. Over the long-term, this tendency could favor the development of a pro-inflammatory environment in the body that sets the stage for chronic diseases like atherosclerosis. However, humiliation was unrelated to systemic inflammatory markers CRP and IL-6, suggesting that such an environment had not yet developed, and there was no significant effect of humiliation on BP. Thus, it may only be after repeated exposures to humiliation that these pathogenic changes emerge in young healthy participants, or that the changes are transitory and not of relevance to disease pathogenesis.

It is unclear why there were no significant effects for loss and danger. In general, major events with high emotional ratings were relatively infrequent. For example, only 10 percent of the sample experienced a major humiliation event at baseline. Thus, the study may have lacked the statistical power to detect effects, let alone differentiate the biological responses of loss, humiliation, and danger. Indeed, previous studies that have tested specificity models have done so in the controlled environment of the laboratory. For instance, Tomaka and colleagues (1993) demonstrated differential effects of threat versus challenge appraisals by looking at patterns of arousal using multiple measures (e.g., cardiac output, total peripheral resistance, heart rate, and pre-ejection period), rather than by focusing on which participants were generally the most reactive. Moreover, most of the evidence for the social self-preservation theory comes from laboratory studies that measure cortisol and immune responses to situations that do and do not involve social-evaluative threat (Dickerson & Kemeny, 2004; Dickerson, Gable, Irwin, Aziz, & Kemeny, 2009). Thus, it may necessary to design observational studies geared specifically toward answering this question. As in the current studies, future work should use in-depth interviews to capture the nuances of stressful experiences, including their emotion-eliciting
properties. In addition, a comprehensive set of biological endpoints should be assessed within days or weeks of the stressful event. Moreover, a high degree of statistical power will be needed to draw any definitive conclusions about biological response specificity. One way to increase statistical power would be to recruit participants who are likely to experience major life events more frequently than they did in the current study, a point which is discussed in more detail below.

**Limitations.** As noted above, Study 3 did not provide a rigorous test of the specificity hypothesis. First, given the significant overlap between loss, danger, and humiliation, it was difficult to disentangle their unique effects. In particular, the intercorrelations between the emotion ratings ranged from approximately .3 to .6, with the greatest overlap between loss and humiliation. Thus, in the future, it may be helpful to differentiate between pure loss and complicated loss (which also has an element of humiliation). An example of a pure loss event would be a friend moving to another city or the death of a close relative, whereas complicated loss event has an element of humiliation, like being cheated on by a romantic partner or being fired from a job. It may be possible to design a study that focuses on the dissolution of romantic relationships. Specifically, the biological impact of participant-initiated break-ups (pure loss) could be compared to that of other-initiated break-ups (complicated loss), although detailed information about each participant’s specific situation would be needed to determine if the event actually falls into the expected category. Second, Study 3 did not include HPA indices, such as cortisol output and the expression of GR mRNA, because these data were not available across the 6 time-points. However, the study would have benefited from these measures because the HPA axis may be especially sensitive to loss and humiliation. For example, the social self-preservation theory predicts HPA activation following acute threats to the social self.
(Gruenewald, Kemeny, Aziz, & Fahey, 2004), which are thought to elicit the same kinds of emotional responses as humiliation events. Moreover, there is mounting evidence that childhood loss experiences are associated with altered HPA dynamics (Luecken, 1998; Luecken, 2000; Luecken & Appelhans, 2006; Hagan, Luecken, Sandler, & Tein, 2010). Thus, future work on response specificity in the real world should incorporate measures of HPA dynamics. The third issue relates to the time-lag between event occurrence and biological assessment, which also arose in Studies 1 and 2. In particular, there is a potential lag of up to 6 months between events with significant loss, danger, and/or humiliation and the biological assessment. Thus, exposed adolescents may have reacted to the events and recovered (both emotionally and biologically) before biological measures were taken. Future work should measure biological outcomes within weeks of the exposure.

**Future directions.** Future studies should recruit large samples of children and adolescents who are likely to experience increased stress levels (e.g., youth from low income neighborhoods). This will increase the frequency of life events that occur across the study. Moreover, studies could focus on particular types of life events. For example, a focus on young people who are apart of stigmatized groups (e.g., racial minorities, individuals with physical disabilities) may increase the frequency of events involving emotions like humiliation and shame.

**The Moderating Effects of Dispositional Interpersonal Sensitivity**

The fourth and final study hypothesis was that the association between major life events and biological stress mediators would be moderated by dispositional interpersonal sensitivity. Moreover, it was expected that there would be specificity in terms of the types of events that would be most salient to interpersonally sensitive individuals. In particular, interpersonally
sensitive individuals are sensitive to rejection, disapproval, and/or loss of status, thus it was hypothesized that interpersonal sensitivity would amplify the effects of major humiliation and/or major loss. However, given that danger events have an element of uncertainty that may also be threatening to interpersonally sensitive individuals, a secondary hypothesis was that interpersonal sensitivity would amplify the effects of danger events. Indeed, the findings that emerged in Study 3 were most consistent with this second hypothesis. Results indicated that interpersonal sensitivity moderated the association between danger events and both DBP and CRP, such that individuals high on interpersonal sensitivity showed elevated DBP and increased concentrations of CRP at times when they had been exposed to danger compared to times when they had not. This suggests that, for interpersonally sensitive adolescents, it’s the increased likelihood of future social threat, or the uncertainty surrounding whether it will materialize, that relates to changes in biological processes involved in CVD risk. Moreover, interpersonally sensitive individuals’ heightened vigilance to the social environment in the aftermath of danger may be an important mediator of this association.

In addition to the danger findings, study results indicated that interpersonal sensitivity can accentuate the biological response to themes of loss, although the evidence was weaker than expected. In particular, results indicated that the association between loss events and IL-6 production was moderated by interpersonal sensitivity, such that adolescents high on interpersonal sensitivity showed increased IL-6 production in the aftermath of major loss, whereas adolescents low on this dimension did not. Although it’s unclear why this effect emerged for loss events and not humiliation events, it may be related to the fact that significant loss was almost twice as likely to occur as significant humiliation.
This pattern of findings is somewhat inconsistent with current thinking about dispositional interpersonal sensitivity and the types of events that would be most threatening to interpersonally sensitive individuals (Marin & Miller, under review). One explanation relates to the types of events that come up in adolescent life. In particular, the bulk of events reported in Study 3 were interpersonal in nature, thus the emotion-eliciting themes of loss, humiliation, and danger may not differentiate between events that are likely to bring about perceptions of negative social evaluation versus those that are not. However, these themes do differentiate between events that have already occurred (loss and humiliation) and those that signal the potential for future threat (danger). As mentioned above, interpersonally sensitive individuals may have a difficult time dealing with the possibility of future social threat.

These findings provide insight into the relationship between interpersonal sensitivity and increased medical risk. In particular, a recent review of the literature showed that constructs related to interpersonal sensitivity (e.g., social inhibition, introversion, rejection sensitivity) are associated with increased risk of morbidity and mortality (Marin & Miller, under review). Thus, interpersonally sensitive persons’ exaggerated biological responses to danger and loss events provides a plausible pathway by which this interpersonal style exerts its effects. Although the magnitude of the biological changes indicated in this study are unlikely to have immediate medical consequences, repeated exposures may take an allostatic toll on the cardiovascular system over time. Moreover, the current work suggests that vigilance in the aftermath of danger may be an important aspect of the interpersonally sensitive disposition.

Limitations. There are a number of study limitations that may have influenced that pattern of results that emerged. First, it’s possible that an unmeasured third variable provides an alternative explanation for the interaction between life event dimensions and interpersonal
sensitivity. For example, hostility is related to aspects of dispositional interpersonal sensitivity and biological outcomes associated with preclinical CVD (Kiesler, 1991; Kiesler, 1996; Smith, Glazer, Ruiz, & Gallo, 2004). Thus, future work should differentiate the effects of these personality variables. Second, the study would have benefited from measures of HPA activity, a point which has already been made above. Specifically, interpersonally sensitive individuals are sensitive to social rejection and cues of disapproval, and it’s these types of situations (i.e., social evaluative threat) that elicit robust cortisol responses in the lab (Dickerson & Kemeny, 2004). Therefore, it seems important to examine whether interpersonal sensitivity prolongs cortisol responses to social threat in the real world. Finally, the study design may have influenced which emotional themes were most strongly associated with biological outcomes. Danger events involve the potential for future threat, thus their effects may be more prolonged than those of loss and humiliation. In particular, the study visits were spaced 6 months apart and life events could occur at any point in between them, thus the biological response may have been stronger in the aftermath of danger, compared to loss and humiliation. This is because danger events were often still unfolding during study visits, whereas other types of events had been resolved for weeks or months. That said, the Study 3 findings may be revealing something that is of major theoretical importance - that the danger events are the ones that are really going to stay in the body for a while and drive the processes related to disease. In contrast, people may recover from loss and humiliation more rapidly, making these events less toxic.

Future directions. The current findings indicate that danger events elicit increases in resting DBP and CRP among adolescents high on interpersonal sensitivity, and that these resting levels return to normal by the subsequent visit (if no other exposures have occurred). However, to do a more rigorous examination of the role of interpersonal sensitivity in the biological stress
response, it will be necessary to measure biological activity more frequently in the aftermath of an event. This is important because few studies have examined biological trajectories of reactivity and recovery in the context of real-world stressors. For example, most studies examine BP reactivity in response to acute laboratory stressors (Kamarck & Lovallo, 2003) or they examine the association between chronic stress and resting BP (e.g., Brady & Matthews, 2006). As a result, little is known about changes in resting BP (or changes in systemic inflammation) following major life events. In addition, the results of Study 3 suggest that fearful vigilance following danger events may prolong the biological stress response among highly sensitive adolescents. Thus, future work should test this hypothesis directly by examining whether vigilance mediates the association between danger and reactivity. Moreover, moderated mediation models could be used to assess differences in this pathway among individuals high and low on interpersonal sensitivity.

Methodological Issues in the Measurement of Stress

The studies in this dissertation used interviewer ratings of stressful events, rather than relying on participants’ reports of their perceptions of stress. The interviewer-based approach was used to minimize the problems associated with self-report measures, including reporting biases like exaggeration and social desirability. This approach also allows the interviewer to rate stressors along the dimensions identified by Lazarus and other appraisal theorists, like controllability and goal threat (Lazarus & Folkman, 1984). Participants may not have access to these appraisals, or they may not consider these dimensions when making their stress ratings. Indeed, studies that have used more objective approaches to the measurement of stress have shown stronger correlations with biological outcomes compared to those that used self-report measures. Specifically, a recent meta-analysis examining the link between stress and parameters
of the immune system showed that brief naturalistic stressors (e.g., exams) and chronic stressors were reliably associated with immune outcomes, whereas subjective reports of stress generally did not associate with immune change (Segerstrom & Miller, 2004). In addition, an earlier meta-analysis examined the role of self-reported negative emotion in the association between acute laboratory stressors and cardiovascular reactivity (Feldman et al., 1999). Results indicated that negative emotion was generally associated with increased cardiovascular responses, yet it accounted for limited variability across tasks. The authors speculate that it may be difficult for participants to detect and report on these subjective states accurately.

However, there are limitations associated with the interviewer-based approach. The major drawback is that it fails to capture an individual’s personal experience of the stressor. In particular, two people may appraise the same event in very different ways, and while perceived stress measures capture these differences, interviewer ratings do not. That said, individual differences in the appraisal process could reflect stable personality factors like neuroticism and negative affectivity. This can complicate the interpretation of study findings because any associations between perceived stress and biological outcomes may be better explained by individual difference variables.

The use of objective rather than subjective ratings may have influenced the pattern of findings across studies. For example, the subjective component of stress may dictate who is likely to show a heightened biological response in the aftermath of an event. Thus, it’s possible that the use subjective ratings would have yielded stronger associations between major life events and biological outcomes. This may be especially relevant to study 3, in which the emotional dimensions of life events were considered. It may be that experiences of loss, danger, and humiliation are highly personal, and that interviewer ratings along these dimensions fail to
capture participants’ experiences. This could explain why the study 3 findings were largely inconsistent with study hypotheses. Future work should measure subjective ratings of loss, danger, and humiliation following major life events, and test whether they are more reliably associated with biological outcomes.

Strengths of the Thesis

There are several noteworthy strengths of this set of studies. First, studies in this dissertation used in-depth interviews to measure life stress. The goal of the interviewer-based ratings is to capture how the average person under similar biographical circumstances would respond to the event. This process is meant to eliminate the influence of reporting biases (e.g., social desirability and exaggeration), which can influence study findings and complicate their interpretation. In particular, interviewer based ratings eliminate the chance that it is idiosyncracies in people’s appraisals and reporting, rather than the events themselves, that are contributing to the outcome. An additional benefit of this interview is that it captures the nuances of stressful experiences. Thus, the current studies were able to examine various features of stressful situations (like their duration and emotion-eliciting properties), as well as their associations with biological outcomes. In contrast, the usual self-report measures of perceived stress and life event checklists do not have these advantages. Second, 2 of the 3 studies in this dissertation used multi-wave prospective designs. Thus, relations between stress and biological processes were tested at the within-person level of analysis. This analytic strategy precludes most of the alternative explanations for associations between stress and biological outcomes (e.g., between-subjects factors like personality and lifestyle factors). Therefore, a compelling case can be made for temporal associations between variables. This is especially important in human stress research because it is not feasible (or ethical) to manipulate long-term stress. Thus,
repeated measures designs and longitudinal designs provide the best tests of causal models. Third, these studies took research questions that had only been addressed in the laboratory and examined them in the context of real life stressful experiences. For example, the majority of the work on biological specificity has come from laboratory studies, thus Study 3 represents a first step in applying these ideas to stressful experiences that really matter to children and adolescents. Finally, these studies used biological outcome data, which provide insights into stress-induced changes across multiple biological systems. Even more, the biological changes observed can have meaningful implications for children and adolescents’ health, including increased symptom expression in asthma and pathogenic changes that set the stage for CVD.

Contributions to the Research Field

These studies make several important contributions to the existing literature. First, the results of these studies point to the importance of accounting for specific features of stressful experiences to delineate the pathways from stressful experiences to biological processes. The most consistent finding across studies was that the combination of acute life events and chronic interpersonal stress (whether in the family or the larger social network) is associated with biological alterations, including elevated cortisol output, decreased expression of GR mRNA, heightened production of asthma-relevant cytokines production, and greater symptom expression in asthma. Second, the asthma paper replicated and extended the findings of the first study in a medical population. This replication provides compelling evidence that double-exposure to acute and chronic stress is a strong elicitor of biological change, which also has implications for the course and severity of disease. Third, these findings provide insights into biological pathways linking stress to pathogenic changes. For example, the HPA alterations shown in double-exposed adolescents in Study 1 provide a possible explanation for why we see heightened
inflammatory profiles in double-exposed asthma patients in Study 2. Fourth, study findings suggest that a dispositional factor like interpersonal sensitivity can influence the kinds of life events that adolescents find most threatening, as well as the magnitude of the biological response in the aftermath of these events. Taken together, these findings point to the need for increased specificity in models of stress and health. In particular, models should account for features of the stressor (e.g., its duration and emotion-eliciting properties), features of the individual (e.g., dispositional factors), and possible interactions between the two.

In sum, the findings of this dissertation exemplify the complexities of stressor impact. Stressful life events may be associated with negative outcomes in some contexts but positive outcomes in others. Moreover, two people may respond to the same event very differently, such that one person shows prolonged biological activation and the other gets through the event relatively unscathed. If future work can clarify the specifics of who is most vulnerable and under which circumstances, then it may be possible to create psychosocial interventions that enhance the health of young people and set them on positive health trajectories for the years to come.
References


# Certificate of Approval

**Principal Investigator:** Miller, G.  
**Department:** Psychology  
**Number:** B04-0567

**Institution/Where Research Was Conducted:** UBC Campus

**Sponsoring Agencies:**
- Canadian Institutes of Health Research

**Title:** Stress and Health During the Transition to Adulthood

**Approval Date:** Aug 31, 2004  
**Term (in years):** 1  
**Documentation Effective Until:** Aug 20, 2004, Consent forms

**Certification:**

The protocol describing the above-named project has been reviewed by the Committee and the experimental procedures were found to be acceptable on ethical grounds for research involving human subjects.

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*Approval of the Behavioural Research Ethics Board by one of the following:*

Dr. James Frankish, Chair,  
Dr. Cay Holbrook, Associate Chair,  
Dr. Susan Rowley, Associate Chair  
Dr. Anita Hubley, Associate Chair

This Certificate of Approval is valid for the above term provided there is no change in the experimental procedures.
# Certificate of Approval

**Principal Investigator:**
Chen, F.

**Department:**
Psychology

**Number:**
B03-0540

**Institution Where Research Will Be Conducted:**
UBC Campus

**Co-Investigators:**
Cohen, Sheldon; Miller, Gregory; Psychology; Strunt, Robert

**Sponsor:**
National Institutes of Health

**Title:**
Socioeconomic Status, Stress, & Asthma Biological Markers

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