PSYCHOLOGICAL, SOCIAL, AND IMMUNOLOGICAL OUTCOMES FOLLOWING MARITAL SEPARATION

by

Karen Hasselmo

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PSYCHOLOGY

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

2017

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Karen Hasselmo, titled Psychological, Social, and Immunological Outcomes Following Marital Separation, and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

_____ Date: 06/10/2016
David A. Sbarra

_____ Date: 06/10/2016
John J. B. Allen

_____ Date: 06/10/2016
Mary-Frances O'Connor

_____ Date: 06/10/2016
Matthias Mehl

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

_____ Date: 06/10/2016
Dissertation Director: David A. Sbarra

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SIGNED: Karen Hasselmo

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Abstract

Close relationships play an integral role in human health (Coan & Sbarra, 2015). It follows, then, that the dissolution of an important relationship can have a variety of negative health consequences (Amato, 2010; Kitson & Morgan, 1990; Sbarra, Law, & Portley, 2011), and social loss confers vulnerability to a range of morbidities and early mortality. Disrupted marital status is one of the strongest sociodemographic predictors of stress-induced physical illness (Somers, 1979) and marital disruption has long been reported as one of life's most stressful events (Bloom, Asher, & White, 1978). Robust evidence links divorce or separation to poorer health outcomes; however, the exact mechanisms through which relationship dissolution influences our health so profoundly are not yet fully elucidated (Sbarra, Hasselmo, & Bourassa, 2015). The current study investigated how changes in psychological responses to divorce are associated with changes in immune responding in recently-separated adults (N = 55). I followed participants over an average of five months, collecting psychological distress measures at three visits, each one month apart, and immune measures at two visits, five months apart. To assess how variability in social engagement is associated with immunological responses following the end of a marriage, I incorporated naturalistic, observational data using a new methodology. I found that an objectively derived composite of social behaviors including (a) time spent with others; (b) time spent socializing/entertaining; (c) time spent in substantive conversation; and (d) time spent receiving positive support predicted concurrent immune outcomes over and above the effects of psychological distress and/or loneliness, and that psychological distress may exert indirect influence on immune outcomes through social integration. Furthermore, attachment style revealed differential longitudinal associations between social integration and immune outcomes. This research expands current knowledge on the immune-relevant outcomes of divorce and

separation, and includes new methodology for naturalistically-derived measures of social engagement in determining how this common life stressor is associated with health over time.

Introduction

High quality close relationships are associated with increased life satisfaction and physical well-being (Robles et al 2013; Uchino, 2009). The dissolution of an intimate relationship, however, is reliably associated with increased morbidity and mortality across the lifespan (Sbarra, Law, & Portley, 2011; Shor, Roelfs, Bugyi, & Schwartz, 2012). In fact, the impact of having insufficient social relationships on increased risk for mortality is as strong as many recognized public health risk factors, including smoking, lack of exercise, and obesity (Holt-Lunstad, Smith, & Layton, 2010). Attempts to alleviate the effects of this increased risk are stymied by both a lack of research on biologically-plausible mechanisms that link marital separation and health outcomes (Miller, Chen, & Cole, 2009). These issues remain largely unexplored in the literature.

Because immunological responses represent a major pathway through which stressful life events may impact morbidity and mortality (Uchino, Cacioppo, & Kiecolt-Glaser, 1996; Uchino, 2006), the current study evaluated changes in immune system parameters over five months following marital separation. This work was guided by three specific aims to: (1) explore cross-sectional relationships between psychological distress and concurrent immune outcomes, (2) investigate a novel measure of objective social integration and its relationship with concurrent immune outcomes, and (3) study a prospective mediational model linking psychological distress, social integration, and longitudinal immune outcomes.

I begin by reviewing psychoneuroimmunological studies suggesting a robust relationship between changes in the immune system and psychosocial stress, followed by a review of the current literature on marital separation and health, the measurement and importance of social behavior following divorce, and health-relevant immunological responses.

Psychosocial Stress and Biological Responding

The general literature examining the association between life stress and immune functioning is extremely robust. The stress response system involves the coordination of several bodily systems, including the endocrine, autonomic, and immune systems in order to produce an orchestrated response to an environmental threat or challenge (McEwen, 1998). It follows that mounting an inadequate stress response to such a threat could result in infectious disease, which remains one of the top ten causes of death worldwide (WHO, 2014). In order to successfully cope with an environmental stressor, the stress response must be well matched to the threat in intensity, duration, and specificity (Kaye & Lightman, 2007). Following an environmental stressor, exposure to chronic distress may over-activate the immune system as part of the stress response system (Kiecolt-Glaser et al., 1987; Segerstrom & Miller, 2004). Chronic elevation of inflammation levels within the body, a key pathogenic mechanism in many diseases (Libby & Theroux, 2005), links the hyper-activation of the immune system to the increases in morbidity and mortality highlighted above. This can occur through the release of cortisol from the endocrine system and/or through the dysregulation of the proinflammatory response. During the stress response, the endocrine system releases cortisol, a hormone that plays an important role in preparing the body to respond to environmental stressors (Segerstrom & Miller, 2004). While this is adaptive in the short term, if levels of cortisol are persistently elevated it can lead to the development of chronic inflammatory diseases such as arthritis and asthma, among others (Hänsel, Hong, Cámara, & von Känel, 2010).

Another important part of the immune-mediated stress response includes chemical messengers known as cytokines. Although chronic inflammation can be problematic in and of itself, a side effect of hyperactivation of the immune system is desensitization to the negative

feedback of the proinflammatory signal. Similar to cortisol levels, initially the proinflammatory signal, propagated by releasing the cytokine interleukin-6 (IL-6) and C-Reactive Protein (CRP), is adaptive, but if the signal becomes dysregulated, the duration and intensity of inflammation levels within the body remain elevated beyond a healthy level. With prolonged exposure to stress, the body can become desensitized to the anti-inflammatory signal of cytokines, which carry in their signal a negative feedback mechanism to down-regulate the proinflammatory response (Miller, Cohen, & Ritchey, 2002). When the immune system becomes desensitized to this negative feedback signal, the proinflammatory response can run unchecked. Over time, this elevated inflammation results in increased risk for inflammatory diseases including type II diabetes, autoimmune disease, and cardiovascular diseases (Cohen et al., 2012).

An additional indicator of immune system health involves the antibody response to latent viruses. Antibodies are proteins produced following exposure to an antigen, such as a virus (Vedhara & Wang, 2005). Antibodies work by binding to the invading antigen and recruiting other immune cells to help counteract the pathogenicity of the antigen. Measuring circulating levels of antibody titers to a specific antigen, such as human Cytomegalovirus (CMV) and Epstein-Barr virus (EBV), provides another index of immune function. The titer levels depend on viral activity, or how much the virus is replicating, such that if the body is having difficulty controlling a latent virus, more antibodies will be present than if it was successfully regulating the viral replication, implying underlying immune dysfunction (Vedhara & Wang, 2005).

Given that the activity of the stress response system is linked to morbidity and mortality across the lifespan, and that divorce is consistently identified as one of life's most stressful events, divorce can be used as a model system for investigating the effects of psychologically stressful life events on health and the immune system (Sbarra et al., 2012).

Marital Disruption and Immune Functioning

The link between divorce and immunological responding has remained largely unexplored. Early work by Kiecolt-Glaser and colleagues (1987) found several significant immunological differences between a sample of married women and their separated counterparts. The separated/divorced sample of women evidenced poorer control of latent viruses, lower percentages of natural killer cells, and a decreased lymphocyte response to stimulation by a mitogen (Kiecolt-Glaser et al., 1987). In the more than three decades since this work, our understanding of the psychosocial responses and health-correlates of divorce has expanded enormously, but very few investigations have integrated this work in an update to the psychoneuroimmunological study of divorce.

Social epidemiological findings reveal a broad-based association between marital separation and health outcomes, with elevated estimates of relative risk of mortality for divorced or separated individuals in comparison to their married counter parts (Donrovich, Drefahl, & Koupil, 2014; Manzoli, Villari, Pirone, & Boccia, 2007; Matthews & Gump, 2002; Sbarra et al., 2011; Shor et al., 2012). Psychophysiological research can illuminate the mechanisms of action and the individual difference factors that may make subsets of people particularly vulnerable to poorer health following a separation. Preliminary investigations of divorce and immune functioning reveal that several parameters of immune response are affected following a recent separation, including a reduced percentage of available natural killer cells and a poor lymphocytic proliferative response in response to an antigen (Kiecolt-Glaser et al., 1987). Separated and divorced individuals from the National Social life, Health, and Aging Project (NSHAP) demonstrated significantly higher levels of CRP when compared to their married counterparts, and also reported greater levels of psychological stress than the married

participants (Sbarra, 2009). In a study of women who were undergoing a separation event compared with continuously married women, the separating women exhibited elevated salivary cortisol, demonstrating the significance of studying the link between divorce and the inflammatory pathway (Hänsel et al., 2010). This inflammatory pathway has yet to be studied specifically in relation to the psychological stress and social upheaval that typically follows marital separation.

Furthermore, incidence of infection and viral reactivation may also represent important pathways connecting relationship dissolution and health. Research reveals that when compared to married participants, recently divorced adults exhibit higher antibody titers to latent EBV (Kiecolt-Glaser et al., 1987). Other researchers followed hospital contacts for over 5.5 million people over the course of three decades, and revealed that divorced patients had a 48% greater change of contracting a hospital-diagnosed infectious disease when compared to married patients (Nielsen, Davidsen, Hviid, & Wohlfahrt, 2014). Divorce is further linked to absence from work due to sickness in a study of administrative personnel in Norway (Nielsen et al., 2014). For those individuals who divorced during the course of the study, the rates of illness increased in the year before the divorce, peaked in the months surrounding the divorce, and did not return to the baseline levels observed six years before the divorce occurred. Authors concluded that divorce has a lasting impact on risk of infectious disease. Taken together, these results suggest that studying infection and viral reactivation as a method of investigating immune functioning following divorce will be fruitful.

Although past research clearly establishes the link between personal relationships and immune function, much remains to be known about the mechanisms that underlie this association (Kiecolt-Glaser, Gouin, & Hantsoo, 2010). Studying changes in the immunological health

outcomes of divorced adults as a function of changes in social behavior may reveal important information about why and how marital separation is linked to risk for poor health and earlier death. The current research will attempt to study inflammatory markers and a functional assessment of the immune system's ability to regulate latent viruses as immunological outcomes following separation, determining if divorce is indeed uniquely related to changes in health-relevant immune system activities.

Daily Social Behaviors and Divorce

A unique contribution of the current research is to examine how objective measures of daily social functioning is associated with immunological biomarkers following marital separation. The Electronically Activated Recorder (EAR) provides information about how participants select certain social environments for themselves, and how they interact in these chosen environments (Mehl, Robbins, & große Deters, 2012), and, in this way, it serves as an excellent tool for tracking objective social behaviors following the end of marriage. The EAR is a reliable measure of objective daily social behavior (e.g. Mehl et al., 2001; Mehl & Pennebaker, 2003). Social functioning is highly relevant to adult perceived psychological distress, which is commonly studied as the outcome variable representing adjustment to and/or recovery from divorce (e.g. Booth & Amato, 1991; Kitson, Babri, Roach, & Placidi, 1989). The variables collected by the EAR (discussed in further detail below) are measures of adult daily social functioning, represented by categories including time spent alone, time spent with others, and time spent in substantive conversations.

Social support is linked with a range of positive health-relevant outcomes (Cohen & Wills, 1985; Uchino, 2004) and is considered a critical resource for coping following a divorce (McKenry & Price, 1991; Wallerstein & Kelly, 1980). Previous research suggests that a relative

dearth of social support is associated with poor long-term health outcomes (Uchino, Bowen, Carlisle, & Birmingham, 2012). Marriage may function as a source of social support, providing quality psychosocial resources to the partners involved (Kessler & Essex, 1982; Sbarra & Hazan, 2008); research suggests that divorced individuals, in addition to sustaining the loss of a spouse, also suffer from more disrupted social networks (Gerstel, Kohler Riessman, & Rosenfield, 1985; Hughes Jr, Good, & Candell, 1993; Menaghan & Lieberman, 1986; Wilcox, 1981). These networks exert influence on well-being and adjustment following the dissolution of the marital relationship (Hughes Jr et al., 1993; Wilcox, 1981). A meta-analysis of post-divorce adjustment and social relationships found that social support following a separation is associated generally with higher levels of positive adjustment and lower levels of maladjustment (Krumrei, Coit, Martin, Fogo, & Mahoney, 2007). The social support literature differentiates between subjective and objective forms of social support as perceived social support and social integration, respectively (Holt-Lunstad, Smith, Baker, Harris, & Stephenson, 2015; Uchino, 2004). Importantly, specific relationships (e.g. one-on-one interactions with a close friend or family member) appear essential in creating a buffer against maladaptive adjustment, defined by depression, distress, mental illness, loneliness, and identity crisis (Krumerei et al., 2007).

One problem with the social support literature is that it relies primarily on retrospective self-report measures of network interactions. Objective measure of daily social behavior, assessed in a prospective way during a period of relationship upheaval would be a notable contribution to the stress and coping literature. In this respect, the EAR provides an ideal tool for assessing time spent interacting with an ex-partner, friends, family, and strangers, as well as time spent engaging in social activities and time spent alone.

As an observational assessment tool, the EAR also may be ideally suited to studying adults' post-divorce interactions, which is a notoriously difficult area of study (Sbarra & Emery, 2008). For example, contact and conflict with an ex-partner, assessed by self-report, is negatively associated with overall psychological adjustment to divorce, depending on the type of contact and degree to which people are accepting of the end of marriage (Kiecolt-Glaser et al., 1987; Mason, Sbarra, Bryan, & Lee, 2012; Symoens, Bastaits, Mortelmans, & Bracke, 2013). Furthermore, individuals who report establishing a relationship with a new partner are found to exhibit positive psychological outcomes following divorce (Bowen & Jensen, 2015; Chase-Lansdale & Hetherington, 1990; Gustavson, Røysamb, von Soest, Helland, & Mathiesen, 2012; Symoens et al., 2013; Wang & Amato, 2000). The EAR allows for the evaluation of the participant's local environment for the existence and frequency of positive and negative social contacts beyond what retrospective self-reports can tell us.

Loneliness and Health Outcomes

In contrast to objective measures of social behavior and social isolation, another important outcome following marital dissolution is subjective reports of loneliness. Subjective loneliness is defined as a discrepancy between an individual's ideal social relationships and their actual relationships (Peplau & Perlman, 1982), which leads to the negative feeling of being alone. Divorced adults are reportedly more lonely than their married counterparts (Chase-Lansdale & Hetherington, 1990; Kalmijn & van Groenou, 2005; Kiecolt-Glaser et al., 1988; van Tilburg, Aartsen, & Pas, 2014; Wallerstein, 1986), but importantly, lonely people are not by definition socially isolated. In fact, they may feel alone even when surrounded by people (Cacioppo, Grippo, London, Goossens, & Cacioppo, 2015), rendering this emotional state accessible only by self-report (Holt-Lunstad et al., 2015). The subjective state of loneliness

appears to activate a variety of systems in the body (e.g. neuroendocrine, behavioral, and immunological) that support short-term survival in the face of threat (Cacioppo, Cacioppo, Capitanio, & Cole, 2014). Loneliness is positively associated with a variety of immune parameters in particular, including CMV titers (Glaser et al., 1985), proinflammatory biomarkers (Loucks et al., 2006), susceptibility to respiratory infection (Cohen, Doyle, Skoner, Rabin, & Gwaltney, 1997), altered gene expression profiles (Cole et al., 2007), and mortality more generally (Holt-Lunstad et al., 2015).

In contrast to the subjective state of loneliness, social isolation is an objective measure of how much time people spend by themselves, marked by living alone, having few relationships, and interacting rarely with social network ties (Holt-Lunstad et al., 2015). A recent meta-analysis of social isolation indicates that both subjective and objective measures predict increased mortality risk, and evidence suggesting one as more influential for health than the other remains mixed (Holt-Lunstad et al., 2015). In light of this research, changes to the social network of recently divorced adults that render them lonely and/or isolated may play a role in their adjustment to the separation as well as future morbidity and mortality. An important question in the literature on social connectedness and health is whether subjective (i.e., loneliness) or objective isolation differentially predict outcomes of interest. A unique feature of the proposed research is the assessment of self-reported loneliness in combination with the EAR-indexed social behaviors (e.g., time spent alone, time spent with others) and I will therefore be able to assess whether loneliness explains variance in the immune outcomes over-and-above these objective indicators of social functioning.

Aims and Hypotheses

Based on the literature reviewed above, the proposed dissertation study is organized around the following aims:

Aim 1: Cross-sectional associations between subjective distress and immunological outcomes. I will examine the magnitude of the association between self-reported psychological distress at visit 1 (V1) and immune outcomes at V1. I expect (**H1**) participants who report higher levels of psychological distress at V1 (as indexed by subjective self-report measures) will evidence significantly poorer immunological outcomes at V1. Said differently, high self-reported distress in response to the divorce will predict concurrent higher antibody titers and higher levels of circulating inflammatory markers. Five self-report measures will index psychological distress, including the Impact of Events Scale- Revised (IES-R), a measure of self-identity disruption (LOSROS), a measure of grief and longing for the ex-partner (ICG), a measure of depression (CES-D) and a three item loneliness scale (Hughes, Waite, Hawkley, & Cacioppo, 2004).

Aim 2: Cross-sectional associations between objective daily social behaviors and immunological outcomes. Aim 2 explores the cross-sectional association between immune outcomes and EAR-assessed daily social behaviors. In order to better understand how social behaviors function in adjustment following divorce, the EAR device collects participants' positive daily social behaviors over three separate weekends during their five-month participation. The primary EAR variables of interest include time spent in the presence of others (one-on-one, in a group), time spent socializing or entertaining, time spent engaging in substantive conversations with others, and time spent receiving positive social support. These behaviors were coded and will comprise the objective dimension of social behaviors. I expect (**H2a**) that daily social behaviors derived from the EAR at V1 will predict concurrent

immunological functioning at V1 such that higher levels of positive social behaviors (e.g. time spent with others, and/or in substantive conversation) will be associated with lower levels of inflammatory biomarkers and antibody titers.

A second analysis under Aim 2 is to determine which social behaviors, in particular, are the strongest predictors of immune outcomes following divorce. To investigate this aim, I will estimate the individual effect sizes of selected daily social behaviors to evaluate their unique contributions to the variance in health-relevant biomarkers.

Finally, I will investigate whether self-reported loneliness is a unique predictor of immunological outcomes over-and-above objectively assessed social isolation, as indexed by EAR-recorded time spent alone. I expect (**H2b**) that the effect size of self-reported loneliness will be non-zero after controlling for objective social isolation, indicating that subjective loneliness uniquely influences immunological functioning following a marital separation.

Aim 3: Prospective mediation analyses linking subjective distress, objective behavior, and immunological outcomes. To explore how measures of psychological and daily social functioning impact recovery trajectories after marital dissolution, I will use subjective psychological distress and daily objective social behaviors to predict immune outcomes at V5 in a pair of meditational models, controlling for the mediator and outcome measures at V1 by residualization. The first model will evaluate daily social behaviors, collected at V3, as the mediator of the association between subjective psychological distress at V1 and immune outcomes at V5. I hypothesize (**H3**) that positive daily social behaviors will mediate the relationship between psychological distress and immune outcomes. A second, exploratory model will switch the mediator and predictor variables, allowing for the analysis of psychological distress at V3 as a mediator of the relationship between daily social behaviors at V1 and immune

outcomes at V5. This analysis is exploratory in nature as this is one of the first studies to consider longitudinal changes in health-relevant outcomes following a separation experience. Few studies that evaluate the long-term effects of divorce on objective measures of physical health in adults exist (see: Kiecolt-Glaser et al., 1987).

Method

Participants

This study involved 55 adults (n = 20 men, mean age = 42.61, SD = 11.20) who reported a recent marital separation (mean months since separation = 3.68 months, SD = 2.42) and who took part in a larger NIH-funded study of psychosocial responses to marital separation. The average length of participants' prior relationship was 12.14 years (SD = 7.90). Separation in this case was defined as the date of permanent physical separation from a spouse. Sixty-three percent of the sample reported their race as Caucasian, 19.4% reported they were Hispanic, 3.2% reported being African American, 3.2% reported their race as Native American, 1.6% reported they were Asian, while 9.7% indicated their race was "Other." Participants were phone screened prior to participation and required to have been married to their ex-partner for at least three years, and lived with them for at least two years. Participants were required to be 18 years of age or older, and free of diagnoses for schizophrenia, bipolar disorder, suicidal ideation, or uncontrolled medical conditions. Participants were further questioned about sleep disorders and shift work. A comparison of the subsample (n = 55) to the full study sample (N = 128) revealed no significant differences in age, gender, length of marriage or time since separation (all ps > .05).

Adults interested in participating in the immunological sub-study (n = 71; see Figure 1) were further screened for inclusion on the following criteria: no history of uncontrolled medical

conditions, not currently pregnant, no history of psychotic disorders, no excessive caffeine intake (> 400mg of caffeine a day), no history of blood or needle phobias, and no recent history (prior three months) of active immunosuppressive treatments, surgeries, or autoimmune diseases. Eight people who did not meet these requirements were excluded, and eight declined to participate.

Participants who were included in the subsample were asked to refrain from ingesting anti-inflammatory agents during the 24-hour period preceding the day of their draw, and to refrain from caffeine and tobacco for the 4-hour period preceding the draw. Three participants declined to participate in the second half of the sub-study. Participants who provided two venous blood samples (n = 52) received $50 for each blood draw.

Procedure

The current study examined the association between immunological functioning and daily social behaviors over a five-month period following a recent marital separation. The Divorce, Sleep, and EAR study (DSE) conducted the initial recruitment of participants and the majority of data collection. For those participants who were interested, the option to participate in the immunological sub-study was offered contingent upon eligibility; these participants comprise the sample for the current study.

Upon entry into the larger DSE study, participants completed a set of questionnaires at home assessing various aspects of their psychological response to the separation. Participants brought their completed questionnaire packets to the first study visit (V1) where a sleep diary was assigned, and a DNA buccal swab was also collected. A follow-up questionnaire packet with a reduced number of measures was administered and collected at each of the subsequent visits, which included measures of Body Mass Index (BMI) in addition to a seven-day sleep diary. These visits occurred approximately one month apart, and on visits 1 (V1), 3 (V3) and 5 (V5),

participants were given instructions concerning the EAR device and took the device home for data collection over the course of a weekend. On these visits, waist-to-hip ratio (WHR) measurements were collected and a stream of consciousness task asking them to reflect on their divorce was completed. Sleep actigraphy data was collected at V1, V3 and V5. Within two weeks of V1 and V5, those participants who were interested and eligible to participate in the immune sub-study were contacted and scheduled for their blood draws. A final visit scheduled a week after V5 allowed participants to review their EAR data files and delete any sound files that they did not want to be included in the research study.

For collection of immune parameters, participants were directed to the Clinical and Translational Sciences Center at the University of Arizona Medical center for both of their blood draws. Here participants provided informed consent specific to the immune parameters sub-study, and completed a short psychological assessment battery. Participants were then escorted to a private room where a certified phlebotomist drew approximately 8 milliliters of venous blood. Participants were then paid and instructed to return for their second blood draw within two weeks of their DSE V5 (final) study assessment. The same procedures were followed for the second draw. After completing the second draw, participants were debriefed and thanked for their participation. Following each draw, the sample was immediately delivered to the Infectious Disease Research Core laboratory for processing and evaluation, including a complete blood count, baseline CRP and cytokine assessment, and cryopreservation of mononuclear cells for future functional cell specific analysis.

Measures

Self-report Measures

The Demographic, Health Behaviors, and Relationship History Questionnaire (see Sbarra, Law, Lee, & Mason, 2009). This questionnaire consists of basic demographics (age, socioeconomic status, children, number of people in home), health behaviors, and objective relationship and relationship history questions that may affect immune outcomes (O'Connor et al., 2009). Participants were asked about prescription and illicit drugs, alcohol, tobacco, and caffeine use and frequency, medical compliance, exercise, diet, sleep efficiency, and perceived health. We also collected health history information to use as additional control variables when evaluating inflammatory outcomes. Time since the separation (in months), previous marriages, new relationship(s), number of children and the existence of any ongoing custody disputes, and initiator status (i.e., whether participants initiated the separation or felt left by their partners) was also assessed.

Assessment of Health Status and Health Behaviors. A questionnaire asking for details on the consumption of tobacco, alcohol, and other medications for the 48 hours prior to the time of the draw was administered to assess compliance with study requests. This measure also assesses exercise within the 24 hours prior to the draw and self-reported sleep quality the night before the draw.

Interpersonal Social Support Evaluation List (ISEL). Participants' self-reported perception of functional social support resources was assessed using the Interpersonal Social Support Evaluation List- Short Form (Cohen & Hoberman, 1983). This 12-item measure is composed of four subscales, Appraisal Support, Tangible Assets Support, Belonging Support, and Self-Esteem Support, and includes items such as "There is someone I can turn to for advice

about handling problems with my family" and "I don't often get invited to do things with others," others" which are answered on a Likert Scale (0 = "*definitely true"* to 3 = "*definitely false"*). The mean of participants' responses was used (α = .89; *M* = 3.07, *SD* = 0.59, range = 1.75 - 4.00).

Attachment Style. The Experiences in Close Relationships- Short Form (ECR-SF) is a reliable, 12-item short version of the Experiences in Close Relationships Scale (Fraley, Waller, & Brennan, 2000) measuring insecure attachment styles in close relationships (Wei, Russell, Mallinckrodt, & Vogel, 2007). The anxiety subscale is composed of 6 items such as "I get frustrated if romantic partners are not available when I need them," while the attachment avoidance subscale has 6 items including "I am nervous when partners get too close." Likert scale responses range from 1 = "*Strongly Disagree*" to 7 = *"Strongly Agree.*" The mean of participants' responses was used. (α = .77).

Psychological Composite. The Psychological Composite score was created by taking the z-scored mean of each of the following self-report measures and averaging them into a composite score for each visit (V1, V3, and V5). Participants who had less than 4 of the 5 scale scores were excluded. A Principal Components Analysis of the various measures revealed they all loaded on a single component (which explained 56.24% of the variance). This was confirmed by visual inspection of the scree plot. The reliability of the Psychological Composite was acceptable (α =.75).

Revised Inventory of Complicated Grief (ICG). The original ICG is a factor-analytically derived measure of complicated grief, where individuals with high scores are associated with higher levels of depression and emotional distress (Prigerson et al., 1995). We revised the basic ICG to make all questions applicable to divorce, and the 15-item measure taps the participant's maladaptive symptoms of loss, including grief, avoidance, and trouble accepting the end of the

relationship. The revised ICG contains items such as "I think about my ex-partner so much that it is hard for me to do the things I need to do." Responses ranged from 0 (*never*) to 4 (*always*). (α= .94).

The Center for Epidemiological Studies Short Depression Scale (CES-D 10). The CES-D 10 is a ten-item version of the original CES-D scale (Radloff, 1977), and serves as a self-report measure of depressive symptoms over the past week. Responses range from *Rarely = 0* to *All of the time = 3* to items such as "I felt depressed." Higher scores indicate more depressive symptomology, with a cut off score of 10 and above considered depressed. (α = .88).

Impact of Events Scale- Revised (IES-R). The Impact of Events Scale – Revised (IES-R; Creamer, Bell, & Failla, 2003) is a widely used measure of subjective responses to stressful events and assesses several dimensions of responding following a stressful event, including intrusive thoughts, hyper-arousal, emotional numbing, avoidance, and total subjective distress. Respondents report on the degree of distress of a given symptom over the last seven days on a scale (from 0 = *Not at all* to 4 = *Extremely*). Sample items include statements such as "Any reminder brought back feelings about it" and "I thought about it when I didn't mean to." Higher scores reflect greater self-reported emotional distress following the separation (α=.94).

Loss of Self - Rediscovery of Self (LOSROS). The Loss of Self - Rediscovery of Self Scale (Lewandowski & Bizzoco, 2007) is a 12-item measure that assesses the extent to which individuals feel they have "lost" or "rediscovered" their sense of self following their separation. Questions to evaluate loss of self-concept include "I do not know who I am" and "I feel as though I am missing a part of me." Questions that evaluate self-rediscovery include "I have done the things I once enjoyed that I could not do while I was in my relationship," and "I have

regained my identity." Participants respond on a 7-point Likert scale (from 1 = *Not at all* to 7 *A great deal*). (α=.90)

UCLA Loneliness Scale, Short Form. The three-item version of the UCLA Loneliness Scale was used to assess subjective loneliness, the subjective experience of social isolation (Hughes et al., 2004). Items include "How often do you feel that you lack companionship," "How often do you feel left out," and "How often do you feel isolated from others?" Respondents indicate the frequency of these feelings on a scale (1 = *Hardly ever* to 3 = *Often*). (α=.86)

Objective Measures

Electronically Activated Recorder (EAR; Mehl et al., 2001). The EAR device is a real-time data capture method composed of voice recording software placed on a pre-programmed iPod Touch that records ambient sounds for 30 s seconds every 12 minutes, or about five times an hour. Over the course of the weekend, files were sampled beginning at approximately 6:00pm on Friday evening with black out periods on Friday and Saturday nights during sleep (as indicated by self-report), and ending on Sunday at 11:59pm. Each participant wears the EAR during the first, third, and fifth visits of their participation for a full weekend. Participants are instructed that it is okay to not wear the EAR during certain activities; they are asked to indicate in a diary when they did not wear the EAR. Because it samples only a fraction of participants' days (~5%) as they unfold, it makes large naturalistic observation studies viable. Participants are given the opportunity to listen to and delete any sound files they do not want researchers to hear.

EAR Derived Social Functioning Behaviors. Trained research assistants coded participants' remaining EAR sound files for aspects of their momentary behaviors using a behavioral coding or counting strategy based on the presence ('1') or absence ('0') of a target

behavior using a standardized coding system called the Social Environment Coding of Sound Inventory (SECSI; Mehl et al., 2006). The SECSI captures acoustically detectible aspects of participants' social environments and interactions in four broad categories.

There are several categories of daily social behavior that are relevant to this study. A distinction of critical importance for this investigation is whether the participant appears to be alone or with others during the observation period. While the participant is alone, a distinction is made between sound files where the participant is physically alone, and sound files where the participant is surrounded by people but not engaged in any interaction, and when the participant is alone and (presumably) talking to themselves. For sound files where the participant appears to be with or talking to others, distinctions are made between sound files containing dyadic (one other person) interactions and interactions with a group of multiple people. In addition, conversations can be classified as to their content, and pertinent categories to the current study include substantive conversation and positive social support received.

If a sound file contains one or more of these behaviors, raw binary codes are assigned within each category, and these codes are converted into a relative frequency variable, indicating the number of waking EAR recordings in an hour in which a coding category applied (e.g. the percentage of time over the course of the weekend during which the participant was performing the target behavior). Two research assistants code each participant, and these assistants are regularly supervised for reliability (see Mehl, Robbins, & Deters, 2012).

Social Integration Composite. The positive social behaviors composite was constructed by taking the mean percentage score (i.e. the percentage of sound files over the course of a weekend where the behavior was indicated as present) for each chosen social behavior to create a composite score. The social behaviors include: (a) time spent with one other person and time

spent with a group of people, (b) time spent socializing or entertaining, (c) time spent in substantive conversation, (d) time spent receiving positive social support. I calculated the inter-rater reliability for each of the social behaviors separately in the subsample. For each participant, I averaged each coder's ratings of the individual social behaviors across all of their Visit 1 EAR files, which provided two average ratings per participant (one from each coder) for each of the social behaviors assessed. Individually, the inter-coder reliability for each is as follows: (a) time spent with one other person, ICC[1,2] = .94, and time spent with a group of people, ICC[1,2] =.92, (b) time spent socializing or entertaining, ICC[1,2] = .30, (c) time spent in substantive conversation, ICC[1,2] =.81, and (d) time spent receiving positive social support, ICC[1,2] = .60. Altogether, the social integration composite displayed acceptable reliability within the subsample (α = .70).

Blood Samples. All samples were collected between 8am and 12pm to control for known diurnal variations in a number of markers of inflammation (Steptoe, Hamer, & Chida, 2007). Six milliliters (mL) of plasma serum was collected and stored in a vacutube containing clotting factors, kept on ice to clot for 10-30 minutes, and then spun at 2000g 4C for 20 minutes. Each sample was then split into 10 microtubes containing 0.5 mL aliquots of plasma and stored at -80 degrees for further analysis (see below). Two mL of whole blood was collected for complete blood counts and held at room temperature for further processing. Four mL of blood was collected and delivered on ice to the University of Arizona Genomics Core immediately following the draw, to be stored in a 4C degree environment. Logged IL-6, CRP, and antibody titers were used to correct for skewed distributions.

***Serum IL-6 Levels*.** Banked frozen plasma was thawed and IL-6 levels were quantified in duplicate using an Elisa kit (Quantikine HS; R&D Systems, Inc., Minneapolis, MN) according to

manufacturer instructions. The intra-assay coefficients of variation were all less than 5%, which falls under the intra-assay variance of 20% as specified by the manufacturer. All standard curves correlated at r = .98 or greater.

Serum CRP Levels. Banked frozen plasma was thawed and CRP levels (high sensitivity) were quantified (in duplicate) at Banner University Medical Center's clinical pathology lab. Because serum CRP >10 mg/L can reflect acute infection, adults scoring above this cutoff (n = 2) were excluded from the analyses (Pearson et al., 2003).

Antibody Titers to CMV and EBV. Plasma samples with high IFA-scored antibody titers (i.e., 2560), obtained from prior studies, were used as the top standards for CMV and EBV. Seven two-fold serial dilutions of the top standards (2560, 1280, 640, 320, 160, 80, 40, and 20) were made with PBS in separate tubes. One hundred microliters of positive and negative controls, standards, and diluted patient samples (all dilutions were at 1:101 with PBS) were pipetted in duplicate into individual microplate wells followed by a 30 min incubation (all steps were carried out at room temperature). The plates were then washed 3 times with 350ul wash buffer using an Embla microplate washer (Molecular Devices, Menlo Park, CA). Next, 100ul of enzyme conjugate (peroxidase labeled anti-human IgG) was pipetted into the wells followed by another 30 min incubation period. The plates were then washed 3 times, and 100ul of chromogen substrate (TMB/H_2O_2) was pipetted into the wells. The plates were then covered to protect from direct light and incubated for 15 min. One hundred microliters of 0.5 M sulphuric acid was added to each well to stop the reaction. Absorbance was then read at 450nm (reference wavelength 620nm) using a SpectraMax Plus 384 (Molecular Devices). The values of the unknown samples were assigned in relation to the standard curve.

Data Analytic Plan

Aim 1: Cross-sectional associations between subjective distress and immunological outcomes. Aim 1 assesses variability in immune responding at V1 as a function of psychological distress at V1. Criterion variables include CRP levels, levels of IL-6, and levels of antibody titers, and these outcomes are predicted in four separate regression models. In all models, covariates include age, sex, WHR, and anti-inflammatory drug (NSAID) use. H1 holds that levels of divorce-specific psychological distress at V1 will predict immune outcomes at V1 over-and-above these general immune- and stress-relevant covariates, and this model is specified as follows:

Immune Outcome = B0 + B1-4 Covariates + B5 Psychological Distress + ε;

In this model, the parameter of interest is B5, which reflects the strength and significance of the association between our target immune outcome at V1 and self-reported, divorced-specific psychological distress at V1. Given the relatively small sample, I first examined whether the psychological distress outcomes were associated with the target immunological outcomes (e.g., using zero-order correlations), then determined if these associations persisted after including the relevant covariates.

Aim 2: Cross-sectional associations between objective daily social behaviors and immunological outcomes. Aim 2 is conceptually similar to Aim 1 but assesses variability in immune functioning at V1 as predicted by the social integration composite at V1. Criterion variables and covariates remain the same as in Aim 1. **H2a** holds that levels of social integration at V1 will predict immune outcomes at V1 over-and-above these general immune covariates, and this model is specified as follows:

Immune Outcome = B0 + B1-4 Covariates + B5 Social Integration Composite + ε;

In this model, the parameter of interest is B5, which indicates the strength and significance of the association between our target immune outcome at V1 and an objective measure of social integration at V1.

A second regression analysis assessing the individual effects of the primary daily social behaviors of interest, as opposed to the social composite, will allow for the evaluation of each as a unique predictor of immune functioning.

A corollary analysis of Aim 2 (**H2b**) will compare the EAR-derived social behavior time spent alone with self-reported loneliness to determine whether loneliness predicts the target outcomes of interest after accounting for the effects of objective reports of social isolation.

Aim 3: Prospective mediation analyses. Aim 3 assesses potential mediating processes among self-reported psychological distress, social behaviors, and immune functioning. There are two hypothetical mechanisms for longitudinal associations between these domains. Models for these pathways can be specified through hierarchical regression or multiple mediator models (Preacher & Hayes, 2008). **H3** evaluates whether social integration at V3 functions as a mediator of the association between divorce-specific psychological distress at V1 and immune outcomes at V5 controlling for immune outcomes at V1 by residualization (see Figure 2). In a follow-up to **H3**, a second meditational analysis will determine if reciprocal influences exist such that divorce-related psychological distress at V3 acts as a mediator of the relationship between social integration at V1 and immune outcomes residualized at V5 (see Figure 2).

Standard mediation analysis regresses the dependent variable (Y) on the independent variable (X), yielding path c; second, they regress the mediator (M) on the independent variable (X), yielding path a; and third, regress the dependent variable (Y) on both the independent variable (X) and the mediator (M) yielding path b and c’ (Baron & Kenny, 1986). For mediation

to occur, path c must indicate that the independent variable (X) and the dependent variable (Y) are correlated. See Figure 2 for specification of pathways with the social integration composite as the mediator.

1. Estimate path c: Immune Outcomes$_{(resid)}$ = B0 + B1 Psychological Distress V1+ ε
2. Estimate path a: Social Integration V3 = B0 + B1 Psychological Distress V1 + ε
3. Estimate path b: Immune Outcomes$_{(resid)}$ = B0 + B1 Psychological Distress V1 + B2 Social Integration V3 + ε

The aforementioned covariates will be included in all analyses to serve as controls for general immune- and stress-related predictors of residualized immune outcomes to determine if divorce-specific stress and social engagement are predictive of said outcomes over-and-above these covariates, however, they have been removed from the modeled equations for clarity. Important parameters include: (a) depicting the association between social integration at V3 and psychological distress at V1, and (b) reflecting the relationship between immune outcomes at V5 and social integration. To investigate the hypothesized mediator relationship, path c will be examined and if it is not equal to zero after controlling for the mediator, partial mediation is supported. To test the significance of the indirect mediated pathway, one then uses a bootstrapping approach to derive a sampling distribution for the *ab* pathway and, from this, an estimate of the 95% confidence interval for the population value of the indirect effect (Preacher & Hayes, 2004).

A second analysis specifies the reverse situation in an exploratory analysis, where psychological distress at V3 functions as the mediator between social integration at V1 and immune outcomes residualized at V5.

4. Estimate path c: Immune Outcomes$_{(resid)}$ = B0 + B1 Social Integration V1 + ε

5. Estimate path a: Psychological Distress V3 = B0 + B1 Social Integration V1 + ε
6. Estimate path b: Immune Outcomes$_{(resid)}$ = B0 + B1 Social Integration V1 + B2 Psychological Distress V3 + ε

Results

Tables 1-3 display descriptive statistics and a correlation matrix of the main variables in the study. As shown, none of the self-report psychological distress variables correlate with any of the individual immune parameters. Time spent alone, time spent with others, time spent socializing or entertaining, and time spent in substantive conversation were not associated with any of the individual immune outcomes, however time spent receiving positive support and CRP were correlated (r = -.31, p < .01).

Aims 1 and 2: Cross-sectional Associations with Immune Outcomes.

Under Aim 1, the first hypothesized model evaluates the association between psychological distress and concurrent immune parameters at V1 in a series of cross-sectional hierarchical regression analyses. I hypothesized that high levels of psychological distress would be associated with poor immune outcomes in the form of higher levels of circulating inflammatory markers and antibody titers to CMV and EBV (**H1**). After controlling for age, sex, V1 WHR, and NSAID use, the composite psychological distress variable did not predict any of the individual immune outcomes at V1 (all *p*s > .05).

Under Aim 2, I hypothesized (**H2a**) that higher levels of social integration, as objectively measured by the EAR, would predict better immune responses, as indicated by lower levels of circulating inflammatory markers and lower antibody titers. In models evaluating the relationship between the social integration composite and immune outcomes. I found the same pattern of null results as observed for H1 (all *p*s > .05). Figure 3 presents scatterplots of the psychological

distress and social integration composites and selected concurrent immune variables 1L-6, CRP, and EBV antibody titers, respectively.

Following my assessment of the individual immune parameter analyses and the formal examination of Aims 1 and 2, I conducted a series of exploratory analyses to investigate whether the psychological or social variables were associated with a composite score of immunological risk. First, I computed an average composite outcome variable using the standardized scores of each individual raw immune measures (i.e., a mean of z-scored levels of raw IL-6, CRP, CMV and EBV antibody titer levels, all at V1). Cross-sectional hierarchical models controlling for the aforementioned covariates were re-run using this composite immune variable as the outcome and separately assessed the effects of the psychological distress and social integration composites. The psychological composite predicting the immune composite revealed no significant results; in contrast, the social integration composite was a significant predictor of the V1 immune composite, and in the predicted direction, B = -0.22, p =.04, 95% CI [-0.41, -0.01], (see Table 4). In this case, recently separated adults who evidenced greater levels of objectively measured social behaviors—i.e., more time spent with others, more socializing/entertaining, more substantive conversations, and more time receiving positive social support—also evidenced lower overall immune activity.

Following this, I added the psychological distress composite to the model so both the social integration and psychological distress composites predicted the immune composite in step 1, with relevant covariates in step 2. When included simultaneously, the psychological distress composite exerted no significant effects, and, although the effects of the social integration composite were significant without covariates, these effects became non-significant in the full model. In a similar analysis, I specified a model where step 1 predictors included the social

integration composite and self-reported loneliness with health covariates in step 2, and results indicate that when including the effects of loneliness, social integration no longer significantly predicts the immune composite. These models are displayed in Table 4.

Individual social behaviors and immune outcomes. I next considered the specific, EAR-assessed behaviors included in the social integration composite as individual predictors of immune outcomes, as dictated by the second part of Aim 2. It is possible that the composite score of positive social behaviors obscures significant contributions from specific behaviors, or that different behaviors may serve as better or worse predictors of concurrent immune outcomes. For this analysis, I entered the individual social behaviors that make up the composite measure of objective social integration in step 1 of a regression model (these include the amount of time over the course of V1 weekend that participants' EAR files were coded as having: (a) time spent with one other person and/or time spent with a group of people, (b) spent time socializing or entertaining, (c) spent time in substantive conversation, (d) spent time receiving positive social support), and in step 2 I entered all relevant health covariates. I specified this model for each immune outcome, including IL-6, CRP, antibody titer levels to CMV and EBV, and the immune composite variable. When included as predictors separately, received positive support was a significant predictor of V1 CRP, B = -.21, p = .01, 95% CI [-.37, -.05] in the hypothesized direction, such that people who spent more time receiving positive social support had lower levels of the circulating inflammation marker CRP (see Table 5). The rest of the individual social behaviors were not significantly associated with immune parameters.

Subjective and objective social isolation. I next explored differences in the relationships between objective and subjective measures of social isolation, as indexed by the EAR-derived measure of time spent alone and self-report measures of loneliness, with individual immune

parameters and the immune composite at V1. In **H2b**, I hypothesized that, after accounting for objective social isolation, loneliness would remain a significant predictor of concurrent immune outcomes. Hierarchical regression models with focal predictors entered at step 1 and covariates entered in step 2 revealed neither time spent alone nor self-reported loneliness were significant predictors of concurrent immune outcomes.

Subjective and objective social support. Based on the previous individual results suggesting EAR-derived positive social support may be associated with immune responses, especially CRP, I ran a similar analysis comparing the predictive power of objective time spent receiving social support to a subjective report of positive support (mean scores on the ISEL). Results indicated that for various immune measures, each measure of support played a different role (see Table 6). Self-reported positive support, as measured by the ISEL, was a significant predictor of the composite measure of all V1 immune variables, $B = -0.30$, $p < .01$, 95% CI [-0.49, -0.10]. This effect persisted above-and-beyond the effects of all covariates and the EAR derived measure of positive social support, and indicates that the presence of more self-reported support is associated with reduced concurrent immune composite scores.

For time spent receiving social support (i.e., the total percentage of EAR files that were rated for the presence of *received* positive support) significant effects emerged when predicting concurrent CRP, $B = -0.17$, $p = .02$, 95% CI [-.31, -.03]. Again, this effect remains after controlling for covariates and the predictive effects of self-reported support received. In the case of objectively rated positive support received, more time spent receiving positive support is linked to lower levels of concurrent circulating CRP even when self-reported social support is included in the model.

Concurrent mediation analyses. Based on the finding that social integration at V1 predicts the concurrent immune composite, the next logical step in this analysis was to determine if the objectively derived social integration composite predicts immune outcomes over and above the psychological distress composite. I entered both the psychological distress and social integration composites in step 1 and relevant health covariates in step 2 to predict the immune composite. When both composites are entered simultaneously, the significant effects of social integration disappear. Given that there is a significant association between EAR-derived social behavior and immune outcomes, and a relationship between psychological distress and these social behaviors, I next specified several exploratory models investigating concurrent mediational pathways. I first used V1 social integration as a mediator of the relationship between V1 psychological distress and V1 immune outcomes. I report standardized regression weights (β) for ease of interpretation, as they indicate the amount of standard deviation (*SD*) unit change in the outcome variables predicted by a 1 *SD* change in the predictor variable. No indirect effects were indicated, though *a* paths for V1 psychological distress predicting V1 social integration were significant for both IL-6, β = -.29, p = .04, 95% CI [-.56, -.02], and the composite immune variable, β = -.29, p = .04, 95% CI [-.56, -.02], both in the expected direction suggesting that higher psychological distress at V1 is associated with lower social integration at V1.

I next specified models to explore the alterative possibility that V1 psychological distress may mediate the relationship between V1 social integration and individual immune outcomes (IL-6, CRP, and antibody titers) and/or the composite immune outcome. In the models predicting concurrent IL-6 and the immune composite, the *a* path between psychological distress and social integration was significant (β = -.31, p = .04, 95% CI [-.59, -.02] and β = -.30, p = .04, 95% CI [-.58, -.02], respectively), however there were no other significant paths and no indirect effects

emerged. In this case, both *a* paths suggest effects in the anticipated direction, such that high social integration at V1 is linked with lower psychological distress at V1.

Aim 3: Prospective Mediation Analyses.

Aim 3 of this study focused on exploring potential mediation models to determine if social integration at V3 might mediate the relationship between psychological distress at V1 and immune outcomes at V5 **(H3)**. For all V5 individual immune outcome variables (after controlling for V1 levels), no mediation model was significant (all *p*s > .05). No significant indirect effects emerged, and neither the *a* nor *b* paths were significant in predicting an IL-6 change score, a CRP change score, or change scores in either of the antibody titers.

I next specified models exploring the alternative possibility, that V3 psychological distress may mediate the relationship between V1 social integration and longitudinal immune outcomes. Again, when predicting changes in IL-6, CRP, and antibody titer scores, none of the models' *a* or *b* paths were significant, and no significant indirect effects emerged.

Exploratory Analyses: An Immune Risk Phenotype Approach.

Thus far, I have studied my outcome variables as either individual markers of risk (as related to the specific dimension of functioning assessed by each variable—e.g., greater EBV titer levels reflecting a difficulty in suppressing the latent EBV virus), or as part of a single, continuous risk measure. Another way to think about how markers of inflammation and viral reactivation might operate together is in a manner conceptually similar to how researchers think about assessing multisystem biological risk, often referred to as allostatic load. Allostatic load represents the cumulative physiological wear-and-tear on the body as a result of maintaining homeostasis in response to environmental challenge (McEwen & Stellar, 1993). The process of making changes to restore homeostasis after a challenge (called allostasis) can be measured

across multiple physiological systems (e.g. cardiovascular, endocrine, and immune) and scores across systems may be combined to yield a measure of allostatic load (see Seeman, Singer, Rowe, Horwitz, & McEwen, 1997). Conceptually, it is reasonable to explore whether dysfunction across multiple parameters *within* a given physiological system might operate in a manner similar to general allostatic load, and, in this way, serve as a critical marker of biological risk. Allostatic load predicts declines in cognition and physical functioning, cardiovascular disease, and mortality (Karlamangla, Singer, McEwen, Rowe, & Seeman, 2002; Seeman et al., 1997). In the current sample, a summary risk index may therefore better represent the potential dysfunction than any single measure or a cumulative index that relies exclusively on the continuous immunological outcome variables. In a study focusing on allostatic load in older adults, researchers created an index of selected immune variables (high CD8 and low CD4 numbers, and poor proliferative response), and called it an Immune Risk Phenotype (Wikby et al., 2005). Following this approach, I combined the current study's immune outcomes into a summary immune variable or Immune Risk Phenotype (IRP), rather than using them as continuous variables. I calculated two indices of Immune Risk Phenotype, one combining the four immune measures individually, and the other combining outcomes into a 3-item index. The first IRP sums across participants' status on all four immune measures. I calculated a quartile cutoff for each of the immune measures, CRP, IL-6, and antibody titers to both CMV and EBV, and participants who fell within the top quartile of a category received a one, while participants falling outside of the highest quartile received a zero (see Seeman et al., 1997). Summing the binary scores on these four parameters results in the 4-item IRP score, ranging from 0 to 4, with higher scores indicating a greater immune-related allostatic load, or a riskier immune phenotype. A second, 3-item index was created employing the quartile cutoff criteria for both inflammatory

measures (CRP and IL-6), and being sero-positive for both CMV and EBV received a one (see Wikby et al., 2005), whereas being sero-positive for only one or neither of these latent viruses received a zero. This index ranged from 0 to 3. The two risk phenotypes correlated at $r = .72$.

Aims 1 and 2: Cross-sectional associations with immune outcomes. All cross sectional hierarchical models using psychological distress and social integration composites at V1 were re-run using these IRPs as outcome variables controlling for age, sex, WHR, and NSAID use. Based on my previous hypothesis for the individual immune outcomes, (**H1**) that higher scores on the psychological composite would predict higher levels of concurrent immune parameters, I expected that higher levels of psychological distress would be associated with increased IRP indices. Results indicated that the psychological distress composite remained non-significant for both the 4- and 3-item risk indices (all *p*s > .05). However, the model including the EAR-assessed social integration composite revealed significant main effects of objective social integration predicting both the 4-item risk phenotype, $B = -0.40$, $p = .01$, 95% CI [-0.71, -0.10], and the 3-item risk phenotype, $B = -0.36$, $p < .01$, 95% CI [-0.61, -0.12], after accounting for health-relevant covariates. These results indicate that as social integration increases, concurrent immune risk scores decrease, as hypothesized (**H2**). Importantly, in models including the psychological distress composite as a simultaneous predictor, the social integration composite remained a significant predictor of both the 4-item and 3-item IRPs. Furthermore, in models including the social integration composite and self-reported loneliness in step 1 and health-relevant covariates in step 2, the social integration composite retains significant predictive power for both immune phenotypes. The details of each of these models predicting the 4-item IRP are presented in Table 7 (predicting the 3-item IRP resulted in a similar significant pattern of results).

Individual social behaviors and immune outcomes. To explore specific contributions of individual social behaviors to immune outcomes, I entered each component of the social composite separately in step 1 of the model predicting each of the IRPs, accounting for relevant health covariates in step 2. No individual social behaviors were significant predictors of risky phenotype (all *p*s > .05). Positive support received approached significance (B = -.32, p = .08) for the 4-item index, and in the expected direction. Figure 4 displays the scatterplots of individual social behaviors and 4-item IRP associations.

Subjective and objective social isolation. I next assessed the significance of time spent alone and self-reported loneliness using both concurrent IRPs as outcomes. I hypothesized (**H2b**) that after controlling for time spent alone, the effects of loneliness on immune outcomes would remain significant. These models revealed no significant effects (all *p*s > .05).

Subjective and objective social support. When exploring the effects of time spent receiving positive social support and self-reported social support, differential effects appeared. The effect of self-reported social support is significant in the 3-item index of IRP, B = -.26, p < .05, 95% CI [-0.51, -.01]. EAR-derived time spent receiving positive social support was negatively associated with the 4-item IRP, B = -0.30, p = .04, 95% CI [-0.59, -0.02]. The effects of both subjective and objective social support appear to operate independently to predict the 3- and 4-item risk variables, respectively, such that an increase in either measurement of support is associated with lower concurrent risk phenotype scores. Table 8 displays the full results of these analyses.

Concurrent mediational analyses. In order to assess the possibility of concurrent mediational processes, I specified a model where V1 psychological distress predicting V1 IRP indices with V1 social integration acting as a mediator of this relationship. In the case of the 4-

item IRP at V1, there was a non-significant *a* path (β = -0.25, *p* = .10) and a significant *b* path (β = -0.35, *p* = .03, 95% CI [-0.66, -0.05]) as well as a non-zero indirect effect (β = 0.09, 95% Bootstrapped CI [0.01, 0.25]; see Figure 5). Results of this model indicate mediation, such that participants who differ by one *SD* unit in their self-reported psychological distress are estimated to differ by .09 *SD* units in their IRP as a result of being less socially integrated. In other words, reporting higher psychological distress is associated with an increased risky immune phenotype through lower levels of objectively derived social integration. The same pattern of results was repeated for the 3-item IRP (see Figure 5).

In assessing the alternative possibility that V1 psychological distress may mediate the relationship between V1 social integration and immune risk scores, results indicate no significant *a* or *b* paths, and a bootstrapped indirect effect that was not different from zero.

Assessing change in IRP over time. In a final set of longitudinal analyses, I wanted to explore change in the IRP variables from V1 to V5. There is no guiding theory on changes in allostatic load, so in looking at longitudinal change in IRP variables I chose to use change scores, as allostatic load is theoretically continuous.

***Aims 1 & 2: Longitudinal associations with immune outcomes*.** To explore longitudinal associations between psychological distress and change in IRP over time, I regressed IRP change scores on psychological distress composite scores at V1 in step 1, accounting for relevant covariates in step 2 of a hierarchical regression. Neither the model with V1 psychological distress predicting the 4- or the 3-item change scores in IRP produced significant results (all *p*s > .05). The social integration composite, however, was a significant predictor of change scores for the 4-item IRP over time in step 1 (*B* = .23, *p* = .04, 95% CI [.01, .44], though this effect became non-significant after accounting for covariates.

Given the multiple indicators of a relationship between social integration and immune outcomes at V1, and the weak indication that V1 social integration predicts change in the 4-item IRP variable before entering covariates, I further examined the relationship between V1 social integration on change in IRP risk over time. The initial hierarchical regression results indicated that the effects of social integration on change in 4-item risk phenotype are positive, which runs contrary to my general hypotheses. The results thus far suggest that for individuals who begin with relatively high social integration following their divorce, they evidence lower concurrent immune parameters; as time unfolds, however, having higher levels of objective social integration at the start of the study is associated with *increased* change scores in the 4-item IRP over time. Because this runs contrary to my predictions and much of the established research literature, I wondered whether this main effect reflected the action of an unmeasured moderator, which would explain why certain adults are suffering negative immune related consequences *over time* when starting with high levels of social integration.

In an attempt to explain these seemingly paradoxical results, I next created a model using self-reported avoidant attachment (from the ECR-R attachment avoidance subscale) as a potential moderator of the relationship between objective social behaviors and changes in immune phenotype. Attachment theory (Bowlby, 1969) provides a framework for describing the biobehavioral systems underlying attachment relationships from birth to adulthood. In adulthood, close relationships are organized around seeking support and emotion regulation from a relational partner (Hazan & Shaver, 1987). Attachment theory suggests that there are individual differences in regulatory strategies for dealing with attachment-related distress, and as relationship dissolution is likely to cause attachment-related distress, I targeted avoidant attachment as a potential moderator; people high in attachment avoidance tend to rely on

deactivating emotion regulatory strategies, similar to response-focused emotional suppression (see Mikulincer & Shaver, 2007) and in part by a high degree of self-reliance. I reasoned that for people who have characteristic tendencies to avoid or suppress emotions and cognitions (in response to relational threats), being highly socially integrated may be unwelcome and, in this case, present people with a degree of stress that maintains heightened immunological activation. Although I have no way to test this specific hypothesis, a (EAR) Social Integration × Avoidance interaction predicting change in the IRP measures would be generally consistent with this idea.

Following this logic, in a model including V1 social integration and attachment avoidance main effects predicting change in 4-item IRP and controlling for relevant health variables, I observed a significant Social Integration × Avoidance interaction, $B = .26$, $p = .02$, 95% CI [.05, .47], (see Table 9). A decomposition of the simple slopes revealed significant positive effects of attachment avoidance exist for those participants reporting high avoidance (at one SD above the mean), $B = .53$, $p = .01$, 95% CI [.17, .89]. Results are displayed in Figure 6, and suggest that for participants reporting high levels of attachment avoidance, being high in social integration at the beginning of the study is associated with having a higher V5 IRP than at V1. Said differently, for participants who are both highly socially integrated (as indexed by the EAR) and who also *report* avoidant attachment levels 1 *SD* above the mean for subsample, their IRP change score is .53 *SD* units higher than participants who are less socially integrated and/or less avoidant. Parallel models predicting change in 3-item IRP indicate that a similar pattern of results exist, though the interaction in this case is not significant (see Table 9).

***Aim 3: Prospective mediation analyses*.** I specified mediational models predicting changes in the 4- and 3-item IRPs over time first using the V1 psychological distress composite as a predictor of change and V3 social integration composite (controlling for V1) as a mediator

(**H3**). Accounting for age, sex, V5 WHR, and NSAID use, results for the 4-item change score IRP model indicate non-significant *a* and *b* paths, and there was no significant indirect effect in this model. Following this approach, I reversed the model to explore the alternative possibility that psychological distress at V3 (controlling for V1) might mediate a relationship between V1 social integration and changes IRP outcomes. Neither the change score for the 4-item or 3-item IRP models resulted in significant effects for *a* or *b* paths, and no indirect effects emerged.

Discussion

The primary purpose of this dissertation research was to explore associations among objective and subjective measures of psychological adjustment and social integration on concurrent and longitudinal immunological outcomes in a sample of recently separated adults. Based on a large literature demonstrating that self-reported psychological variables and measures of social integration are associated with immunological health (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Holt-Lunstad et al., 2015, 2010), I hypothesized (**H1 and H2a)** that psychological distress and objective measures of social integration would be associated with inflammatory markers and control of latent viruses. In addition to individual measures of immune responses (i.e., IL-6, CRP, latent virus antibody titer levels), I also computed a composite concurrent immune variable by taking the mean of the standardized levels of each individual immune parameter. Finally, I modeled an alternative metric for immune outcomes, conceptually similar to a common index of multisystem biological risk (allostatic load), by using quartile cutoff scores to create two immune risk phenotype (IRP; Wikby et al., 2005) scores for each participant. To investigate longitudinal associations in this metric, I used change scores (by subtracting V1 IRP scores from V5 IRP scores) as my primary outcome.

Aims 1 and 2: Cross-sectional Associations with Immune Outcomes.

Aim 1 assessed immune outcomes as a function of self-reported psychological distress. I predicted (H1) that higher levels of psychological distress would be associated with higher immune parameters of inflammation and viral reactivation. Using cross sectional hierarchical regression, the composite of subjective distress (composed of self-reported levels of depression, loneliness, loss of self, grief, and a measure of the impact of the separation event) did not predict any of the individual, composite, or IRP outcomes.

Aim 2 assessed the effects of social integration on immune outcomes, and I predicted (H2a) that objectively derived measures of daily social behavior, combined into a composite social integration variable (made up of (a) time spent with others, (b) time spent in substantive conversation, (c) time spent socializing or entertaining, and (d) time spent receiving positive support), would predict concurrent immune responses at V1. This hypothesis was partially supported in that the social integration composite predicted the composite immune measure, though when including the psychological distress composite this effect was no longer significant. The social integration composite was, however, a significant predictor of both measures of concurrent IRPs, and this effect remained when including the psychological distress composite in the model, as well as in a model including self-reported loneliness as a simultaneous predictor. Higher levels of objectively-assessed social integration were associated with concurrent lower phenotype risk in each model, and this main effect is over-and-above the effects of self-reported loneliness and an overall measure of self-reported psychological distress. The main effect of social integration on immune outcomes is consistent with a robust literature linking the positive effects of social relationships on health. Social integration is related to beneficial effects across cardiovascular, endocrine, and immune system health (Uchino et al., 1996) and increases

probability of survival (Holt-Lunstad et al., 2010). Social support is further associated with positive health habits (Uchino, 2004), buffers against the negative effects of stress (Cohen, 2004), and increases feelings of personal control (Berkman, Glass, Brissette, & Seeman, 2000).

Individual social behaviors and immune outcomes. In a more detailed regression analysis of the individual effects of the social behaviors of interest, I evaluated each as a unique predictor of immune functioning in models using individual V1 immune outcomes, the composite immune outcome, and the two risk phenotypes as outcomes. Of the four behaviors, time spent receiving positive support was the only variable that emerged as a unique predictor of concurrent immune outcomes over-and-above the other behaviors. Objective positive support received predicted concurrent levels of CRP in the expected direction, such that high levels of positive support received were associated with lower levels of CRP. All other associations were non-significant.

Objective and subjective social isolation. A corollary of Aim 2 was to explore the effects of self-reported loneliness and objective time spent alone on the various immune measures. I predicted (H2b) that loneliness would predict immune outcomes over and above the effects of social isolation. These measures were not significant in predicting any concurrent immune outcomes, which stands in opposition to strong evidence that the absence of social relationships has a deleterious effect on health (Holt-Lunstad et al., 2015). Lack of social bonds is associated with higher morbidity and mortality rates on par with several other major indicators of health, such as obesity, smoking status, and lack of physical activity (Holt-Lunstad et al., 2010). More specifically, loneliness is linked to higher levels of circulating cortisol (Pressman et al., 2005), poorer control of latent viruses (Glaser et al., 1985; Pressman et al., 2005) lower natural killer cell activity and natural killer cell functioning (Glaser et al., 1985), infection

(Cohen et al., 1997), inflammation (Loucks et al., 2006), and the regulation of certain genes important for immune response (Cole, Hawkley, Arevalo, & Cacioppo, 2011). Given these previous findings, it is surprising that self-reported loneliness was not associated with any immune measures in the current study.

Objective and subjective social support. However, based on indications that positive social support influences immune responses, specifically CRP, I compared two additional constructs simultaneously, self-reported social support (as determined by the ISEL), and objective time spent receiving positive support. Self-reported social support predicted the immune composite and 3-item IRP score after accounting for the health-relevant covariates and EAR-derived positive social support received. EAR-assessed positive social support received was a significant predictor of CRP levels beyond the effects of self-report social support and health covariates and predicted the 4-item immune phenotype. Both measures independently predicted concurrent immune outcomes in all models such that higher levels of social support – regardless of the measurement method – predicted reduced immune parameters. The presence of social support is linked to increases in natural killer cell cytotoxicity in patients with ovarian cancer (Lutgendorf et al., 2005), stronger antibody response to vaccine (Glaser et al., 1992), lower levels of cortisol (Rosal, King, Ma, & Reed, 2004), and studies consistently conclude that higher social support is associated with better immune functioning (Uchino et al., 1996). In the current study, the correlation between self-report and EAR-assessed social support is significant, $r = .27$, $p < .01$, suggesting the two are tapping similar constructs. However, these two measures evaluate very different aspects of social support. The ISEL asks about the existence of someone the respondent could *hypothetically* turn to if they needed something (i.e. "There is someone I can turn to for advice about handling problems with my family,") whereas the EAR-assessed

support measure is measuring the literal receipt of positive social support. These measures clearly represent the important difference between perceived (subjective) and actual (objective) social support. In the current study, the average percent of EAR files during the V1 weekend that contained receipt of positive support was about 2%, and it ranged from 0 to 10% of files. Given the low percentage of positive support received in the sample, the strength of its effects on immune outcomes is telling of how a little support can go a long way.

Aim 3: Concurrent and Prospective Mediation

A third aim of the current study hypothesized the presence of mediational effects. I expected that social integration at V3 would mediate the relationship between psychological distress at V1 and immune outcomes at V5 (**H3**), such that more psychologically distressed participants might be less likely to be socially integrated, and thus suffer the double negative of distress and isolation, revealing heightened levels of risky immune parameters. Alternatively, it could be that being socially integrated over the course of divorce recovery might serve as a buffer against the effects of psychological distress. Concurrent mediation models indicated mediation of the relationship between psychological distress and immune outcomes by social integration for models using immune phenotypes as the outcome. No total effect existed between psychological distress and immune outcomes. Non-zero indirect effects reveal that higher levels of psychological distress have a positive indirect effect on IRP via their negative effect on social integration.

I also explored the competing possibility that psychological distress at V3 might explain the relationship between social integration at V1 and immune outcomes at V5. No mediational models specified this way yielded significant results with any individual residual immune parameters or the residual IRP variables.

Longitudinal Assessment of Immune Outcomes

In series of additional, exploratory analyses, I investigated the effects of V1 social integration on changes in immune phenotypes over time. Although social integration at V1 only significantly predicted change in IRP before including covariates, suggesting that these covariates better accounted for the phenotype changes, the negative (though non-significant) association between social integration and IRP was intriguing. In exploring these surprising findings, I determined that individual differences in attachment avoidance may play an important role in shaping how social behaviors (following a separation or divorce) are associated with immune activity. Results revealed the interaction of social integration and self-reported attachment avoidance significantly predicted *increases* in 4-item IRP change scores. Simple slopes indicate that this association was significant only for those reporting high levels of attachment avoidance (one *SD* greater than the mean). For individuals reporting high levels of avoidance, a construct marked by a desire to suppress or deny thoughts and feelings associated with the ex-partner, being highly socially integrated at V1 is associated with increases in 4-item IRP from V1 to V5. One-way speculative interpretation of this finding is that for people high in attachment avoidance, social integration may result in constant bombardment of thoughts about the separation and their ex-partner; in this way, social involvement may force people high in attachment avoidance to engage in emotion regulatory strategies of suppression and avoidance that can have an effect on inflammation and control of latent viruses. For example, in intact couples, participants reporting higher levels of avoidant attachment evidenced an increased inflammatory response following a conflict related discussion with their partner compared to more securely attached participants (Gouin et al., 2009). The activation of such avoidant strategies is also associated with changes in heart rate variability (Sbarra & Borelli, 2013), which

is an important predictor of cardiovascular health. In light of this prior work, the current finding suggests an important avenue for continued research with a particular focus on the match between social context and adults' preferred means of regulating strong emotions around interpersonal events.

Social Behaviors and Health Outcomes

As one of a handful of studies to explore immune outcomes in recently separated or divorced individuals longitudinally, these results have implications for developing a deeper understanding of the health trajectories following the dissolution of marriage. Importantly, initially engaging in positive social behaviors such as spending time with others and cultivating relationships that yield positive support may decrease the risk for elevated levels of inflammation and the reactivation of previously latent viruses. The positive physical effects of social support and integration considered one of the most well-documented predictors of physical health outcomes (Uchino et al., 2012), however the current study established this effect independent of the contributions of self-reported loneliness, and independent of a composite measure of self-reported psychological distress which included loneliness. This general finding is tempered by differential implications of social integration over time. For people who self-report having avoidant attachment styles, more regular engagement over time with one's social support network may increase the need for avoidant coping strategies associated with this style, thus increasing the probability of maintaining higher scores on the immune phenotype over the five-month study. For these people, it is possible that the typical buffering effects of social integration serve to activate their avoidant tendencies and the regulatory effort associated with suppressing thoughts and emotions. Cacioppo and colleagues (2015) make mention of a possible dark side to social bonds, suggesting some relationships may be viewed as caring or supportive while others

may be seen as negative or even isolating. For example, evidence from investigations of marital quality in predicting longevity indicate that not all social bonds are purely positive (Tucker, Friedman, Wingard, & Schwartz, 1996), and negative relationships predict increased mortality risk (Friedman et al., 1995). A positive association between greater family strain (as measured by self-report ratings of criticism, demands, and disappointment from one's family) and allostatic load provides further evidence that not all social bonds assert a positive influence on health outcomes (Priest et al., 2015). The current results lend credence to the idea that the quality of social relationships may moderate their influence on health outcomes, and this perception may be informed by previous experiences and personal preferences for amount of social contact (Cacioppo et al., 2015). The current study implies that, for avoidant individuals, too much social integration or the wrong *kind* over time may hurt instead of help.

Beyond the differential benefits of social relationships, this research contributes to a better understanding of the impact of *objective* social behaviors on health. The EAR-derived positive social support received variable predicted circulating levels of CRP even after controlling for self-report measures, and was an independent predictor of immune-related risk phenotype when including self-reported social support in the model. This is an innovative addition to the literature on social relationships and health. Furthermore, this is the first study of its kind demonstrate a mediated effect across multiple disparate methodologies. The indirect effect of self-reported psychological distress on health-relevant immune phenotypes through an objectively measured social integration composite suggests that psychological distress operates through social behaviors to effect concurrent immune markers, and not the other way around. This effect was strong enough to persist across multiple methods of data collection, which lends

support for the robustness of the association when the predictor and mediator share no method variance.

This study makes additional contributions to the literature on subjective and objective measures of social isolation. Despite the lack of findings when comparing objective time spent alone and self-reported loneliness, the social integration composite made up of EAR-assessed behaviors predicted immune outcomes over and above self-reported loneliness and the psychological distress composite variable, which includes loneliness. This general approach suggests that it is feasible and potentially informative to compare the potentially different effects of perceived loneliness and levels of objective social integration. In combination, the findings from measures of objective social behaviors add important new evidence to the ongoing debate in the literature about whether and how subjective and objective reports of social integration and isolation predict health outcomes. Considerable research efforts have focused on elucidating differences between objective and subjective social isolation and integration on health (Holt-Lunstad et al., 2015; Luo, Hawkley, Waite, & Cacioppo, 2012; Shankar, McMunn, Banks, & Steptoe, 2011), but the "objective" measures used by these studies are quite limited. Using marital status or number of persons in the household as an objective measure of isolation leaves much to be desired, and other measures such as number of social activities, "Do you have friends/relatives living nearby", and frequency of contact with others are, at best, a mediocre measure of actual behavior and, at worst, still relying on self-report. The EAR is able to unobtrusively collect ambient sound in the participant's environment, thereby allowing coders, blind to the hypotheses of the study, to code for the presence of target behaviors, as opposed to relying on the memories and biases of participant's self report. This method of observation is ideal for recording individual idiosyncrasies in interaction style, and provides an accurate

portrayal of the frequency of naturalistic behavior outside the confines of the laboratory and outside the demand characteristics that bias traditional self-report (Mehl et al., 2012). The current study clearly improves the strength of the work in this area by adding daily, objective methodology for measuring social isolation and integration. The potential contributions of the EAR methodology in this area are enormous (Mehl, 2007), and as of yet there are no findings linking EAR-assessed social behaviors with immune outcomes.

Failures to Replicate

There are several potential explanations for the lack of support for some of the hypotheses, both general to psychoneuroimmunology and more specific to the design of this study. First and foremost, the sample size of this study was small, as it was a pilot study and designed to demonstrate that this data could be successfully collected. A *N* of 62 for biological specimen collection is not in and of itself unheard of (see *N*s in Herbert and Cohen's 1993 meta-analysis of immunology studies ranging from 53 to 555 and Segerstrom and Miller's 2004 meta-analysis ranging from 56 to 1,026), but reduction in *N*s occurred due to participants declining to come in for their second sample, attrition from the bigger divorce and sleep study, raw values outside of established healthy ranges, and idiosyncratic failures in biological assays. Given these events, the sample size for individual immune parameters varied from 52 to 61 at V1, and from 42 to 51 at their second measurement, V5. With these constraints in mind, creating variables modeled on allostatic load summaries made sense, as missing data could be scored as a zero and summary scores could still be calculated. The resulting immune risk phenotype scores can be considered conservative, and may provide further explanation for discrepancies between the current study and previous findings.

The lack of association between psychological distress and any immune measures (concurrent or change-scored) was particularly unexpected, as previous research indicates marital dissolution is associated with increases in depression (Kiecolt-Glaser et al., 1987) and loneliness (Van Tilburg, Aartsen, & Van Der Pas, 2015). These increases, coupled with the findings that measures of inflammation are robustly associated with depression (Dantzer et al., 2008), immune differences have been found in studies of grief following bereavement (Schultze-Florey et al., 2012), and the perception or appraisal of stressful events is associated with immune measures (Herbert & Cohen, 1993; Segerstrom & Miller, 2004), suggests that my basic study design was positioned to replicate these significant relationships.

One potential explanation for lack of findings is that psychoneuroimmunological investigations often look at multiple measures (e.g. IL-6 production, numbers of T cells, natural killer cell cytotoxicity, to name only a few), and although some may change significantly following divorce, many studies report no differences between married and divorced individuals on various immune parameters. For example, Kiecolt-Glaser et al. (1987) reported significant immunological differences between married participants and their divorced counterparts (e.g. group differences in EBV antibody titers, natural killer cell percentages, and on proliferative response to the mitogen PHA in the expected direction), but found no differences in percentages of several types of T lymphocytes or proliferative response to the mitogen Con A. Segerstrom and Miller (2004) point out that the type of stressor (i.e. chronic vs. acute) and the type of immune response investigated (i.e. enumerative measures such as the number of circulating natural killer cells vs. functional measures of the immune system including natural killer cell cytotoxicity) matter. Chronic stressors tended to be related to more functional measures in their meta-analysis, but this was the exception, as inconsistent immune system response is the general

rule. In this way, the current study's disparate results are not unprecedented, but pin-pointing the exact immune mechanisms that are most involved in pathways to health from a given stressor remains difficult.

A more study-specific limitation may be that while divorce is generally accepted as a stressful life event, it does not present all adults with a high degree of psychological distress (Amato & Booth, 1991). In fact some people experience an increase in life satisfaction following divorce (Bourassa, Sbarra, & Whisman, 2015; Gustavson et al., 2012), and thus while many participants may exhibit some distress, a large group of people were likely quite resilient following their separation. This resilience may have washed out the effects of self-reported psychological distress on immune outcomes, and this sub-study was notably under-powered to examine the possibility that immune dysregulation would be observed only among people experiencing more extreme negative reactions to their separation. Additionally, meta-analyses by Herbert and Cohen (1993) and Segerstrom and Miller (2004), results indicated that subjective reports of psychological stress were generally not associated with immune outcomes, whereas objective stressors were reliably associated with immune alterations. As the psychological distress composite was made up entirely of self-reported distress, it is possible that these effects were simply not strong enough in this sample to produce significant results. This may also explain why the objective measures of social integration made up of coded daily behaviors were successful in predicting some immune outcomes.

Limitations

Findings from the present study must be viewed in light of several limitations. These results should be considered preliminary, due to small sample size. Furthermore, these findings may over-emphasize the experiences of women, as the sample included an unequal sex

distribution. Women who reported an avoidant attachment style are likely to exhibit increases in cortisol following a conflict conversation with relational partners compared to less avoidant women, while this pattern of increased cortisol was demonstrated only by anxiously attached men (Powers, Pietromonaco, Gunlicks, & Sayer, 2006). This sample, being skewed female, may be providing results that are are most generalizable to women. More generally, another limitation (from the perspective of immune assessment) is the relatively young age of the sample. Many studies indicate that age plays a large role in immune outcomes, noting that as people age their immune system undergoes a process of senescence, and becomes more vulnerable to negative life events (Segerstrom & Miller, 2004). The mean age for the sample in the present study was 42.61 years (ranging from 21 to 64 years), and generally "older adults" are defined as being at least 50 years, if not older (<65, in many studies). In this sample, about 70% of participants fell under the conservative "older adult" definition of 50, and the immune systems of the participants may have been more likely to be resilient given the sample's youth. In addition, the sample is relatively healthy, as they were selected on the basis of being free of most major mental disorders (e.g. bipolar disorders or schizophrenia) and being free of any uncontrolled medical conditions. Disease status is another moderator of immune system vulnerability and senescence (Segerstrom & Miller, 2004).

Future Directions

Further research is necessary to replicate the disparate findings noted in the current study, as some aspects of the results differ from those in the published literature. In particular, more studies employing the EAR-derived social behaviors as measurement of objective social isolation are necessary to solidify the methodology and elucidate the current equivocal understanding of the contributions of objective and subjective social isolation to health

outcomes. There is no argument that objective and subjective isolation are relevant for health, but the importance of one over the other, or their equal import, remains to be seen. This type of research is important because current interventions for social integration to improve health have not been particularly successful (Cattan, White, Bond, & Learmouth, 2005), therefore a better understanding of the most important variables involved in the pathway between relationships and health may serve to identify the most potent targets for future trials. The mediational findings of the current study shed light on the possibility of developing interventions to target psychological responses to divorce by altering social behaviors which have downstream effects on immune functioning and future health outcomes.

Regarding longitudinal outcomes, future studies might address the specifics of the social integration × attachment avoidance interaction. Measuring self-reported attachment avoidance is a partial test of this effect, but indexing actual avoidant behaviors following marital separation, or, better yet, inducing this behavior in the lab and testing effects on immune outcomes over time represents an important future direction for this research. Elucidating the differential effects of social integration by individual differences is likely important in determining for whom particular social behaviors are the most beneficial, or perhaps, the most harmful.

Conclusion

The primary findings of the current study add much to the literature concerning social integration and physiological health. The present analysis found that an objective composite of positive social behaviors predicted concurrent immune markers in recently separated adults, and individual measures of objective and subjective positive social support were associated with immune responses as well. These social behaviors may operate indirectly to mediate the association between psychological distress and immune outcomes. Further, I discovered that,

over time, there are differential effects of objectively measured social integration, such that for some individuals there are fewer benefits, or more risks, associated with spending more time embedded in social networks based on attachment style. These results emphasize the individual variation in response to a major life event such as divorce, and illuminate new avenues for understanding how such events may "get under the skin" to affect relevant biological pathways leading to morbidity and mortality following marital transitions.

APPENDIX A - TABLES

Table 1
Demographics and Correlation Table for Demographics and Immune Variables

Variables	1	2	3	4	5	6	7	8
Age (1)	1.0							
Sex (2)	.22	1.0						
Waist-Hip-Ratio (3)	.13	.24	1.0					
NSAID Use (4)	.19	.24	-.02	1.0				
IL-6 (5)	.26*	.06	.05	-.10	1.0			
CRP (6)	-.06	-.06	.01	-.06	.20	1.0		
CMV (7)	.18	-.12	-.08	-.06	.51**	.12	1.0	
EBV (8)	.16	.08	-.20	0.20	-.06	.14	.09	1.0
Mean	42.61	0.32	0.90	0.10	1.05	1.50	214.18	452.47
SD	11.31	0.47	0.15	0.30	0.92	1.85	507.19	491.62

Note. Sex coded 0 = female, 1 for male; NSAID Use = Non-steroidal anti-inflammatory drugs, and is coded 0 = no, 1 = yes; IL-6 = Interluekin-6; CRP = C-Reactive Protein; CMV = antibody titers to Cytomegalovirus; EBV = antibody titers to Epstein Barr Virus. All immune parameters are in raw units.
*p <.05; **p<.01.

Table 2
Demographics and Correlation Table for Psychological Distress and Immune Variables

Variables	1	2	3	4	5	6
Grief (1)	1.0					
Depression (2)	.21	1.0				
IES-R (3)	.22	.76**	1.0			
Loss of Self (4)	.24	.66**	.53**	1.0		
Loneliness (5)	.09	.61**	.57**	.57**	1.0	
Psych Com (6)	.47**	.85**	.80**	.86**	.72**	1.0
IL-6 (7)	.17	.11	-.03	.18	.19	.17
CRP (8)	.08	-.07	-.02	-.10	.20	.00
CMV (9)	.10	.24	.07	.06	.10	.14
EBV (10)	.21	-.10	-.01	-.15	-.14	-.06
Mean	2.21	2.13	1.43	3.10	2.00	2.17
SD	0.90	0.68	0.89	1.40	0.66	0.67

Note. IES-R = Impact of Events Scale- Revised; Psych Com = Psychological Distress Composite; IL-6 = Interluekin-6; CRP = C-Reactive Protein; CMV = antibody titers to Cytomegalovirus; EBV = antibody titers to Epstein Barr Virus. All psychological distress variables are scale means; the Psychological Composite is the mean of these means. All immune parameters are in raw units. *p <.05; **p<.01.

Table 3
Demographics and Correlation Table for Social Behaviors and Immune Variables

Variables	1	2	3	4	5	6
Others (1)	1.0					
Soc/Ent (2)	.55**	1.0				
Sub. Conv. (3)	.68**	.53**	1.0			
Pos. Support (4)	.22	.50**	.18	1.0		
Social Com (5)	.81**	.80**	.80**	.60**	1.0	
Alone (6)	-1.0**	-.54**	-.67**	-.21	-.81**	1.0
IL-6 (7)	-.17	-.20	-.20	-.15	-.22	.16
CRP (8)	-.25	-.13	-.13	-.31*	-.28	.24
CMV (9)	-.13	-.23	-.18	-.14	-.22	.10
EBV (10)	.02	-.12	.09	-.26	-.08	-.03
Mean	0.41	0.13	0.17	0.02	-0.01	0.59
SD	0.22	0.13	0.12	0.02	0.76	0.22

Note. Others = Time spent with others; Soc/Ent = Time spent socializing/entertaining; Sub. Conv. = Time spent in substantive conversation; Pos. Support = Time spent receiving positive support; Social Com = Social Integration Composite; IL-6 = Interluekin-6; CRP = C-Reactive Protein; CMV = antibody titers to Cytomegalovirus; EBV = antibody titers to Epstein Barr Virus. For all social behaviors, means represent the mean percentage of EAR files over the course of the weekend that participants were coded as having been engaged in the behavior, the Social Integration Composite is the mean of those standardized variables. All immune parameters are in raw units.

*p <.05; **p<.01.

Table 4

Unstandardized Coefficients from Regression Models Predicting Immune Composite

Outcome: Imm Com	*B*	95% CI	*B*	95% CI
Intercept	0.05	[-0.13, 0.24]	0.37	[-1.04. , 1.78]
Social Composite	-0.22*	[-0.41, -0.04]	-0.21*	[-0.41 , -0.01]
Age			0.01	[-0.01 , 0.03]
Sex			-0.18	[-0.61 , 0.25]
NSAID Use			-0.05	[-0.67 , 0.58]
Waist-Hip-Ratio			-0.70	[-2.19 , 0.78]
Intercept	0.04	[-0.15 , 0.22]	0.32	[-1.05 , 1.68]
Social Composite	-0.20*	[-0.39 , -0.01]	-0.15	[-0.36 , 0.05]
Psych Composite	0.02	[-0.16 , 0.21]	0.12	[-0.09 , 0.32]
Age			0.01	[-0.01 , 0.03]
Sex			-0.36	[-0.80 , 0.09]
NSAID Use			-0.03	[-0.64 , 0.58]
Waist-Hip-Ratio			-0.78	[-2.22 , 0.67]
Intercept	0.03	[-0.16 , 0.22]	0.24	[-1.12. , 1.59]
Social Composite	-0.19	[-0.39 , 0.00]	-0.14	[-0.35 , 0.07]
Loneliness	0.05	[-0.14 , 0.24]	0.14	[-0.07 , 0.34]
Age			0.01	[-0.01 , 0.03]
Sex			-0.39	[-0.85 , 0.07]
NSAID Use			-0.02	[-0.62 , 0.59]
Waist-Hip-Ratio			-0.65	[-2.07 , 0.78]

Note: IL=6 =logged Interleukin 6. CRP = logged C-Reactive Protein. Imm Com = Immune Composite. CI = confidence interval. Social Composite = Social Integration Composite. NSAID = Non-steroidal anti-inflammatory drugs and is coded 0 = no, 1 = yes; Sex coded 0 = female, 1 for male; Psych Composite = Psychological Distress Composite.

* $p < .05$.

Table 5
Unstandardized Coefficients from Regression Models Predicting CRP

	B	95% CI	*B*	95% CI
Intercept	-0.05	[-0.17, 0.08]	-0.23	[-1.15 , 0.69]
Other	-0.08	[-0.25, 0.09]	-0.11	[-0.32 , 0.10]
Soc/Ent	0.05	[-0.12, 0.09]	0.08	[-0.10 , 0.27]
Sub Conv	0.02	[-0.14, 0.19]	0.07	[-0.11 , 0.26]
Pos Support	-0.18*	[-0.33, -0.02]	-0.21**	[-0.37 , -0.50]
Age			0.00	[-0.01 , 0.02]
Sex			-0.13	[-0.42 , 0.16]
NSAID			-0.34	[-0.82 , 0.15]
Waist-Hip-Ratio			0.15	[-0.80 , 1.09]

Note: CRP = C-Reactive Protein; CI = confidence interval; Others = Time spent with others; Soc/Ent = Time spent socializing/entertaining; Sub Conv = Time spent in substantive conversation; Pos Support = Time spent receiving positive support; NSAID = Non-steroidal anti-inflammatory drugs and is coded 0 = no, 1 = yes; Sex coded 0 = female, 1 for male.
* $p < .05$. ** $p < .01$.

Table 6

Unstandardized Coefficients from Regression Models Predicting Immune Variables

Outcome: CRP	*B*	95% CI	*B*	95% CI
Intercept	-0.04	[-0.16, 0.08]	-0.31	[0.16 , 0.08]
ISEL	0.02	[-0.11, 0.15]	0.00	[-0.14 , 0.15]
Pos Support	-0.17*	[-0.31, -0.31]	-0.17*	[-0.31 , -0.03]
Age			0.00	[-0.01 , 0.02]
Sex			-0.14	[-0.44 , 0.16]
NSAID Use			-0.19	[-0.61 , 0.24]
Waist-Hip-Ratio			0.20	[-0.86 , 1.26]
Outcome: Imm Com	*B*	95% CI	*B*	95% CI
Intercept	0.01	[-0.17, 0.20]	0.28	[-0.96 , 1.51]
ISEL	-0.17	[-0.36, 0.03]	-0.30**	[-0.49 , -0.10]
Pos Support	-0.16	[-0.37, 0.05]	-0.16	[-0.36 , 0.03]
Age			0.02	[0.00 , 0.04]
Sex			-0.51	[-0.92 , -0.10]
NSAID Use			-0.07	[-0.62 , 0.47]
Waist-Hip-Ratio			-1.05	[-2.49 , 0.39]

Note: CRP = C-Reactive protein; Imm Com = Immune Composite; CI = confidence interval; ISEL = mean of Interpersonal Support Evaluation List; Pos Support = Time spent receiving positive support; NSAID = Non-steroidal anti-inflammatory drugs and is coded 0 = no, 1 = yes; Sex coded 0 = female, 1 for male.

* $p < .05$.

Table 7
Unstandardized Coefficients from Regression Models Predicting IRP Variables

Outcome: 4-IRP	*B*	95% CI	*B*	95% CI
Intercept	0.93**	[0.67, 1.20]	2.00	[-0.05 , 4.03]
Social Composite	-0.40**	[-0.65, -0.13]	-0.40*	[-0.71 , -0.10]
Age			0.00	[-0.03 , 0.03]
Sex			0.05	[-0.59 , 0.68]
NSAID Use			-0.02	[-1.00 , 0.95]
Waist-Hip-Ratio			-1.35	[-3.44 , 0.75]
Intercept	0.89**	[0.63 , 1.16]	1.80	[-0.18 , 3.79]
Social Composite	-0.37**	[-0.63 , -0.10]	-0.35*	[-0.66 , -0.05]
Psych Composite	-0.00	[-0.27 , 0.26]	0.06	[-0.23 , 0.35]
Age			0.01	[-0.02 , 0.04]
Sex			-0.16	[-0.82 , 0.51]
NSAID Use			0.03	[-0.92 , 0.98]
Waist-Hip-Ratio			-1.33	[-3.34 , 0.71]
Intercept	0.90**	[-0.64 , 1.16]	1.76	[-0.21 , 3.73]
Social Composite	-0.34*	[-0.61 , -0.08]	-0.33*	[-0.64 , -0.02]
Loneliness	0.07	[-0.20 , 0.35]	0.13	[-0.17 , 0.43]
Age			0.01	[-0.02 , 0.04]
Sex			-0.22	[-0.90 , 0.45]
NSAID Use			-0.02	[-0.92 , 0.96]
Waist-Hip-Ratio			-1.28	[-3.29 , 0.74]

Note: IRP = Immune Risk Phenotype; 4-IRP = 4-item IRP; CI = confidence interval. Social Composite = Social Integration Composite; NSAID = Non-steroidal anti-inflammatory drugs and is coded 0 = no, 1 = yes; Sex coded 0 = female, 1 for male; Psych Composite = Psychological Distress Composite.
* $p < .05$. ** $p < .01$.

Table 8
Unstandardized Coefficients from Regression Models Predicting IRPs

Outcome: 4-IRP	*B*	95% CI	*B*	95% CI
Intercept	0.87**	[0.61, 1.14]	1.43	[-0.45 , 3.32]
ISEL	-0.14	[-0.43, 0.15]	-0.31	[-0.63 , 0.01]
Positive Support	-0.31*	[-0.60, -0.02]	-0.31*	[-0.59 , -0.02]
Age			0.02	[-0.00 , 0.05]
Sex			-0.41	[-1.06 , 0.25]
NSAID Use			-0.27	[-1.15 , 0.61]
Waist-Hip-Ratio			-1.62	[-3.83 , 0.58]
Outcome: 3-IRP	*B*	95% CI	*B*	95% CI
Intercept	0.97**	[0.74 , 1.20]	0.91	[-0.73 , 2.54]
ISEL	-0.18	[-0.43, 0.06]	-0.26*	[-0.51 , -0.00]
Positive Support	-0.24	[-0.50, 0.02]	-0.23	[-0.48 , 0.03]
Age			0.02	[0.00 , 0.05]
Sex			-0.23	[-0.77 , 0.31]
NSAID Use			-0.61	[-1.38 , 0.15]
Waist-Hip-Ratio			-0.92	[-2.82 , 0.98]

Note: 4-IRP = 4-item Immune Risk Phenotype; 3-IRP= 3-item Immune Risk Phenotype; CI = confidence interval; ISEL = mean score from Interpersonal Support Evaluation List; Positive Support = Time spent receiving positive support; NSAID = Non-steroidal anti-inflammatory drugs and is coded 0 = no, 1 = yes; Sex coded 0 = female, 1 for male.
* $p < .05$. ** $p < .01$.

Table 9

Unstandardized Coefficients from Regression Models Predicting Change in IRP

Outcome: Ch 4-IRP	*B*	95% CI
Intercept	1.80	[-1.15, 4.75]
Social Composite	-0.57	[-1.25, 0.11]
Avoidance	-0.31**	[-0.53, -0.09]
Interaction	0.26*	[0.05, 0.47]
Age	0.00	[-0.02, 0.03]
Sex	0.34	[-0.34, 1.02]
NSAID Use	-0.04	[-0.93, 0.85]
Waist-Hip-Ratio	-1.31	[-4.91, 2.28]
Outcome: Ch 3-IRP	***B***	**95% CI**
Intercept	1.08	[-1.06, 3.22]
Social Composite	-0.42	[-0.95, 0.12]
Avoidance	-0.23*	[-0.41, -0.06]
Interaction	0.16	[-0.01, 0.32]
Age	-0.00	[-0.02, 0.02]
Sex	0.10	[-0.41, 0.61]
NSAID Use	0.20	[-0.50, 0.89]
Waist-Hip-Ratio	-0.35	[-3.00, 2.30]

Note: Ch 4-IRP = Change in 4-item Immune Risk Phenotype from V1 to V5; Ch 3-IRP= Change in 3-item Immune Risk Phenotype from V1 to V5; CI = confidence interval; Social Composite = Social Integration Composite; Avoidance = Attachment Avoidance; NSAID = Non-steroidal anti-inflammatory drugs and is coded 0 = no, 1 = yes; Sex coded 0 = female, 1 for male.

* $p < .05$. ** $p < .01$.

APPENDIX B - FIGURES

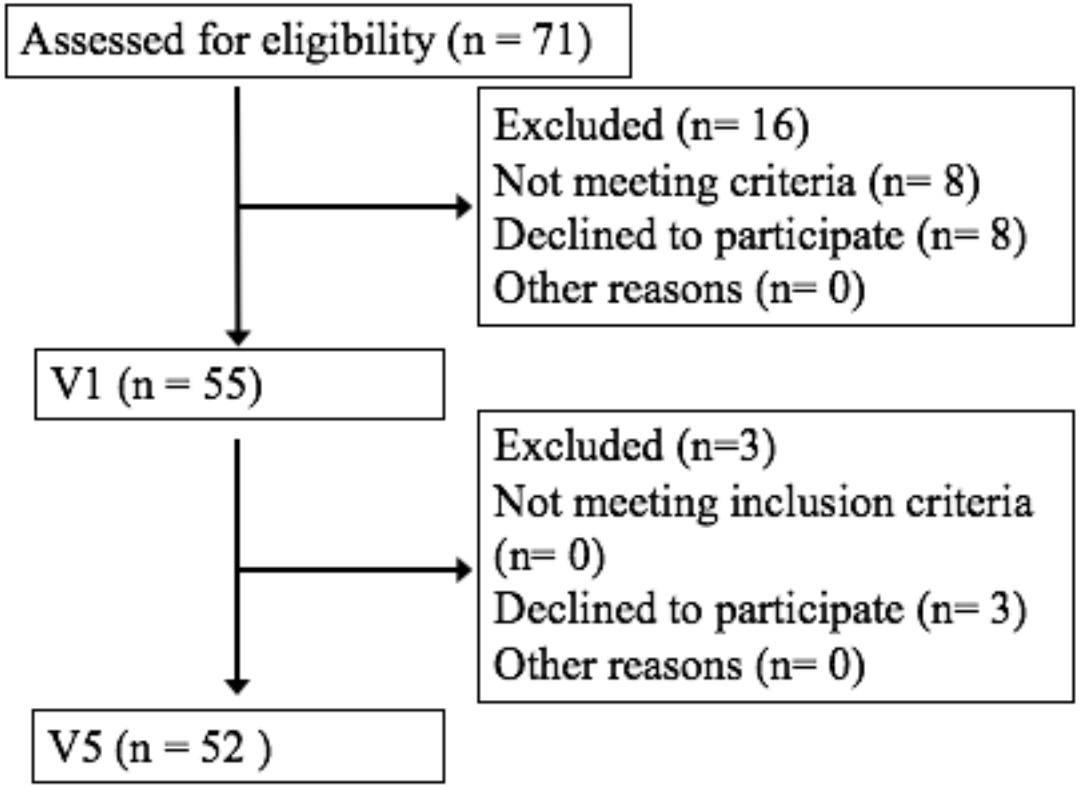

Figure 1. Flowchart showing selection and attrition of participants. V1 = visit 1; V5 = visit 5.

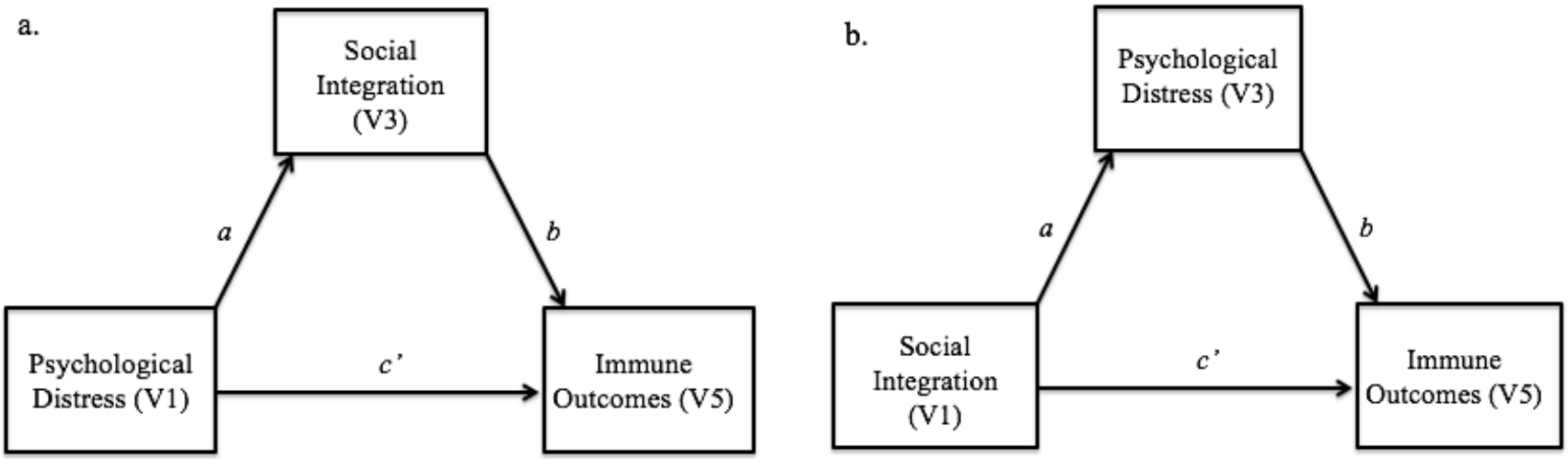

Figure 2. Mediation models for Aim 3. Panel A indicates the hypothesized direction where psychological distress influences immune outcomes through social integration. Panel B indicates the alternate possibility, that social integration influences immune outcomes through psychological distress.

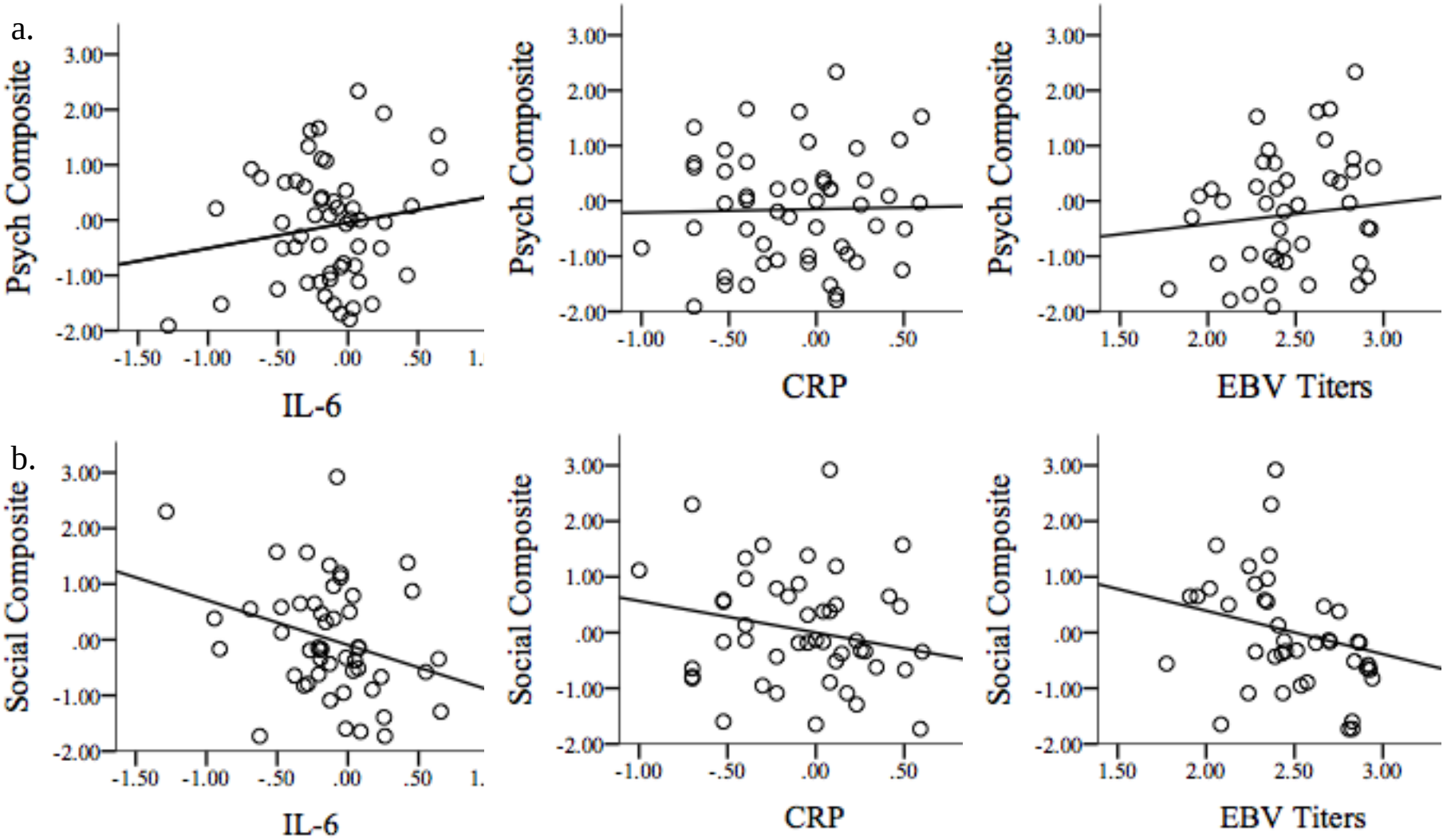

Figure 3. Panel A displays scatterplots of the standardized Psychological Composite plotted against selected logged immune variables. Panel B displays scatterplots of the standardized Social Integration Composite plotted against selected logged immune variables. Note: IL-6 = Interluekin-6; CRP = C-Reactive Protein, EVB = Epstein-Barr Virus.

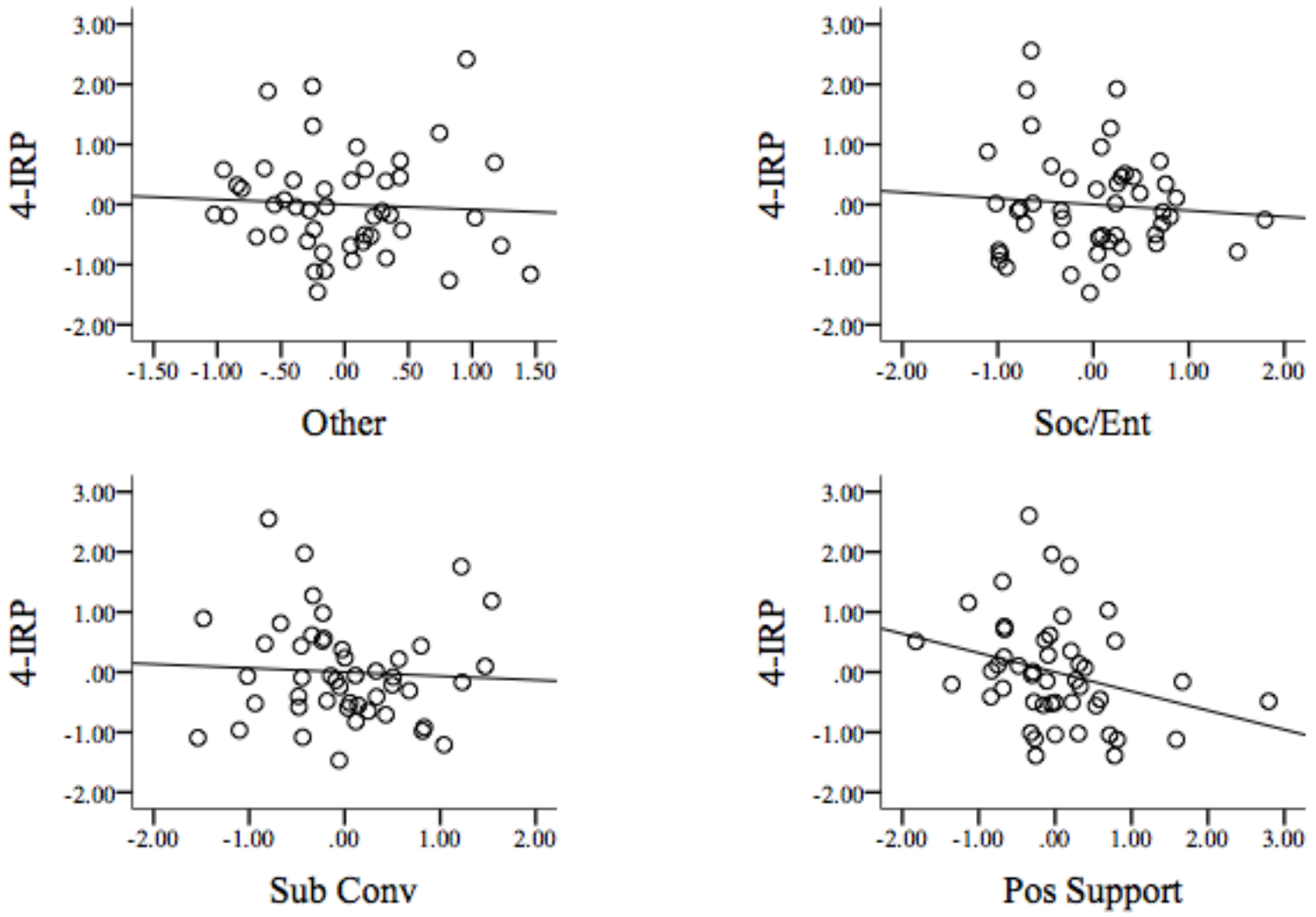

Figure 4. Scatterplots of the standardized individual social behaviors plotted against the 4-item Immune Risk Phenotype. All social behaviors are standardized. 4-IRP = 4-item Immune Risk Phenotype; Other = Time spent with others, Soc/Ent = Time spent socializing/entertaining; Sub Conv = Time spent in substantive conversation; Pos Support = Time spent receiving positive support.

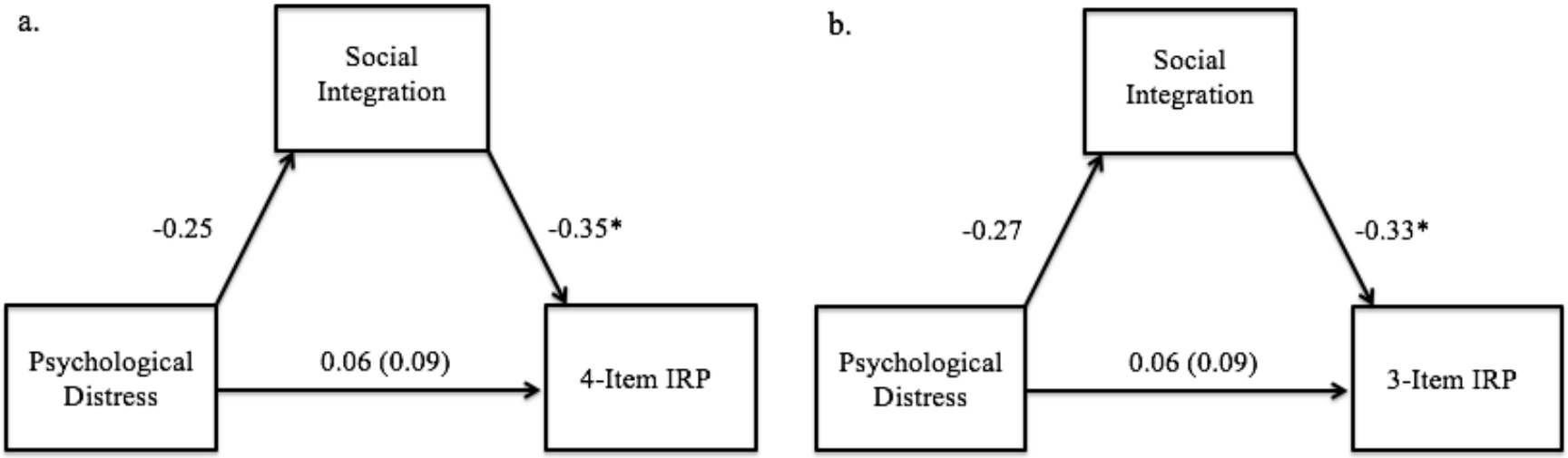

Figure 5. Concurrent mediation models predicting Immune Risk Phenotype variables. Panel A indicates the hypothesized direction where psychological distress influences the 4-item Immune Risk Phenotype through social integration. Panel B indicates the alternate possibility, that social integration influences immune outcomes through psychological distress. 4-IRP = 4-item Immune Risk Phenotype; 3-IRP = 3-item Immune Risk Phenotype.

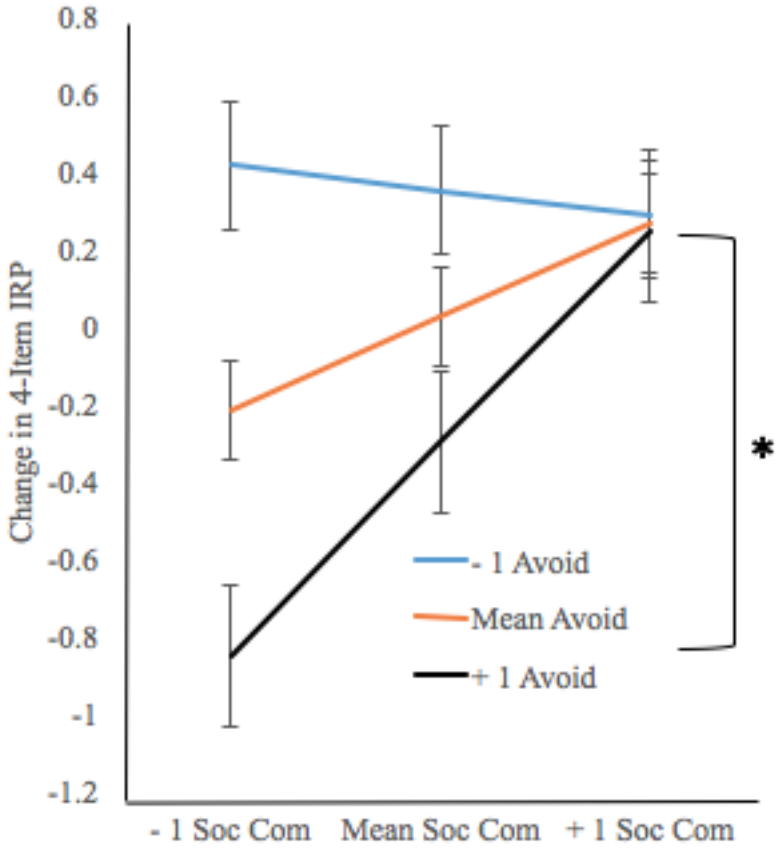

Figure 6. Simple slope decomposition of the interaction between Social Integration and Attachment Avoidance (after accounting for relevant covariates) predicting change in the 4-item Immune Risk Phenotype. Simple slope decompositions are illustrated for peopling scoring 1 *SD* +/- the mean of Attachment Avoidance.

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