SOCIAL RELATIONSHIPS, DAILY SOCIAL INTERACTIONS, AND INFLAMMATION

By

Amoha Bajaj

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This thesis was presented

by

Amoha Bajaj

It was defended on

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and approved by

Sheldon Cohen, Professor, Department of Psychology

Anna L. Marsland, Associate Professor, Department of Psychology

Thesis Director: Thomas W. Kamarck, Professor, Department of Psychology

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Amoha Bajaj, M.S.

University of Pittsburgh, 2015

Social integration (SI) and perceived social support (SS) are associated with reduction in premature mortality, while poor marital quality (MQ), and social conflict (SC) are associated with negative health outcomes. Systemic inflammation has been proposed as a mechanism accounting for these associations. However, the literature exploring the association between aspects of social relationships and inflammation has yielded inconsistent findings. The extent to which daily social interactions may play a role in the association of SI, SS, MQ, and SC with inflammatory markers in humans is currently unknown. The literature also shows stronger evidence for links between inflammation and SC, than between inflammation and positive relationship features, although these two sets of associations have rarely been compared in the context of a single study. Using ecological momentary assessment of social measures, this project aims to examine the relationship between daily social interaction characteristics and inflammatory markers, CRP and IL-6, and to compare negative interactions with positive interactions in their association with inflammatory biomarkers, in a sample of 494 men and women, using a cross-sectional design. This results of this study show no significant associations between global measures of social integration, social support, and marital quality, and either inflammatory biomarker. There was also no association found between the frequency of social interactions and the proportion of negative social interactions with inflammatory biomarkers. However, in this sample, the proportion of positive interactions was positively associated with

CRP level. Additional exploratory analyses were conducted to test the robustness of this finding and it was found that this association existed in married individuals, females, and particularly, married females, suggesting that this finding may not be robust and should be interpreted with caution. And lastly, in a subsample of married individuals, there was no association found between the frequency and quality of marital interactions and either inflammatory marker.

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PREFACE

I would like to acknowledge Dr. Kamarck’s extensive guidance in this thesis and I would like to thank all members of the committee for their support and feedback in this project.

1.0 INTRODUCTION

Accumulating evidence suggests that individuals who perceive themselves as more supported by others, who are better socially integrated in their social networks, and experience less social conflict in their relationships are at lower risk for premature mortality (Blanchard et al., 1985; Kroenke et al., 2013; Stringhini et al., 2012; Steptoe et al., 2012; Holt-Lunstad et al., 2010). Social support, social integration, and social conflict pertain to different characteristics of social relationships, with social support referring to the perceived availability of emotional and informational resources, social integration referring to the number of social roles in one's social network, and social conflict referring to interpersonal stress. There is evidence to suggest that social conflict, in addition to social support and social integration, is one of the features of social relationships that is uniquely associated with health outcomes, such as susceptibility to infection, and negative and positive relationship characteristics may influence health through different mechanisms (Cohen et al., 2004). Although these three aspects of social relationships have each been linked to health outcomes, the physiological mechanisms accounting for these effects are not well understood. One proposed pathway linking social relationships with health outcomes that has garnered interest involves the association of social relationships in reduced chronic, systemic inflammation (Kiecolt-Glaser et al. 2010; Uchino 2006).

Inflammation can be seen in the body in a local and systemic fashion. Local inflammation is an adaptive process that takes place in response to physical injury or infection

and is characterized by redness, swelling, rising temperature, pain, and perhaps loss of function (Hansel et al., 2010). These signs reflect increased blood flow and capillary permeability, release of inflammatory mediators, and leukocyte migration to the site of infection/injury. These processes are well orchestrated to resolve tissue damage. However, prolonged presence of these proinflammatory agents can lead to chronic inflammation. The systemic elevation of proinflammatory cytokines in chronic inflammation is associated with increased risk for health conditions, such as cardiovascular disease (Black & Garbutt, 2002).

Interleukin (IL)- 6 is a proinflammatory cytokine that can be reliably detected in peripheral blood and is widely assessed as a marker of systemic inflammation. IL-6 stimulates the production of acute phase proteins, including C-reactive protein (CRP), by the liver. CRP is another widely measured marker of chronic inflammation. Care must be taken in assuming the source of circulating IL-6 because it is produced by many different cells in addition to immune cells. For example, adipocytes, muscle, and endothelial cells all release IL-6 and contribute to circulating levels. Regardless of source, circulating levels of IL-6 and CRP are widely used as indicators of general systemic inflammation and can provide information regarding the links between social relationships and disease etiology. A positive association of circulating levels of IL-6 with psychosocial stress has been relatively consistently found (Hansel et al., 2010). In addition, elevations in IL-6, as well as CRP, have both been associated with increased risk for cardiovascular disease (Kaptoge et al., 2010).

Two different measures of inflammatory markers are reported in the literature. The first provides a measure of the level of the inflammatory marker that is in peripheral circulation. Circulating measures provide an index of current levels of systemic inflammation; relatively consistent evidence has linked circulating levels of inflammatory mediators to health risk. Other

studies employ a different measure of inflammation, measuring magnitude of inflammatory response to ex vivo stimulation. This process entails exposing the immune cells to an immune stimulant (e.g., endotoxin) and observing the increase in the concentration of proinflammatory cytokines over a period of incubation. These stimulated measures examine the ability of immune cells to respond to endotoxin and are a measure of immune competence. These methods are conceptually measuring two different indices of inflammation, are often unrelated, and should be interpreted quite differently.

Tissue injury is one important stimulus of acute inflammation, but even in the absence of injury, studies have shown that stress alone can induce an inflammatory response, characterized by fever, sickness, and increased production of proinflammatory cytokines by immune cells stimulated by endotoxin (Black & Garbutt, 2002). By activating various stress responses in the body (e.g. sympathetic nervous system, hypothalamic-pituitary-adrenal axis, renin angiotensin system), social stress and other psychosocial processes may contribute to acute and chronic inflammation (Black, 2002; Black & Garbutt, 2002; Miller et al., 2009; Kiecolt- Glaser et al., 2010).

1.1 SOCIAL SUPPORT, SOCIAL INTEGRATION, AND INFLAMMATION

There is some evidence that social isolation and lack of social support may be associated with chronic inflammation, although findings are not consistent. Seven notable studies in the epidemiological literature have examined this question and with the exception of one study discussed later (Marsland et al., 2007), all of them use circulating levels of proinflammatory mediators as their outcome. The first was a longitudinal study of 3 community-based cohorts of

older adults, ages 70-79, that explored the association between social integration and circulating levels of CRP and IL-6 (Loucks et al., 2006a). Social integration was indicated by a social network score that summed 6 measures: presence of spouse, number of close relatives, number of friends, participation in religious services, participation in other religious activities other than religious services, and participation in clubs and voluntary activities. These measures were dichotomized as above or below a threshold (e.g. participation in $\leq$ 2 religious services). Covariate- adjusted cross-sectional analyses revealed that social integration was inversely associated with plasma CRP concentration only in men and not in women, suggesting that differences may exist between men and women in the biological pathways linking social integration with health. A second epidemiological study used a younger population with a similar measure of social integration to study the association between social integration and CRP levels in a community sample of participants aged 20 or older (Ford et al., 2006). Consistent with the previous findings, this study also reported an inverse association between social integration and CRP levels only in older men, aged 60 or older.

A third epidemiological study tested the association between social integration and a number of circulating inflammatory markers, IL-6, CRP, sICAM-1 (a soluble intercellular adhesion molecule), and MCP-1 (monocyte chemoattractant protein-1), in a sample of 3,267 participants with a mean age of 62 years (Loucks et al., 2006b). Social integration was measured through the Social Network Inventory, which is a measure of diversity and frequency of participation in various social roles. Models adjusted for demographic, biobehavioral, and medical risk factors, showed that social integration was inversely associated with IL-6 in men only.

These sex-specific effects in the links between social relationships and inflammation were demonstrated once again in a fourth cross-sectional study, this time, examining perceived social support (Mezuk et al., 2010). Participants (ages 45-84 years) were administered an emotional social support index, a measure of perceived availability of emotional support, to test whether social support would be associated with circulating inflammatory markers, CRP, IL-6, and fibrinogen, which is a soluble protein present in blood plasma that has been found to contribute to the initiation and maintenance of thrombosis (Land et al., 2009). Stress was measured by a composite of five self-report items concerning on-going stressors in multiple domains (personal health, health of relative, work-life, finances, etc.). Fully adjusted models showed that low social support was associated with high CRP concentrations among men. In middle-aged women, social support moderated the association between stress and CRP, such that associations were stronger for those with low ratings of social support, suggesting that the association between social support and inflammation may vary by age and gender.

However, a fifth epidemiological study asked a similar question but reported inconsistent results. Measures of social support and integration were collected in the same sample in an effort to examine their differential associations with multiple inflammatory markers, using community-based samples from the U.S. and Taiwan (Glei et al., 2012). The social integration measure used in this study was a network score based on the diversity of social roles (e.g. married, participation in church, etc.) and frequency of interactions. The perceived social support measure was based on questions regarding the availability and quality of care and support from friends and family. In adjusted models, social integration was only weakly inversely associated with CRP levels in the Taiwan sample and, contrary to the hypothesis, perceived social support was associated with increased CRP and sIL-6R, an IL-6 receptor, in the U.S. sample.

Another study also contributed to these inconsistent results. McDade et al. (2006) used a sample of 188 participants, ages 52-70, to explore the association between a variety of behavioral/psychosocial factors, including social support, and CRP concentrations. The Interpersonal Support Evaluation List (ISEL) measured social support. There was no significant association found between social support and CRP concentration.

Generally, studies assessing the association between social support and social integration and circulating measures of proinflammatory cytokines report inconsistent results, but studies that use stimulated markers as outcomes should be considered separately, due to their measurement of immune competence. In particular, one study reported an inverse association between perceived social support, also measured by the ISEL, and the LPS-stimulated production of proinflammatory chemokine, IL-8, in a sample of 183 participants (Marsland et al., 2007), suggesting that perhaps an association exists between low social support and greater stimulated levels of proinflammatory mediators.

In sum, the epidemiological literature is characterized by some conceptual replications but a number of mixed results. When findings are shown, they tend to be age- and gender-specific in the association of social support and social integration with systemic levels of CRP and IL-6, with social support and social integration being more strongly associated with CRP in men, than in women. With one exception (Marsland et al., 2007), all studies examined circulating, rather than stimulated measures. Whereas circulating measures of IL-6 and CRP have been associated with risk for cardiovascular disease (Ridker et al., 2005; Ridker et al., 2003), stimulated measures of cytokine production assess the physiological ability to fight injury or infection (Parkin & Cohen, 2001). Increasingly, there is a distinction being made between support and conflict, with conflict potentially being a stronger predictor of inflammatory

markers. The literature investigating the associations between social conflict and inflammatory markers is explored in more depth next.

1.2 SOCIAL CONFLICT AND INFLAMMATION

When compared with positive relationship qualities, negative aspects of social interactions may be more strongly associated with measures of distress and wellbeing. In a sample of older adults, ages 65-90, it was found that negative social exchanges (such as interactions including unwanted advice, failure to provide needed help, unsympathetic behavior, rejection/neglect), were inversely associated with psychological well-being, as assessed by a 6-item questionnaire about life satisfaction, and positively associated with psychological distress, as measured by depressive symptoms endorsed on the CES-D (Newsom et al., 2005). Positive social exchanges (such as information support, instrumental support, emotional support, and companionship) were associated positively with psychological well-being but were not related to distress. This finding was replicated in another study of older women, ages 60-89, where the number of social problems was inversely associated with psychological well-being, whereas the number of social supports was unrelated (Rook, 1984).

Because of its differential associations with measures of distress and well-being, social conflict has also been explored as a potential correlate of inflammation. The literature on social conflict and inflammation consists of 2 types of research designs: an experimental social disruption (SD) model in rodent samples and correlational studies using measures of interpersonal stress in human samples. This literature is also characterized by both stimulated markers of immune function and systemic measures of chronic inflammation, as outcome

variables. First, the literature using stimulated markers of immune function will be presented, and then the literature using systemic markers of inflammation.

Rodent studies generally use the social disruption (SD) model as a form of social stress to test its association with stimulated inflammatory markers. The SD model involves introducing an aggressive intruder mouse in a cage of male mice that have already established a stable dominance hierarchy (Avitsur et al., 2006). This form of SD is repeated once a day for multiple days as a model of chronic stress. The aggressor normally starts to attack the cage residents within 5–10 min from the beginning of each session and the residents attempt to escape and/or display the characteristic behavioral signs of fear and submissiveness. If one or more of the residents attack the intruder, the intruder is replaced with a new intruder. Typically, attacks last 20–30 s, after which the intruder rests for 1-2 min.

Rodent studies have generally shown that mice that were subjected to the SD model show an increase in stimulated measures of inflammation, such as an increase in the percentage of activated neutrophils and increased production of IL-1β, TNF-alpha, and IL-6 from LPS-stimulated splenocytes, compared to controls (Curry et al., 2010; Bailey et al., 2009; Avitsur et al., 2005). One example of this finding is a study using an experimental model of endotoxic shock (Quan et al., 2001). In this model, bacterial endotoxins (i.e. lipopolysaccharide - LPS) induced the expression of high levels of proinflammatory cytokines, TNF- alpha and IL-1, and mice that were subjected to social disruption were more likely to die from endotoxic shock than control animals, who were not subjected to social disruption.

One study in this series compared a sample of younger mice with a sample of older mice, in relation to inflammatory markers and glucocorticoid (GC) sensitivity (Kinsey et al., 2008). Glucocorticoids are stress hormones released by the hypothalamic-pituitary-adrenal axis (HPA);

in humans, the primary GC hormone is cortisol and in rodents, the GC hormone is corticosterone. Although GCs generally can have an anti-inflammatory effect, there is evidence suggesting that the GC resistance promoted by chronic stress may be accompanied by increases in inflammation (Rohleder et al., 2003; Miller et al., 2002). In this study, younger mice were 2 months old and older mice were 14 $\pm$ 1 months of age. All mice were randomly assigned to 4 groups: Younger Defeat (n=6), Younger Control (n=10), Older Defeat (n=15), Older control (n=9) and the SD model was used in the Defeat groups. Results indicated that regardless of age, the defeated mice had significantly more splenic monocytes and neutrophils than controls. Supernatants from cultured splenocytes in older mice contained higher IL-6 and TNF-alpha than in younger mice. The same cells derived from the older defeated mice were hypersensitive to LPS and insensitive to GCs in vitro, as well. These data indicate that repeated social defeats result in a proinflammatory state, shown by an increased immune sensitivity to endotoxin stimulation, and that this may be exacerbated in older mice.

Interpersonal stress is also associated with stimulated markers of immune response in correlational studies using human samples. In a sample of 103 adolescent females, interpersonal stress was measured by the UCLA Life Stress Interview and IL-6 production was measured at baseline and at the 6 month period (Miller et al., 2009). The interview measured stress in various domains of social relationships (e.g. romantic, family, etc.), and produced an aggregate index that was collapsed across all of these domains. High chronic interpersonal stress at baseline was associated with increases in leukocyte mRNA for the proinflammatory transcription factor nuclear factor –kB (NF-kB) over the following 6 months after adjusting for demographics and health behaviors. Chronic interpersonal stress at baseline was also associated with increasingly pronounced IL-6 responses to LPS-stimulation. Given this evidence and the evidence presented

above, these results imply that social conflict may amplify the effects of pathogenic insult on inflammatory response, resulting in greater stimulated levels of proinflammatory biomarkers in animal and human samples.

When studying the association between interpersonal stress and circulating markers of systemic inflammation, many studies have used daily diaries to assess interpersonal stress in human samples. One study explored the association between social conflict and inflammation in a group of 53 caregivers and 77 noncaregivers between the ages of 45-90 (Gouin et al., 2012). A semi-structured interview was conducted to assess the occurrence of daily stressors in the past 24 hours. The study reported that caregivers were more likely to experience multiple stressors in the past 24 hours than noncaregiving controls, and that the occurrence of multiple daily stressors was associated with greater serum IL-6 and CRP levels. The greater occurrence of daily stressors in the past 24 hours, as measured by daily diary report, partially mediated the relationship between dementia caregiving and CRP levels. These results suggest that stressors that occur on a daily basis may be responsible for the effect of interpersonal stress on systemic levels of inflammatory markers, in a chronically stressed sample.

A second study focused on measures of daily social interactions in an adolescent sample (N=69), where participants were asked to complete daily diary checklists each night for 14 days in which they reported their experience of negative interpersonal interactions in the domains of family, peers, and school (e.g. conflict with family and friends, peer harassment) (Fuligni et al., 2009). Blood samples were obtained an average of 8.63 months later and assayed for CRP levels. In adjusted models, adolescents who reported higher interpersonal stress in daily social interactions had higher plasma levels of CRP than those with less interpersonal stress in daily life.

And, lastly, when considering the literature on interpersonal stress and systemic inflammation, a recent study distinguished between interpersonal support and strain and tested their longitudinal association with inflammatory markers, while particularly studying social relationships with family, friends, and spouse, in a sample of 647 participants (ages 24-74) in the Midlife Development in the United States (MIDUS) study (Yang et al., 2014). Support and strain were assessed using survey items. (e.g. for strain, items were if spouse, friend or family "make too many demands of you," criticize you," etc.) Fully adjusted models showed that family strain and total strain were associated with significantly higher odds of elevated fibrinogen, and family strain, friend strain, spouse strain, and total strain were associated with significantly higher odds of having elevated E-selectin. This study extends the findings of the previous study by showing that interpersonal strain was a better predictor of circulating levels of APPs and adhesion molecules than interpersonal support, and that interpersonal stress with close partners, such as family, friends, and spouse, may be particularly important.

There is only one study in the human literature, that we know of, that has included both stimulated and circulating markers of inflammation. This study required 58 rheumatoid arthritis patients to complete up to 30 daily ratings of the stressfulness in their interpersonal relationships via an abbreviated version of the Inventory of Small Life Events (Davis et al., 2008). Participants rated the extent to which negative events occurred in each of four interpersonal domains (i.e. spouse/partner, family, friends, and work) and were stressful on a 4 point scale. Individual differences in chronic stress were derived by averaging participants' daily reports of stressfulness across the four interpersonal domains, and over 30 daily ratings. Final models adjusted for demographics, biological risk factors, current pain, and steroid medication use and showed that higher chronic interpersonal stress was associated with greater IL-6 production in patients, not

circulating levels. This suggests that when there is a direct head-to-head comparison between stimulated and circulating levels of IL-6, perhaps there is a stronger association between interpersonal stress and stimulated levels of IL-6, rather than circulating levels.

In addition to stimulated and circulating measures of immune function, studies have also looked at reactivity measures of inflammatory markers after a stress-task, as another index of immune function. Stress-induced increases in inflammatory markers are interpreted to be reflective of an inflammatory phenotype that may increase risk for inflammatory conditions. But, recently, it has been suggested that while detrimental in the long term, these heightened responses may be of short-term benefit, reflecting physiological preparation for acute challenge, such as infection or injury, and may be potentially catalyzed by the activation of the autonomic nervous system in acute stress (Steptoe et al., 2007). There is little empirical evidence in support of either of these possibilities.

A recent study distinguished between daily positive, negative, and competitive interactions and their association with plasma levels of inflammatory markers, as well as reactivity measures of inflammatory markers after a stress task, involving social stress. One-hundred twenty two college students reported these types of interactions in a daily diary for 8 days (Chiang et al., 2012). Competitive interactions included those in which one competes for another's attention and academic/work-related competition, suggesting a level of social threat. Within 4 days of the daily diary, each participant completed the Trier Social Stress Test (TSST). Results suggested that negative interactions were associated with greater baseline, circulating levels of sTNFαRII, a type II receptor for TNF-α and competitive interactions predicted greater baseline levels of IL-6 and sTNFαRII. Negative interactions also predicted greater levels of IL-6 and sTNFαRII 25-min post-stressor, which are both inflammatory reactivity measures, as

opposed to circulating or stimulated measures. These findings indicate that negative and competitive interactions may be more closely linked with inflammation than positive interactions, and that negative interactions may be positively associated with not just systemic markers of inflammation, but also reactivity measures of inflammation after exposure to acute stress.

In sum, the literature focusing on social conflict and inflammation, as described above, has provided consistent evidence for an association between interpersonal stress and particularly stimulated markers of inflammation and reactivity measures of inflammation. Results have been less consistent when circulating and stimulated markers are included in the same study, with stronger findings in relation to stimulated markers, as opposed to circulating markers (Davis et al., 2008). In addition, the Davis et al., Fuligni et al., and Chiang et al. studies use a novel ambulatory methodology to measure interpersonal stress in daily life, and have reported consistent associations with inflammatory outcomes.

1.3 METHODOLOGY FOR SOCIAL VARIABLES

In the social conflict literature, rodent studies, using the SD model, and human studies, using daily diary measures, consistently report an association between interpersonal stress and inflammation. However, epidemiological studies exploring the association of social support and integration with inflammation, using global trait measures, are less consistent. This may be due to stronger effects of negative social interactions compared to positive social interactions, or it may be due to the use of more precise, event-specific measures of conflict in daily interactions

used in the human social conflict literature as opposed to largely traditional retrospective measures used in social support and integration studies.

To substantiate this latter possibility, there is emerging evidence to suggest that momentary and trait self-reports measure 2 different types of information: episodic and semantic information, respectively. It is argued that multiple ambulatory assessments in naturalistic settings may reflect a more accurate representation of event-specific experiences (Conner & Barrett, 2012; Tulving 1983), whereas trait self-report measures may largely reflect our beliefs about events, which may not correspond as closely to the actual events.

One example of a methodology that is designed to capture event-specific information about daily social interactions is ecological momentary assessment (EMA), which is often used to measure behaviors, affect, and cognitions in real-time and natural settings (Stone & Shiffman, 1994). Four particular qualities define the EMA methodology: phenomena are assessed as they occur, assessments are usually made in the environment that the individual typically inhabits, assessments are dependent upon careful timing, and assessments usually involve a substantial number of repeated observations. When compared to questionnaire assessments, the use of aggregated EMA measures have shown stronger associations with biological stress responses, presumably due to their sensitivity to the event-specific triggers of biological responses in the natural environment. For example, one study looked at the relationship between negative affect and intima-media thickness (IMT), a marker for cardiovascular disease, using EMA and trait measures of negative affect, in a sample of 480 healthy middle-aged adults (Bajaj et al., 2013). All participants completed an electronic diary on an hourly basis for a 4-day period. Results indicated that higher mean momentary negative affect was associated significantly with greater

IMT in fully adjusted models, whereas the trait measure of negative affect yielded no significant association.

Other studies have shown that EMA measures of positive affect better predicted cortisol early in the day and cortisol increase after waking than trait measures of positive affect (Steptoe et al., 2007), and higher mean momentary task demand during work at baseline showed larger 6-year changes in IMT, while traditional measures of job demand did not (Kamarck et al., 2012). This evidence suggests that momentary measures of subjective experiences in daily life show stronger correlations with biological stress responses and markers for disease perhaps due to their ability to capture event-specific information in the natural environment.

1.4 MARITAL INTERACTIONS AND INFLAMMATION

Most of the studies we have described so far include measures of social relationship quality in general. A number of studies have studied interactions in marital relationships as they are linked with the inflammatory process. Two studies, in particular, used a sample of healthy men and women, ages 35-84, to examine the association of partner support and strain with circulating IL-6 levels, but reported conflicting findings (Whisman & Sbarra, 2012; Donoho et al., 2013). Partner support was measured by six supportive items (e.g. How much does your spouse really understand the way you feel about things?) and partner strain was measured by six negative interaction items (e.g. How much does your spouse criticize you?). Whisman & Sbarra (2012) showed that partner support and partner strain scales were significantly associated with circulating IL-6 in younger women only (below age 53). Donoho et al. (2013) showed that marital strain was associated with higher IL-6 in the univariate model, but the association

diminished after the addition of behavioral and psychosocial covariates, including marital duration.

A third study examined the association between marital conflict and inflammatory reactivity measures in a sample of 42 healthy, married couples, ages 22-77 (Kiecolt-Glaser et al., 2005). In the first session, couples had a structured social support interaction, where one partner was asked "to talk about something you would like to change about yourself," while the other partner was instructed "to be involved in the discussion and respond in whatever way you wish." Roles were reversed after 10 minutes. The second session consisted of a conflict resolution task, where the couple was asked to discuss and try to resolve 1 or 2 marital issues that the experimenter judged to be the most conflict producing, based on the couple's ratings on the Relationship Problem Inventory. Rapid Marital Interaction Coding System (RMICS) was used to provide data on behavior during both tasks and has been shown to discriminate between distressed and nondistressed couples, with high reliabilities for the overall system as well as the individual codes (Heymen, 2004). The authors summed the top 3 RMICS codes in the hierarchy: psychological abuse (disgust, contempt, etc.), distress-maintaining attributions ("You were being mean on purpose."), and hostility (criticism, hostile voice tone). Cytokine production was assessed during each session. Results indicated that high-hostile couples, as assessed by these codes, produced larger increases in plasma IL-6 and TNF-alpha levels the morning after a conflict than after a social support interaction, while low-hostile couples showed a 24-hour increase in IL-6 levels that were similar at each visit, and a smaller 24-hour increase in TNF-alpha levels at the conflict visit. Results suggest that marital conflict can lead to heightened reactivity inflammatory responses 24 hours after a negative interaction, at least among high

hostile couples, supporting the idea that the quality of marital interactions may be associated with reactivity measures of inflammatory biomarkers.

Overall, epidemiological studies suggest that low social support and social integration may be associated with higher circulating IL-6 and CRP among older men, but the results are inconsistent and may reflect different immune measures (Glei et al., 2012; Marsland et al., 2007; McDade et al., 2006). Literature on social conflict among animals and humans has yielded some associations with circulating levels of inflammatory markers, but more consistent associations with stimulated markers of inflammation and inflammatory reactivity. Marital quality has been inconsistently associated with circulating levels of biomarkers but has been associated with inflammation reactivity measures. The current study proposes to use momentary measures to study social interactions in daily life as possible mediators in the association between trait measures of social support, integration, and marital quality with inflammatory markers of systemic inflammation, CRP and IL-6.

Our first aim is to replicate previous work in examining whether global measures of social integration, social support, and marital quality may predict inflammatory biomarkers, CRP and IL-6, cross-sectionally. It is hypothesized that all 3 factors will be inversely correlated with inflammatory markers. The second aim is to test whether daily social interactions account for associations between global measures of social integration, social support, and marital quality and inflammatory markers. Previous research suggests that individuals who perceive more social support tend to rate their daily life interactions as more positive, that those who are better socially integrated spend a large proportion of their time engaging in social interactions, and that these daily life correlates are relatively specific to the global constructs they indicate, such that there are stronger associations between social support and the quality of social interactions, and

stronger associations between social integration and the number of social interactions (Kamarck et al., 2004 Society of Behavioral Medicine Abstract; Cohen & Lemay, 2007). Similarly, individuals who show more marital adjustment tend to engage in more positive interactions with their partner in daily life, than individuals who show less marital adjustment (Janicki et al., 2005; Joseph et al., 2014). It is hypothesized that the proportion of positive social interactions will mediate the association between social support and inflammatory markers, that the frequency of social interactions will mediate the relationship between social integration and inflammatory markers, and that the proportion of positive marital interactions will mediate the association between marital adjustment and inflammatory markers. While this project is testing the quality and frequency of daily social interactions as possible mediators, it is acknowledged that there may be other aspects of daily social functioning not measured in this study, that may account for any observed effects of social relationship characteristics on inflammation. A third aim will be to test whether negative interactions will be more strongly associated with inflammatory markers than positive interactions. Based on the literature, it is hypothesized that negative interactions may exert a larger impact than positive interactions, perhaps because of the adaptive value of detecting social and/or physical threat and the consequent mobilization of the immune system to respond to threat.

2.0 RESEARCH DESIGN AND METHODS

Participants were drawn from the Adult Health and Behavior Project – Phase 2 (AHAB-II), a study of psychosocial factors, behavioral and biological risk factors, and subclinical cardiovascular disease. The full study protocol included 7 appointments completed over approximately 4-8 weeks and included medical, demographic and social histories; biomedical measures, psychosocial questionnaires, a structured psychiatric interview; ambulatory monitoring of BP, physical activity, mood and social interactions; cognitive testing; and functional and structural brain imaging. AHAB-II participants were recruited between February 2008 and August 2011 through mass mailings of recruitment letters to individuals selected from voter registration and other public domain lists.

To be eligible to participate in AHAB-II, individuals had to be between the ages of 30-54 years and working at least 25 hours per week outside of the home (a substudy involving this cohort was focused on the association between occupational stress and CHD risk). Individuals were excluded from participation if they (a) had a history of cardiovascular disease, schizophrenia or bipolar disorder, chronic hepatitis, renal failure, major neurological disorder, chronic lung disease, or stage 2 hypertension (BP $\geq$ 160/100 mm Hg); (b) reported drinking $\geq$ 35 portions of alcohol per week; (c) took fish-oil supplements (because of the requirements for another substudy); (d) were prescribed insulin or glucocorticoid, anti-arrhythmic, antihypertensive, lipid-lowering, psychotropic, or prescription weight-loss medications; (e) were

pregnant; (f) had less than 8th grade reading skills; or (g) were shift workers. The study was approved by the University of Pittsburgh Institutional Review Board. Participants signed an informed consent form when enrolled and received compensation up to $410, depending on extent of participation in visits and compliance with the protocol.

At total of 177,415 mailings yielded 8,957 study inquiries (response rate 5%). We were able to reach 3,431 individuals for telephone screening, and 2,751 either declined participation or were ineligible, leading to 680 consented participants. One hundred-fifty additional participants withdrew prior to monitoring due to ineligibility (n=69), time or work constraints (n=78) or missing key data (n=3). Five-hundred thirty participants were scheduled for the protocol, out of which 36 additional individuals withdrew due to ineligibility (n=6), and time/work constraints (n=30), leading to 494 participants that comprise the AHAB-II sample.

2.1 PROCEDURE

Participants completed six visits, some of which are not relevant to the current report. Demographic variables and a fasting blood draw were completed at Visit 1. Global marital quality was assessed at Visit 3 and global social support and social integration were assessed at Visit 4. Ecological momentary assessments (EMA) were completed between Visits 2 and 3 using a 4-day monitoring protocol, i.e., 3 working days and 1 nonworking day. The monitoring protocol consisted of two, 2-day monitoring periods, usually one period at the beginning of the work week and another at the end of the work week, with at least one non-monitoring day in between. During each monitoring day, subjects carried a PDA (Palm Z22) used to collect EMA

data. During waking hours on each monitoring day, participants initiated a 43-item questionnaire on the PDA, on an hourly basis.

Participants were trained to use the EMA device during Visit 2. Training began with a self-paced tutorial. Each subject was required to meet demonstrated competence on the use of all of the equipment before being sent into the field for a practice day. A phone call was made to each subject at the end of the practice day, which presented the subject with an opportunity to detect and correct technical or operational problems that may have arisen during the practice day. See Figure 1 for details on Procedure.

2.2 INSTRUMENTS

2.2.1 Social Support and Social Integration

Perceived social support was measured by the 12-item version of the Interpersonal Support Evaluation List (ISEL), assessing tangible support, belonging support, and appraisal support (Cohen et al., 1985). Each item was scored on a 4-point scale and scores were summed and averaged across the 3 subscales. Social integration was measured by the Social Network Inventory (SNI). The SNI assesses participation in 12 types of relationships ; one point is assigned for each role the individual participates in within their social network at least once every 2 weeks. Both questionnaires have shown adequate validity and test-retest reliabilities (Delistamati et al., 2006; Treadwell et al., 1993; Cohen et al., 2012).

2.2.2 Global marital Quality

Global marital quality was assessed using the widely-used Dyadic Adjustment Scale (DAS) (Spanier, 1976), a 32-item self-report instrument which has been shown to discriminate between distressed and nondistressed married or cohabitating couples and to have adequate test-retest reliabilities (Spanier, 1976; Carey et al., 1993).

2.2.3 Social Interactions

EMA was used to collect information on daily social interactions. Participants were asked to carry a PDA (Palm Z22) that prompted them with a 43-item questionnaire on an hourly basis throughout the waking day. Among the items on this questionnaire were 11 items pertaining to daily social interactions. These items assessed when the most recent interaction was, the length of the interaction, the number of people it involved, types of interaction partners (e.g. spouse, coworker, etc.), and the quality of the interaction. Four of these 11 items assessed information about the quality of the most recent social interaction and one item assessed when the most recent social interaction ended.

Interaction quality was assessed using 4 of the interview items. Two items assessed positive aspects of interactions ("agreeable interaction" and "pleasant interaction") and two assessed negative aspects of interactions ("someone in conflict with you" and "someone treated you badly"). Item responses [**NO!** No no yes Yes **YES!]** were converted to a 1-6 rating scale. Although the positive and negative items were inversely correlated, confirmatory factor analysis (CFA) indicated that they are best treated as indicators of separate constructs, with a two-factor

model fitting the data significantly better than a one-factor model (Joseph et al., 2014). See Appendix B for details on individual EMA items.

Because we were interested in measuring the frequency of positive and negative interactions to assess the quality of interactions, scores of 4-6 (all indicating yes) on the positive quality interactions items were counted as positive interactions and scores of 4-6 (all indicating yes) on the negative quality interaction items were counted as negative interactions. These frequency scores were normalized to the total number of interactions for each person to indicate the proportion of positive and negative interactions (range of 0-100%). To measure frequency of total interactions, the proportion of time that individuals spent interacting was calculated. This consisted of the frequency of interactions that ended 0-10 minutes before the hourly interview and was normalized to the total number of observations (range of 0-100%). The same procedure was repeated for marital interactions, among individuals who reported that they were married.

$$EMA_{\text{positive interactions}} = \frac{\text{\# of positive interaction items that were answered as yes}}{\text{Total \# of interactions}}$$

$$EMA_{\text{negative interactions}} = \frac{\text{\# of negative interaction items that were answered as yes}}{\text{Total \# of interactions}}$$

$$EMA_{\text{frequency of interactions}} = \frac{\text{\# of interactions that ended 0-10 min before interview}}{\text{Total \# of observations}}$$

Inflammatory Measures - Blood samples were drawn for the measurement of circulating levels of CRP and IL-6, during Visit 1. On this occasion, participants were asked to fast for 8 hours, to avoid exercise for 12 hours, and to avoid alcohol for 24 hours before coming into the laboratory during morning hours. Blood was drawn through an antecubital venipuncture into citrate-treated Vacutainer tubes and serum separator tubes. Procedure details are described in Appendix A.

High-sensitivity CRP was measured by the University of Vermont's Laboratory of Clinical Biochemistry Research Lab with the BNII nephelometer utilizing a particle enhanced

immunonephelometric assay. The assay range is 0.175 to 100 mg/L. CRP values above 10 mg/L were assumed to be due to acute infection and were dropped from all analyses. Plasma IL-6 levels were determined by the University of Pittsburgh's Behavioral Immunology Laboratory using the high-sensitivity, quantitative sandwich enzyme immunoassay technique (R&D Systems). Assay standard range is from 0.156 to10 pg/mL. IL-6 levels were extrapolated from a log-linear curve. The study excluded participants with autoimmune connective tissue disorders (e.g. rheumatoid arthritis), HIV/AIDS, inflammatory bowel disease, chronic hepatitis, individuals with asthma using medication for $\geq$ 7 times in 14 days prior to blood draw, chronic lung disease, oral glucocorticoid medication, acute viral or bacterial infection, regular use of allergy shots or recent vaccination, and cold or flu in the past 2 weeks. Participants were asked to refrain from non-steroidal anti-inflammatory medication for 24 hours (e.g. Ibuprofen, aspirin) prior to their visit. For details on blood draw procedure, see Appendix A.

We assessed demographic and biological risk factors for cardiovascular disease.

2.2.4 Demographics

Participants self-reported their age, race/ethnicity, sex, and highest level of education completed. Age was treated as a continuous variable, race/ethnicity was coded into White, African American, and Other groups, sex was dichotomized into male and female, and education was coded into 4 categories (High school diploma, Associate Degree, Bachelor's degree, Advanced degree – Master's or MD/Ph.D./J.D./PharmD) and treated as a continuous variable.

2.2.5 Biological risk factors

Body mass index (BMI) was calculated based on height and weight measured in the clinic (lbs/inches^2 X 703).

3.0 DATA ANALYSIS

All analyses were conducted in SAS 9.3.

3.1 SPECIFIC AIMS

3.1.1 Specific Aim 1

Aim 1 was to test whether traditional self-report measures of social integration (SI), social support (SS), and marital quality (MQ) are associated with inflammatory biomarkers, CRP and IL-6. Using multiple linear regression models (PROC GLM), inflammatory marker measures were regressed on summed scores of SS, using the ISEL, SI, using the SNI, and MQ, using the DAS. The model included age, sex, race, education, and body mass index (BMI) as covariates. Measures of SI, SS, and MQ were entered individually, as well as together, to test for independent main effects of each construct above and beyond the others.

3.1.2 Specific Aim 2

Aim 2 was to test whether daily social interactions account for associations between global measures of social relationships, SI, SS, and MQ, and inflammatory measures, CRP and IL-6.

Product of coefficient analyses were planned to test whether the appropriate measure of social interactions mediates the association between trait measures of SS, SI, and MQ and inflammatory markers. Multivariate regression models were first used to calculate individual regression coefficients and product of coefficient mediation analysis were only to be conducted using significant coefficients. For example, to test the frequency of interactions as a mediator, the regression coefficient of the association between trait SI and the proportion of time spent in social interactions would be multiplied by the coefficient of the association between the proportion of time spent in social interactions and inflammatory markers. The cross-product (αβ) would be tested for significance by dividing it by its standard error ($\sigma\alpha\beta=\sqrt{(\alpha^2\ \sigma\_\beta^2+\beta^2\ \sigma\_\alpha^2\ )}$) and comparing its value to a standard normal distribution to test for significance (H0: $\alpha\beta=0$) (Sobel, 1982).The same process was planned to be repeated for trait SS (using the proportion of positive and negative interactions as mediators) and MQ (using the proportion of positive and negative marital interactions as mediators).

3.1.3 Specific Aim 3

Aim 3 was to test whether negative interactions will be more strongly associated with inflammatory markers, IL-6 and CRP, than positive interactions. Partial correlations, partialling out all covariates, were calculated between the proportion of positive interactions and inflammatory markers, as well as the proportion of negative interactions and inflammatory markers. The significance of the difference between these partial correlations was tested by calculating the Hotelling's statistic (Steiger, 1980).

3.1.4 Exploratory analyses

Exploratory analyses were conducted to test for gender-specific associations between trait and momentary measures of SS, SI, and MQ and inflammation. Gender was dummy coded and was entered as part of a cross-product with the social predictor variables in the regression models to test whether for significant differences between men and women in the association between social variables and inflammatory markers.

4.0 RESULTS

The results from the whole sample and married subsample are presented below.

4.1 SELECT SAMPLE CHARACTERISTICS

The total sample on which analyses were conducted was N=463 for global measures and measures of social interactions, and N=332 for marital interactions in married individuals who had completed the DAS measure. Sample sizes vary due to missing data on independent variables, dependent variables, or covariates. Selected characteristics of the study population are listed in Table 1. IL-6 values were between 0.063 and 9.832 mg/mL. CRP values were between 0.15 mg/mL and 22.4 mg/mL. Approximately 3% (N=10) of the values for CRP were above 10 mg/mL, which is indicative of the presence of an acute infection. Therefore, IL-6 and CRP values for these individuals were excluded from analyses. Approximately 9% (N=41) of the values for IL-6 were below the detection limit of 0.16 mg/mL but were considered valid and were included in the analyses. CRP and IL-6 values were log transformed to reduce skewness and the log-transformed values were used for all analyses.

Sample characteristics for the total sample and the married subsample are presented in Tables 1 and 2. Age, African American race, and BMI were significantly correlated with greater

levels of CRP and IL-6, while education was inversely correlated with these biomarkers. Levels of CRP and IL-6 are also positively correlated with each other (Table 3).

Regression models were used to predict social characteristics. In univariate models predicting social integration, none of the four demographic variables (age, sex, race, and education) or BMI were independent significant predictors. However, when these predictors were entered together in the model, it was found that years of education (b= 0.226, F(1, 489)= 4.27), p=.04) and BMI (b= .037, F(1,489)= 4.19, p= .04) were positively associated with social integration.

When predicting social support using univariate models, sex was a significant predictor (b= 1.266, F(1,490)= 8.61, p= .004), with women reporting greater levels of social support (t(489)= 2.93, p=.004) than men. In models adjusting for all demographic variables and BMI, sex remained a significant predictor of social support (b= 1.38, F(1, 489)= 10.11, p= .002). When predicting marital adjustment using univariate models, age was inversely associated with marital adjustment (b= -.622, F(1,330) = 21.35, p< .0001). Those who self identified as being nonwhite and non-African American reported lower marital adjustment by DAS when compared to Whites (b= -12.82, F(1,330)= 4.30, p= .04). The association between the minority race group and marital adjustment remained significant even when adjusting for demographic variables and BMI (b= -13.24, F(1, 329)= 4.87, p= .03), as did the association between age and marital adjustment (b= -0.591, F(1, 329) = 18.64, p < .0001).

When predicting the total proportion of time spent in social interactions in univariate models, level of education (b= -.014, F(1,493)= 4.69, p = .03) was inversely associated with the proportion of time spent in social interactions, but this association lost significance when adjusting for all other covariates in the model (b= -.013, F(1,492) = 3.50, p= .06).

In the married subsample, univariate models including all individuals who reported having marital interactions show that older individuals (b= -.003, F(1, 331)= 15.47, p= .0001), females (b= -.048, F(1, 331) = 17.08, p<.0001) and those who identified as African-American (b= -.040, F(1, 331)= 5.05, p= .03) were likely to have fewer marital interactions. All of these associations remained significant in fully adjusted models (age: b= -.002, F(1, 330) = 11.91, p= .0006, sex: b= -.038, F(1, 330)= 10.19, p= .002, African-American race: b= -.037, F(1, 330)= 4.21, p=. 04). Additional correlations between global social measures (i.e. social support, social integration, marital adjustment) and EMA measures of social interactions and marital interactions (i.e. frequency and quality) are included in Table 11.

4.2 GLOBAL MEASURES OF SOCIAL SUPPORT, SOCIAL INTEGRATION, AND MARITAL QUALITY AND INFLAMMATION

Using separate multiple regression models with age, race, sex, and education as covariates, measures of IL-6 and CRP were regressed on global measures of social support, social integration, and marital adjustment, as assessed by the ISEL, SNI, and DAS, respectively. None of the global measures of social integration, social support, and marital adjustment predicted either inflammatory marker, CRP or IL-6 (ISEL with CRP: b= -0.001, F(1, 452) = .01, p=.91; ISEL with IL-6: b= -0.005, F(1,453) =.39, p= .39; SNI with CRP: b = 0.013, F(1,452)= .61, p= .61; SNI with IL-6: b= 0.009, F(1,453) = .40, p= .53; DAS with CRP: b= -0.004, F(1, 306) = .97, p=.33; DAS with IL-6: b= -0.000, F(1, 306) = 0.00, p=.94). In a second set of regression models with only age and sex as covariates, none of the results substantially changed (See Table 4), nor were there any changes with additional adjustments for BMI (See Table 5).

In the third set of regression models, scores on the ISEL, SNI, and DAS were entered together, adjusting for demographic variables and BMI, to test for possible independent main effects in predicting levels of CRP and IL-6. Once again, there were no significant results. When ISEL and SNI scores were entered together in the same model, neither measure was associated with levels of CRP (ISEL: b= -0.002, F (1,450) = .03 p=0.86; SNI: b= -0.003, F(1, 450) = .02, p=.90), or IL-6 (ISEL: b= -0.006, F(1, 451) = 1.01, p=.32; SNI: b= .003, F(1, 451) = .06, p=.81). In the marital subsample, when DAS scores were entered along with the ISEL and SNI scores in the fully adjusted model, there were no significant results associations with CRP (ISEL: b= -.000, F(1, 300) = .00, p= .98, SNI: b= .015, F(1, 300) = .25, p= .61, DAS: b= -.003, F(1, 300) = .68, p= .41) or IL-6 (ISEL: b= -.011, F(1, 300) = 1.94, p= .16; SNI: b= .013, F(1, 300) = .59, p= .44; DAS: b= .000, F(1, 300) = .05, p= .68).

4.3 DAILY SOCIAL INTERACTIONS AND INFLAMMATION

The association of frequency and quality of social interactions with inflammatory markers was tested here. In the first set of regression models that adjusted for age, sex, race, and education, there were no significant associations between frequency of interactions or proportion of negative social interactions with either inflammatory marker (frequency of social interactions and CRP: b= .083, F(1,455) = .05 p= .83; frequency with IL-6: b= -.021, F(1, 456) = .01 p= .93; negative social interactions and CRP: b= -.869, F(1, 455) = 1.43, p= .23; negative social interactions and IL-6 :b= .061, F(1, 456) = .02, p= .89). There was also no association between the proportion of positive interactions and levels of IL-6 (b= -.318, F(1,456)= 0.43, p= .51), but there was a significant positive association between the proportion of positive social interactions

and levels of CRP, which surprisingly suggested that individuals who had a greater proportion of positive interactions also tended to have greater levels of CRP (b= 1.63, F(1,455) = 3.87, p= .0497). When only age and sex were used as covariates, none of these results changed (See Table 4). Adjusting for BMI did not alter any of the results reported above, and the CRP association remained significant (b= 1.88, F(1, 454) = 5.96, p= .02). See Table 6.

Partial correlations were used to assess the predictive value of negative interactions versus positive interactions, in predicting levels of CRP and IL-6, while partialing out the effects of age, sex, race, education, and BMI. The proportion of negative social interactions was not significantly correlated with IL-6 (r= .-.005, p=.91)or CRP (r= -.07, p=.12). The proportion of positive social interactions was not significantly correlated with IL-6 (r= -.02, p= .72), but was significantly correlated with levels of CRP (r= .11, p=.02). To test for a significant difference in the magnitude of these correlations, the Hotelling's t-statistic was calculated. The magnitude of the difference between the correlation of positive interactions with IL-6 and the correlation of negative interactions and IL-6 was not significant (t(462) = .143, p> .05), but the magnitude of the difference between the correlation of positive interactions and CRP, and negative interactions and CRP was statistically significant (t(461) = -2.22, p <.05), suggesting that frequency of positive interactions was more strongly correlated with CRP, than the frequency of negative interactions, albeit in the direction opposite of that which was initially predicted.

To test the internal consistency of the positive association between positive interactions and CRP levels, the total sample was divided into various subsamples. First, the sample was divided into males and females. In males (N=219), there was no association between the proportion of positive social interactions and levels of CRP (b= .74, F(1,218) = .57, p= .49), but in the female subsample (N=243), there was a positive association between the proportion of

positive interactions and levels of CRP (b= 3.40, F(1, 242) = 7.86, p= .006). Similarly, when the total sample was divided into married (N=313) and unmarried individuals (N=149), there was no association found between the proportion of positive interactions and CRP level in the unmarried sample (b =1.623, F(1,148) = .33, p=.33), but the positive association remained significant in married individuals (b= 1.79, F(1, 312) = 4.20, p=.04). When the married sample was further divided into married males (N=158) and married females (N=155), there was no association between proportion of positive interactions and CRP levels in married males (b= .451, F(1, 157) = .18, p= .67), but there was a positive association between these variables in married females (b= 4.026, F(1, 154) = 7.45, p= .01).

4.4 DAILY MARITAL INTERACTIONS AND INFLAMMATION

These results used a subset of married individuals (N=332). In models adjusting for only demographic covariates, frequency of marital interactions in daily life was not associated with CRP (b= .62, F(1,307)= 1.08, p=.30) or IL-6 levels (b= .006, F(1,307) = 0.00, p=.98). The proportion of positive marital interactions also did not significantly predict CRP (b= .153, F(1,297) = .09, p=.76) or IL-6 levels (b= -.108, F(1,297) = .16, p=.69). Likewise, the proportion of negative marital interactions did not significantly predict CRP levels (b= .205, F(1, 297) = .18, p=.67), or IL-6 levels (b= 0.024, F(1,297)= .01, p= .92). In models that further adjusted for BMI to test the association between frequent, positive, and negative marital interactions and IL-6 and CRP, all findings remained non-significant. See Table 7.

Since global trait measures of social support, social integration, and marital adjustment were not predictive of inflammatory outcomes, mediation analyses to test the role of daily social interactions to account for the effects of global trait measures were not conducted.

4.5 EXPLORATORY FINDINGS

Moderation analyses were conducted to test whether any of the findings above were moderated by age, sex, or the interaction between age and sex; none of these findings were significant.

The marital interaction findings reported above only included individuals who specifically stated that they were either married and/or living with a partner on the DAS measure. However, there were some participants who did not report being married on the DAS but reported having spousal interactions through EMA measures. These individuals were excluded from the analyses above, but additional exploratory analyses were conducted to study "spousal" interactions in analyses that included these individuals. Therefore, the results presented here test the association between characteristics of interactions with significant others and inflammatory markers in all individuals who reported having spousal interactions, whether or not they reported being married on the DAS or completed a DAS measure. When we re-ran analyses using this larger subgroup, however, all of the findings remained nonsignificant. See Table 8.

Previously, positive and negative interactions were operationalized in terms of frequency of occurrence and assessed using proportion measures. Alternatively, analyses were conducted to assess the association between mean levels of positivity or negativity in social interactions, marital interactions, and inflammatory markers. Ratings of the positivity or negativity of each interaction were averaged across observations and days. In fully adjusted analyses, no significant

associations were found between quality of interactions and either inflammatory marker in the whole sample or in the married subsample. See Tables 9 and 10.

5.0 DISCUSSION

In this middle-aged, healthy sample, there were no associations found between global measures of social integration, social support, and marital adjustment and inflammatory markers, CRP and IL-6. There were also no associations found between EMA measures of frequency and quality of marital interactions and inflammatory markers, but there were mixed findings when testing the association between the quality of total social interactions and inflammatory markers.

There is some evidence to suggest that social integration and social support are inversely associated with chronic inflammation but findings are generally mixed. Therefore, the lack of association found between global measures of social support and social integration with inflammatory markers is not entirely inconsistent with the literature. Studies that have shown an inverse association between social integration and CRP have found these effects generally for older adults, rather than middle-aged adults who were included in the current study (e.g. age 60 or older) (Loucks et al., 2006a; Ford et al., 2006; Loucks et al., 2006b). When studying the association between social support and circulating levels of inflammatory markers, findings have also been mixed, with some studies reporting no association, even in older adults (McDade et al., 2006), and others reporting an unexpected positive association between social support and inflammatory biomarkers, including sIL-6r (Glei et al., 2012).

When studying the association between characteristics of marital quality and inflammatory markers, there have also been mixed findings. For example, Whisman & Sbarra

(2012) reported that in younger women, partner support and partner strain were both associated with circulating levels of IL-6 in the expected directions in younger women (below age 53), whereas Donoho et al. (2013) found an inverse association only between spousal support and circulating levels of IL-6, but only in univariate models. Both studies have included older adults in their sample (age 35-84 and age 25-74, respectively).

Contrary to our predictions, frequent positive interactions were associated with higher CRP levels, rather than negative interactions, in this study. To our knowledge, there is only one recent study that has done a head-to-head comparison between the association of positive and negative interactions with inflammatory markers, using daily diaries (Chiang et al., 2012). They reported a positive association between negative interactions and baseline levels of sTNFalphaRII, and a positive association between competitive interactions and baseline levels of IL-6 and sTNFalphaRII, but no association between positive interactions and circulating levels of inflammatory outcomes, which is inconsistent with the findings of the current study. It is possible that the positive association found in the current study between the frequency of positive interactions and CRP levels may be a chance finding and should be interpreted with caution. The fact that this association was only significant for females, married individuals, and in particular, married females is consistent with this possibility.

The fact that the association between positive interactions and CRP level was not consistent across different types of operational definitions is also consistent with the possibility of a chance finding. The frequency measure employed in this study provides an estimate of how often individuals were engaging in positive or negative interactions throughout the day, rather than the mean level of positivity or negativity in their interactions (i.e. how positive or negative these interactions were). So, although this proportion measure provides information about the

frequency of positive interactions, it does not provide information about how positive these interactions were. When these analyses were repeated using a mean level of positivity in positive interactions, instead of the frequency of positive interactions, the positive association between positive interactions and CRP is not significant in the total sample, females, married individuals, or married females.

Although the association between frequency of positive interactions and CRP may be due to chance, there are also some plausible explanations for this effect. Positive social interactions may be demanding in their own right, leading to immune mobilization. For example, a social interaction about wedding planning or starting a new job can certainly be positive in nature, but those actual events may very well be perceived as stressful. Although this form of stress generally does not contribute to illness, this level of prolonged, high activity can lead to the physiological mobilization of metabolic resources, even when it's regarding a positive event, which could contribute to elevated reactivity measures while facing a stressor. Therefore, more information about the situational context of positive interactions may be needed in order to interpret pathways through which positive interactions may be related to markers of physiological stress.

Secondly, although the analyses controlled for BMI, due to the large contribution of adipocytes in the production of IL-6, which also contributes to the production of CRP, it is possible that engaging in unhealthy behaviors may also contribute to greater circulating levels of CRP. One can imagine that positive social interactions may be more likely to occur in social gatherings, where the use of alcohol or cigarettes may be more common (Collins et al., 1985). Smoking status has been associated with greater levels of CRP, IL-6, and fibrinogen (Glei et al., 2012) and generally, in the literature, adjusting for smoking behavior and excessive alcohol

intake use has often reduced the odds ratio for elevated CRP levels in socially isolated individuals, suggesting that these health behaviors may account for at least part of the observed association social interactions and CRP. However, in this sample, the positive association between proportion of positive interactions and CRP levels remained significant, even after adjusting for alcohol intake and smoking status.

These results also raise an important question of why the quality of interactions is associated with levels of CRP, and not IL-6 in this sample. The cytokine IL-6 is often considered to be multifunctional in nature, such that it can be pro- or anti-inflammatory, depending on other cytokines that are activated in the cascade of events during local or systemic inflammation. This quality is in contrast to that of CRP, which is more closely tied to the activation of the immune system because of its ability to activate the complement system that is responsible for the opsonization of foreign material (i.e. bacteria, viruses) for detection by the host's immune system. CRP is also less influenced by diurnal variation, making it a more stable marker for immune activation (Meier-Ewert et al., 2001). Therefore, the association between frequent positive interactions and greater levels of CRP suggests that mechanistically, the frequency of positive interactions may be more closely tied to the downstream effects of immune system activation.

6.0 LIMITATIONS

Limitations of this study include its cross-sectional design. A longitudinal design would provide clarity about the directionality of these results, as well as allow researchers to study change in inflammatory markers over time. In addition, an experimental design that involves treatment focusing on the quality and frequency of social interactions could better test for a causal relationship between these social factors and inflammation.

Regarding stability of our measurements, it is a limitation that inflammatory markers are only assessed at one time point, especially because there is evidence to suggest that there is considerable intra-individual variability when CRP is measured during multiple times (i.e. daily, weekly, monthly, and tri-monthly measurements) with individuals moving from one CRP risk category to another (Bogaty et al., 2013). Nevertheless, even when measured at only one time point, CRP level has been predictive of negative health outcomes, including future risk of a fatal or nonfatal coronary event (Koenig et al., 1999). It may also be beneficial to include a variety of inflammatory outcomes, in addition to circulating levels of IL-6 and CRP. Although IL-6 contributes to the hepatic synthesis of CRP, TNF-α and IL-1 can also induce CRP production. Therefore, including measures of TNF-α (or receptors of TNF-α) and IL-1 (although difficult to quantify in healthy adults) may provide a more complete depiction of the pattern of circulating cytokines.

The current study uses a sample of middle-aged, healthy adults. These findings may not be generalizable to younger or older populations, although significant associations of social support and social integration, assessed by global measures, with inflammation have been found previously in older populations (Loucks et al., 2006a), and middle-aged samples (Ford et al., 2006). An association between social conflict and inflammation has also been observed in adolescent samples (Fuligni et al., 2009). On a related note, this sample was subject to a wide range of exclusionary criteria so the final sample is remarkably healthy, which may also limit generalizability.

7.0 IMPLICATIONS/FUTURE DIRECTIONS

This study reports that the frequency of positive social interactions in daily life is associated positively with levels of circulating CRP, whereas no association was found between frequency of interactions, in general, or the frequency of negative interactions and inflammatory markers. Previous literature seems to report mixed findings, with greater consistency in the association of negative interactions and stimulated measures of inflammation. Given the variability in methodology and results makes it difficult to compare current findings to those of previous work.

Biological pathways would consist of characteristics of HPA activity, glucocorticoid resistance, and its impact on inflammatory pathways, while psychological and behavioral pathways may consist of affect, appraisal, as well as the implementation of healthy behavioral practices in daily life, such as physical activity, smoking and alcohol use, sleep duration and quality, and adherence to a healthy diet.

Table 1. Demographic and Clinical Characteristics of the Analytic Sample for Social Support, Social Integration, and EMA-assessed Social Interactions (N =494)

Characteristic	Mean (SD) or % (n)
% male (n)	47 (234)
% African American (*n*)	16.8 (83)
% bachelor's degree or higher (*n*)	71.45 (353)
% current smokers (*n*)	13.2 (65)
Mean age (SD)	42.77 (7.34)
Mean BMI (SD)	26.98 (5.27)
Mean CRP (SD)	1.50 (1.83)
Mean IL-6 (SD)	1.09 (.94)

Table 2. Demographic and Clinical Characteristics of the Analytic Sample for Marital Adjustment and Married Interactions (N =332)

Characteristic	Mean (SD) or % (n)
% male (n)	50.1 (168)
% African American (*n*)	12.3 (41)
% bachelor's degree or higher (*n*)	73.1 (243)
% current smokers (*n*)	10.9 (36)
Mean age (SD)	42.42 (7.29)
Mean BMI (SD)	26.83 (5.27)
Mean CRP (SD)	1.37 (1.67)
Mean IL-6 (SD)	1.01 (.82)

Table 3. Correlations between covariates and inflammatory markers and between inflammatory markers.

	Log CRP	Log IL-6
Variable	*r*	*r*
Age	.13**	.20***
	N= 462	N=463
Sex	.05	.05
	N= 462	N=463
Black race	.17***	.16***
	N= 462	N=463
Education	-.17***	-.19***
	N=462	N=463
BMI – Body Mass Index	.40***	.37***
	N=462	N=463
Log CRP	1.0	.49***
	N=462	N=460
Log IL-6	.49***	1.0
	N=460	N=463

Note: p<.001***, p< .01**, p<.05

Table 4. Correlations between global measures of social variables, EMA measures of marital and total social interactions, and inflammatory markers, while partialing out age and sex.

	Log CRP	Log IL-6
Variable	*r*	*r*
Social Support (ISEL)	-.01 N=457	-.04 N=457
Social Integration (SNI)	.00 N=457	.01 N=457
Frequency of social interactions	.02 N=460	.01 N=460
Frequency of positive interactions	.10* N=460	-.02 N=460
Frequency of negative interactions	-.06 N=460	.01 N=460
Marital Adjustment (DAS)	-.06 N=312	-.01 N=312
Frequency of marital interactions	.01 N=303	-.02 N=303
Frequency of positive marital interactions	.02 N=303	-.02 N=303
Frequency of negative marital interactions	.01 N=303	-.01 N=303

Table 5. Coefficients from Regression Models Predicting log IL-6 and CRP from global measures of social support, social integration, and marital adjustment in fully adjusted models

		Log CRP N=459			Log IL-6 N= 460	
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.009	1.96	.16	.012	9.84	.002
Sex	.137	1.94	.16	.06	1.05	.31
Black race	.154	1.24	.27	.058	.49	.49
Education (highest degree)	-.072	1.62	.20	-.058	3.01	.08
BMI – Body Mass Index	.084	70.4	<.0001	.044	53.99	<.0001
ISEL – Social Support	-.002	.05	.83	-.006	.95	.33
		Log CRP N=459			Log IL-6 N=460	
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.009	2.01	.16	.013	10.16	.002
Sex	.134	1.91	.17	.052	.80	.37
Black race	.153	1.22	.27	.057	.48	.49
Education (highest degree)	-.071	1.58	.21	-.059	3.04	.08
BMI – Body Mass Index	.084	70.04	<.0001	.044	53.31	<.0001
SNI – Social Integration	-.004	.03	.86	-0.00	0.00	.99
		Log CRP N=313			Log IL-6 N= 313	
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.009	1.20	.27	.015	10.86	.001
Sex	.139	1.14	.24	.105	2.54	.11
Black race	-.038	.04	.83	-.169	2.82	.09
Education (highest degree)	-.045	.46	.50	-.067	3.16	.08
BMI – Body Mass Index	.095	58.01	<.0001	.046	44.28	<.0001
DAS – Marital Adjustment	-.003	.79	.37	.000	.02	.90

Table 6. Coefficients from Regression Models Predicting log CRP and log IL-6 from EMA-assessed social interactions in fully adjusted models

	Log CRP N= 462			Log IL-6 N= 463		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.009	1.99	.16	.013	10.41	.001
Sex	.133	1.89	.17	.053	.85	.36
Black race	.148	1.15	.28	.056	.47	.49
Education (highest degree)	-.074	1.72	.19	-.06	3.22	.07
BMI – Body Mass Index	.083	69.98	<.0001	.043	54.06	<.0001
Frequency of social interactions	-0.084	.06	.81	-.095	.20	.66

	Log CRP N= 462			Log IL-6 N= 463		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.009	1.68	.20	.013	10.45	.00
Sex	.138	2.02	.16	.051	.79	.38
Black race	.158	1.31	.25	.058	.50	.48
Education (highest degree)	-.069	1.51	.22	-.059	3.08	.08
BMI – Body Mass Index	.084	71.06	<.0001	.043	53.84	<.0001
Proportion of negative interactions	-1.042	2.37	.12	-.044	.01	.91

	Log CRP N= 462			Log IL-6 N= 463		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.009	1.81	.18	.013	10.69	.00
Sex	.14	2.13	.15	.050	.77	.38
Black race	.143	1.09	.30	.057	.49	.48
Education (highest degree)	-.065	1.36	.24	-.060	3.17	.08
BMI – Body Mass Index	.084	72.37	<.0001	.043	53.52	<.0001
Proportion of positive interactions	**1.88**	**5.96**	**.02**	-.168	.13	.72

Table 7. Coefficients from Regression Models Predicting log CRP and log IL-6 from EMA-assessed marital interactions in married couples in fully adjusted models

	Log CRP N= 314			Log IL-6 N= 314		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.011	1.77	.18	.014	9.06	.00
Sex	.150	1.58	.21	.091	1.82	.18
Black race	-.031	.03	.87	-.177	3.03	.08
Education (highest degree)	-.047	.49	.48	-0.07	3.42	.07
BMI – Body Mass Index	.094	56.93	<.0001	.046	42.96	<.0001
Frequency of marital interactions	.190	.12	.73	-0.177	.33	.57
	Log CRP N= 304			Log IL-6 N= 304		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.010	1.72	.19	.014	10.35	.00
Sex	.137	1.36	.25	.103	2.54	.11
Black race	.017	.01	.93	-.14	2.02	.16
Education (highest degree)	-.034	.24	.63	-.057	2.29	.13
BMI – Body Mass Index	.093	52.69	<.0001	.046	44.32	<.0001
Proportion of negative marital interactions	.079	.04	.84	-.015	.00	.95
	Log CRP N= 304			Log IL-6 N= 304		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.011	1.74	.19	.014	10.32	.00
Sex	.144	1.47	.23	.101	2.41	.12
Black race	.023	.02	.90	-.142	2.08	.15
Education (highest degree)	-.030	.19	.66	-.059	2.41	.12
BMI – Body Mass Index	.093	55.33	<.0001	.046	44.27	<.0001
Proportion of positive marital interactions	.216	.22	.64	-.085	.11	.74

Table 8. Coefficients from Regression Models Predicting log CRP and log IL-6 from EMA-assessed marital interactions in whole sample in fully adjusted models

		Log CRP N= 462			Log IL-6 N= 463	
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.008	1.04	.31	.012	8.64	.00
Sex	.118	1.47	.23	.038	.42	.52
Black race	.131	.89	.35	.037	.20	.65
Education (highest degree)	-.071	1.61	.21	-.057	2.89	.09
BMI – Body Mass Index	.083	70.50	<.0001	.043	54.82	<.0001
Frequency of marital interactions	-.399	1.04	.31	-.437	3.52	.06
		Log CRP N= 353			Log IL-6 N= 353	
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.011	2.40	.12	.012	8.35	.00
Sex	.120	1.21	.27	.085	1.96	.16
Black race	.081	.24	.63	-.066	.52	.47
Education (highest degree)	-.008	.02	.90	-.051	2.03	.16
BMI – Body Mass Index	.087	56.95	<.0001	.045	47.29	<.0001
Proportion of negative marital interactions	.138	.16	.69	.102	.29	.59
		Log CRP N= 353			Log IL-6 N= 353	
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.012	2.42	.12	.012	.814	.00
Sex	.123	1.27	.26	.084	1.88	.17
Black race	.083	.26	.61	-.068	.55	.46
Education (highest degree)	-.005	.01	.94	-.051	2.07	.15
BMI – Body Mass Index	.088	57.52	<.0001	.045	47.73	<.0001
Proportion of positive marital interactions	-.015	.00	.97	-.194	.92	.34

Table 9. Coefficients from Regression Models Predicting log CRP and log IL-6 from mean measures of EMA-assessed quality of social interactions in whole sample in fully adjusted models

	Log CRP N= 462			Log IL-6 N= 463		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.008	1.64	.20	.013	10.31	.0014
Sex	.139	2.06	.15	.052	.81	.37
Black race	.157	1.30	.26	.059	.52	.47
Education (highest degree)	-.069	1.55	.21	-.059	3.05	.08
BMI – Body Mass Index	.084	71.15	<.0001	.043	53.95	<.0001
Mean negative interactions	-1.91	2.41	.12	- .235	.10	.75

	Log CRP N= 462			Log IL-6 N= 463		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.010	2.09	.15	.013	10.42	.00
Sex	.129	1.78	.18	.053	.86	.36
Black race	.151	1.18	.28	.056	.47	.50
Education (highest degree)	-.071	1.56	.21	-.061	3.28	.07
BMI – Body Mass Index	.083	69.58	<.0001	.043	54.11	<.0001
Mean positive interactions	.135	.14	.71	-.123	.32	.57

Table 10. Coefficients from Regression Models Predicting log CRP and log IL-6 from mean measures of EMA-assessed quality of marital interactions in married subsample in fully adjusted models

	Log CRP N= 314			Log IL-6 N= 314		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.009	1.32	.25	.014	9.91	.00
Sex	.145	1.53	.22	.098	2.19	.14
Black race	-.032	.03	.86	-.169	2.80	.096
Education (highest degree)	-.042	.39	.53	-.069	3.26	.07
BMI – Body Mass Index	.096	59.47	<.0001	.046	42.65	<.0001
Mean negative marital interactions	-2.85	1.36	.24	-.422	.09	.76
	Log CRP N= 314			Log IL-6 N= 314		
Variable	*b*	*F*	*p*	*b*	*F*	*p*
Age	.011	1.94	.16	.014	8.99	.00
Sex	.157	1.74	.19	.089	1.75	.19
Black race	-.023	.02	.89	-.179	3.09	.08
Education (highest degree)	-.046	.48	.49	-.070	3.43	.065
BMI – Body Mass Index	.094	56.65	<.0001	.046	43.09	<.0001
Mean positive marital interactions	.369	.42	.52	-.216	.45	.50

Table 11. Correlations between global measures of social variables and EMA measures of marital and total social interactions.

	1	2	3	4	5	6	7	8	9
1.Social support (ISEL)	1.00 N=491								
2. Social integration (SNI)	.25*** N=491	1.0 N=491							
3. Marital Adjustment (DAS)	.27*** N=328	.07 N=328	1.0 N=331						
4. Frequency of social interactions	.20*** N= 491	.33*** N=491	.16** N= 331	1.0 N=494					
5. Frequency of positive interactions	.13** N=491	.01 N=491	.20*** N= 331	-.02 N=494	1.0 N= 494				
6. Frequency of negative interactions	-.03 N=491	.15** N=491	-.15** N= 331	.10* N= 494	-.63*** N= 494	1.0 N=494			
7. Frequency of marital interactions	.11* N=329	.02 N= 329	.36*** N=331	.39*** N= 332	-.00 N= 332	.02 N= 332	1.0 N= 332		
8. Frequency of positive marital interactions	.02 N=318	.04 N= 318	.29*** N= 319	.05 N= 320	.52*** N= 320	-.39*** N=320	.12* N=320	1.0 N= 320	
9. Frequency of negative marital interactions	-.03 N=318	-.02 N=318	-.25*** N= 319	-.01 N= 320	-.33*** N=320	.56*** N= 320	-.15* N=320	-.73*** N=320	1.0 N= 320

Figure 1. Procedure

APPENDIX A

BLOOD DRAW PROCEDURE

IMMUNE BLOOD PROCEDURES (MAY 2012)

A.1 CHRONIC MEDICAL CONDITIONS RESULTING IN IMMUNE MEASURE INELIGIBILITY[1]:

- Autoimmune "connective tissue" disorders. This includes rheumatoid arthritis, lupus, psoriatic arthritis, Sjogren's syndrome, SICCA syndrome, scleroderma (also called systemic sclerosis), polymyositis/dermatomyositis, mixed connective tissue disease, anklosing spondylitis, polyarteritis nodosa or other types of vasculitis.
- HIV/AIDS
- Inflammatory bowel disease (Crohn's disease and ulcerative colitis) ("Irritable bowel syndrome" is OK, so collect the immune measures.)
- Chronic hepatitis. This includes hepatitis B and C (not A), autoimmune hepatitis, alpha-1 anti-trypsin deficiency, Wilson's disease, hemachromatosis
- [[Asthma – Anyone with asthma using medication ≥ 7x in past 14 days is ineligible for AHAB2. Asthmatics not taking daily meds are enrolled in AHAB2, PRN meds documented, and immune labs are drawn.]]
- Chronic lung disease (other than asthma). This includes cystic fibrosis, sarcoidosis, and interstitial lung diseases due to asbestosis, silicosis or radiation.
- [[Oral glucocorticoid medication (e.g., prednisone) for any indication – Oral steroid ≥7x on past 14 is an exclusion from AHAB2.]]

[1] Do no collect or store samples for CRP/IL6 or collect a green top for the Immune lab if the subject has any of the following medical conditions:

A.2 OTHER MEDICAL ISSUES AND MEDICATION RESULTING IN IMMUNE MEASURE INELIGIBILITY

- Acute or chronic infection being treated with antiviral or antibiotic – Such as Zovirax (acyclovir) for herpes, PCN for oral infection or Keflex for chronic osteomyeliltis.
- Regular use of allergy shots or recent vaccination (draw if given >21 days ago)
- Cold or flu in past 2 weeks**.** Exclude if symptom score >5.

A.3 BLOOD DRAW PROCESS

Before blood is drawn at the initial AHABII visit the subject completes a medical history and a medication review. The nurse reviews the checklist completed by the subject and further clarifies any diagnoses checked. Additionally, the subject is asked if he/she is taking any medications that require a prescription from a doctor, the name and dose of the medication, reason for the medication, how many days in the last 14 the med was taken[2], and finally when the medication was last taken. The process is repeated for over the counter medications and nutritional supplements. It is also asked if the client has EVER taken medication for mental health or mood, the name and reason of these meds as well as when last taken are recorded. A copy of the medication eligibility list is available in the lab for review and meds are checked for appropriate category. A general list of excluded drugs is a variety of cardiovascular, psychotropic, insulin, asthma/allergy, cholesterol, glucocorticoids, weight-loss, and sleeping meds. Secondary to medical condition or current medications some subjects are excluded at this point. Those that proceed next answer another series of questions.

- The participant is asked several questions related to current infections. The first question asks if the subject has taken any antibiotics or antivirals in the past 2 weeks. If yes, the subject is ineligible for immune labs that day (no green top tubes collected & no CRP/IL-6 samples preserved). Blood may be drawn 2 weeks from last dose taken (exception is Z-Pack where 5 extra days are added) & participant is asked if it would be acceptable to retry for the blood draw on visit 4 of the study. The next question asked is "Do you currently have or have you had an infection in the past 2 weeks." If yes, the subject does not have immune blood drawn (no green top tubes collected & no CRP/IL-6

[2] Medication eligibility coding categories are: 0-permitted daily or prn no restrictions, 1-disallowed daily or prn, and 2-disallowed if taken 7 or more days in the past 14 days.

samples preserved) that day and based on treatment or type of infection, it is determined when participant will be infection free and blood may be drawn 2 weeks from that date. Next, the subject is asked whether or not he/she has had cold or flu in the past 2 weeks. If the answer is yes, a Symptom Severity Scale is assessed. Eight items are assessed using a scale of 0 to 4, 0 being none up to 4 very severe. The items assessed are: congestion, sore throat, runny nose, sneezing, cough, malaise, headache, chills. If the score is <6 the immune labs are drawn. If the score is 6 or more the patient is ineligible to have immune functions drawn that day. The option is then given to have the immune functions drawn at the V4 if the repeat severity score is below 6. If the client has a severity score at the fourth visit of 6 or greater immune functions are not drawn for this subject. Additionally, subjects have the right to refuse the redraw at the 4th visit for immune functions.

- It is also verified that the subject has received no vaccinations or allergy shots in the past month and has not smoked any cigarettes that morning.

A.4 POST-STUDY CHART REVIEW

After study completion, we audited paper and electronic data to confirm adherence to the above guidelines. In several instances, immune sample results were re-coded as invalid because the samples were run despite the fact that an exclusion criterion was present.

Additionally, Drs Muldoon and Marsland decided the following.

1. Code as immune invalid/ineligible subjects who: were taking nasal or inhaled steroids 1-14 times in past 2 weeks. This excluded 7 subjects, and this procedure matches how AHAB1 immune measures were handled.
2. Code as immune invalid/ineligible subjects who took a sedating or non-sedating antihistamine within 2 days of blood draw. This concerns primarily subjects with hay fever or seasonal allergies and was done to exclude subjects whose immune system was currently perturbed by an antihistamine medication. In AHAB1, immune labs were considered invalid if subject reported use of sedating antihistamines (of > 7 in past 14 days), whereas non-sedating antihistamines were permitted. So, the rules were somewhat different in AHAB1 vs AHAB2.
3. Code as immune invalid/ineligible subjects who reported having a current cold/URI/flu with a symptom score > 1 **while a taking cold/flu remedy**.

APPENDIX B

EMA ITEMS REGARDING DAILY SOCIAL INTERACTIONS

- At time of BLOOD PRESSURE
  "In a social interaction?" No, Yes

- If yes, skip to "Think about this most recent interaction…" prompt

- At time of BLOOD PRESSURE

  "When did your most recent social interaction end? 0-10 min before ALARM,
  11-45 min before ALARM,
  45+ min before ALARM

- PROMPT SCREEN: Think about this most recent interaction….
  1. Type of interaction? In person, Telephone, Instant Messaging, Webcam (e.g. Skype)
  2. With how many people? 1 other, 2 others, 3 others, 4 or more
  3. Interacting with whom? Spouse/Partner, Co-worker, other friend, Other family or relative(s), Other acquaintances, Stranger
  4. Pleasant interaction? **NO!** No no yes Yes **YES!**
  5. Agreeable interaction? **NO!** No no yes Yes **YES!**

6. Someone treated you badly? **NO!** No no yes Yes **YES!**
7. Someone in conflict with you? **NO!** No no yes Yes **YES!**
8. I told someone they annoyed me. **NO!** No no yes Yes **YES!**
9. I yelled at someone. **NO!** No no yes Yes **YES!**

Note: Items 3 and 4 used to assess positive interactions and items 5 and 6 are used to assess negative interactions.

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