FOR VA IRB

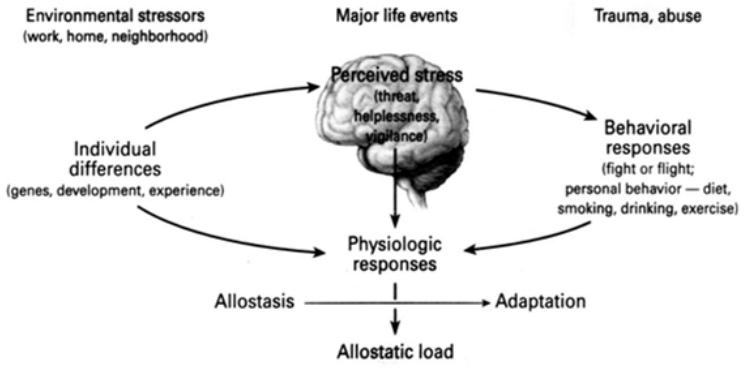
2. RESEARCH PLAN

Background

Over 42 million women in the U.S. have been diagnosed with cardiovascular disease (CVD) and more women than men die each year from CVD.[15] Traditional risk factors for coronary heart disease (CHD), such as obesity, hypertension, and diabetes do not fully explain CHD occurrence.[16] Compelling evidence implicates psychological stress in the etiology and pathogenesis of atherosclerosis and it is increasingly evident that chronic stress can promote inflammatory-based diseases, such as cardiovascular disease and stroke. Furthermore, studies demonstrate that cumulative stressors, such as childhood maltreatment, adverse social environment, and other salient social characteristics contribute to health status through various biological pathways.[17] Others suggest that adverse early life experiences, may "prime" inflammatory pathways predisposing an individual to mount a greater proinflammatory response to future stressors in adulthood.[18]

Evidence demonstrates that Veterans are at greater risk for CVD. For example, the Life Course Socioeconomic Status, Social Context and Cardiovascular Disease Study[2] reported a greater incidence of CHD and stroke among combat Veterans compare to noncombat Veterans and non-Veterans. However, these studies have primarily focused on men. Compared to female civilians, women Veterans have greater histories of abuse[19] and it has been suggested that some women may join the military to escape violent environments.[4] In a study of 238 African American women Veterans, Campbell et al.[4] found *that 59% had been sexually abused as children*; significantly higher than the 24.7% prevalence rate of childhood sexual abuse reported by women in the US(Centers for Disease Control).[20] Data substantiates an independent link between early adverse life events, such as childhood maltreatment, and elevated levels of proinflammatory cytokines.[21] As a whole, women Veterans, given their military service and socio-cultural experience, have significantly more lifetime exposure to stress than non-Veterans and are subsequently at greater risk for adverse stress-related inflammatory disease such as CVD. Therefore, it is crucial that the VA takes steps towards developing interventions to assist women Veterans in improving psychological well-being and reducing CVD risk.

Figure 1: Allostatic Load (McEwen, 1998)



Mindfulness Based Stress Reduction

Few interventions have been examined to reduce psychological distress and decrease CVD risk in women. One rigorously evaluated program found to reduce stress and improve health is Mindfulness Based Stress Reduction. Mindfulness Based Stress Reduction (MBSR) involves intensive training in mindfulness, which promotes positive adaptation to life stress. Mindfulness is a state in which one is aware and accepting of one's thoughts, physical sensations, and emotions in the present moment without analysis or interpretation. Practitioners of MBSR gain increased awareness and insight into the relationship among their thoughts, emotions, and somatic reactivity, which can facilitate change in conditioned patterns of emotional reaction.[7] These skills can be applied to deal with the stress of living with disease or other types of stressors. Instructors of MBSR undergo rigorous training and are certified prior to administering the program.

MBSR has wide appeal to diverse groups and has been adapted to meet specific needs of these individuals. Meta-analysis of MBSR and health benefits reveal consistent and strong effect sizes for psychological benefits in individuals dealing with emotional distress.[22] Although no studies were found that evaluated MBSR in women Veterans, MBSR is reported to reduce symptoms of depression and improve

quality of life (QOL) in Veterans experiencing Post-Traumatic Stress Disorder (PTSD).[6] Studies in nonveteran populations have demonstrated that MBSR decreases anxiety and depression and improves immunologic function and QOL.[23] However, few studies have examined MBSR as an approach to reduce stress in individuals with cardiovascular disease. Tacon et al.[24] found that MBSR reduced anxiety in women with advanced cardiovascular disease. However, that study was limited by a small sample size (N=18) and a wait-listed control group. Other studies have demonstrated that MBSR reduces blood pressure;[25] however overall risk for CVD disease was not examined.

Witek-Janusek et al.[26] (investigators for this proposal) demonstrated that MBSR reverses stress-associated immune dysregulation in cancer patients. In that study, women with recently diagnosed breast cancer who participated in an 8-week MBSR program exhibited improved restoration of cytokine production levels, including reduction of the proinflammatory cytokine interleukin-6 (IL-6), compared to women with breast cancer who received usual care. These findings suggest that MBSR has the potential to minimize inflammation related to CVD. However, MBSR has not been examined to determine its efficacy to reduce CVD risk.

The MBSR program has significant advantages over other stress reduction programs in that it has been widely tested in diverse populations with strong evidence of health benefits*. Importantly, it incorporates a training/certification program for instructors to ensure fidelity in administering the program.* A randomized clinical trial (RCT) testing a MBSR program specifically for women Veterans is needed to provide evidence of its effectiveness within this population. Importantly, MBSR has not been examined within the context of reducing CVD risk. If found effective in reducing CVD risk, MBSR could be used both within and outside of the VA as a safe and cost-effective treatment for addressing one our nation's leading causes of mortality.

Scientific Rationale and Theoretical Framework

Cardiovascular disease refers to diseases of the heart and blood vessels. The four most common types of CVD are coronary heart disease (CHD), ischemic stroke, hypertension, and heart failure. The most common mechanism for the development of CVD is atherogenesis, a complex process by which the artery becomes obstructed with plaque. The process appears to begin early in life with the deposit of small lipoprotein particles in the intima and progresses with leukocyte recruitment, formation of lipid-laden macrophages, and foam cells. Evidence continues to accumulate that implicates a state of chronic, low-grade inflammation in the development of atherosclerosis.[27] Endothelial cells and leukocytes within atherosclerotic plaques produce a variety of inflammatory mediators, such as interleukin-6 (IL-6).[27] Progression of an atherosclerotic lesion from an early fatty streak to an advanced fibroproliferative atherosclerotic lesion is believed to be related to infiltration of T cells and macrophages into the intimal wall. Activated T-cells present in atherosclerotic lesions can secret interferon (IFN)-γ which induces vascular cellular adhesion molecule (VCAM-1).[27]

Identification of psychobiological determinants of disease acknowledges the individual as the center of a dynamic environment where multiple social, psychological, biological, and cultural factors mediate and moderate disease progression and outcomes.[28] Such a view is consistent with *allostatic load,* a concept derived from the term *allostasis*, *meaning the maintenance of stability or "homeostasis."* [13] Allostatic load refers to the fluctuations in health status based on response to stressors and can be thought of as the "wear and tear" on the body that results from acute and chronic stressors. McEwen[13] conceptualized a model of the relationship between perceived stress and physiologic responses and the resulting allostatic load on the individual. This model will provide the basis for the proposed research **(see Figure 1).** Within the context of the proposed study, allostatic load will be considered to be cumulative exposure to stressors across the life span, including prior life adversity, chronic stress, and stressors associated with being in the military. The proposed stress reduction intervention, MBSR is posited to reduce allostatic load by assisting the individual to better cope and adapt to stressors, thereby improving psychological well-being and reducing inflammation associated with CVD.

Significance and Relevance to VA patient care

Heart disease and stroke account for more than one third of all U.S. deaths and are estimated to cost $444 billion per year.[29] It is established that, as a group, Veterans experience greater occurrences of coronary heart disease and stroke than non-Veterans.[30] However, previous studies have primarily focused on male Veterans. According to a recently published report describing the demographic characteristics of women Veterans,[31] the number of women Veterans using VA services has doubled in the past decade and is expected to grow considerably over the next several years. Further, this report found that the peak age of women using VA services was 47 years. As the risk of developing CVD in women increases with age, it is crucial that the VA develop and implement programs to reduce CVD risk in this growing population. Nurses within the VA can play an important role in training Veterans in the techniques of MBSR. **This proposal is responsive to the VA NRI 2012 priority to improve the health of women Veterans through nursing interventions. As well, CVD is identified as a high priority for VA Women's Health Research.[32]**

Psychological Well-Being, Inflammatory Markers, Cardiovascular Risk, and MBSR (Aim 1)

Several psychological variables and inflammatory markers have been associated with cardiovascular risk:

Psychological stress. Stress refers to events or stressors that are threatening to an individual and that evoke behavioral and physiological responses.[33] Numerous studies link chronic stress to CVD.[34] Although the mechanism is not clear, it is suggested that allostatic load contributes to the development of CVD. [35] Although evidence demonstrates that MBSR is effective in reducing psychological stress,[22] no studies were found that examined the extent to which MBSR reduced inflammation related to CVD.

Anxiety. Anxiety is defined as an unpleasant feeling that is typically associated with uneasiness, apprehension, and worry. It is a generalized mood condition that most often occurs without an identifiable stimulus and is distinguishable from fear, which occurs in the presence of an observed threat.[36] High levels of anxiety have been associated with increased risk of myocardial infarction.[37] Although studies have examined anxiety levels in relation to MBSR, no previous research was found that considered anxiety within the context of proinflammatory cytokines, MBSR, and risk of CVD.

Depressive symptoms. A plethora of literature supports the relationship between depressive symptoms and cardiovascular disease.[38] Furthermore, there is evidence to suggest that early life adversity promotes inflammation leading to depression[39] and may contribute to the development of CVD. For the proposed study, depressive symptoms and proinflammatory cytokines will be addressed in terms of CVD risk.

Social isolation. Evidence suggests that social isolation and loneliness is associated with well-being as well as cardiovascular risk. [40-42] Therefore, social isolation will be measured in the proposed study.

Sleep disturbance. Studies have consistently linked sleep disturbance and cardiovascular disease.[43] However little is known about how sleep disturbance may influence CVD and well-being in women Veterans. Therefore, sleep disturbance will be measured in the proposed study

Anger. Anger, an emotion characterized by irritation or annoyance, is predictive of CVD morbidity with one 7-year prospective study demonstrating a 2.66 greater risk of coronary artery events in individuals reporting the greatest levels of anger versus those with the lowest levels of anger.[40] Although MBSR has been shown to decrease anger,[41] anger was not considered in relation to MBSR, inflammation, and CVD risk.

Quality of life (QOL). Several studies demonstrate improvements in QOL post MBSR.[6] Furthermore, since QOL is closely linked to depressive symptoms and anger in women with CVD,[42] we will measure QOL in the proposed study.

Interleukin-6 (IL-6). IL-6 is a cytokine that influences the development of CVD by regulating fibrinogen synthesis.[43] Elevated systemic IL-6 levels are associated with chronic stress and low SES.[44] Evidence also demonstrates that social support attenuates IL-6 and may decrease risk of CHD.[45]

Interleukin-10 (IL-10). IL-10 is an anti-inflammatory cytokine that is induced by psychological stress.[46] However, previous studies have not examined IL-10 in relation to psychological stress and cardiovascular disease risk.

Interferon gamma (IFN gamma). IFN gamma is a cytokine that is strongly associated with perceived stress.[47] No previous studies have examined IFN gamma in relation to CVD and MBSR.

Vascular cell adhesion protein-1 (VCAM-1). Vascular cell adhesion protein-1 (VCAM-1) is a molecule found to be associated with endothelial dysfunction and is marker of early cardiovascular disease.[48] VCAM-1 is also associated with psychological stress and depression.[49] However, no studies were found that examined VCAM-1 in relation to MBSR or stress reduction.

Endothelial dysfunction. Compelling evidence implicates psychological stress in the etiology and pathogenesis of atherosclerosis. Vascular inflammation, particularly in the endothelium, plays a central role in the development of atherosclerosis.[50] and is considered to be an independent predictor of cardiovascular events.[50] Despite the strong body of evidence linking endothelial dysfunction to psychological stress and CVD, endothelial dysfunction has not been considered in relation to MBSR. Peripheral artery tonometry (PAT) of small distal arteries is a noninvasive, highly reproducible technique based on the pulse wave amplitude variations for the digital arteries before and after ischemia.[48] Measurement of endothelial function using PAT, in conjunction with measures of proinflammatory cytokines and sVCAM-1 will provide a novel method to assess the effectiveness of MBSR in reducing inflammation.

Protective and risk factors posited to moderate the effect of MBSR on psychological well being, inflammatory burden and cardiovascular risk in women Veterans (Aim 2)

Social support. Several studies demonstrate the important role of social support in diminishing CVD.[10] Therefore, social support will be measured as a moderator among psychological well-being and cardiovascular risk.

Positive emotions. Evidence supports that positive emotions are associated with better physical health outcomes.[55] Beliefs in one's capacity to savor positive experiences may be related to well-being and physical health. [56] Therefore, beliefs about savoring will be measured in this study.

Health behaviors. It is well-established that health behaviors, such as diet and exercise are related to cardiovascular health. Therefore, health behaviors will be measured as a moderator in the proposed study.

Acculturation. Acculturation is "the level at which an individual shares the values, language, and cognitive style of their own ethnic community versus those of the dominant culture."[51,(p. S11)] Cultural beliefs can affect how individuals feel about their health, when and by whom they seek healthcare, and how they respond to suggestions for health and lifestyle changes.[11] Therefore, acculturation will be considered as a moderator of the effects on MBSR on study outcomes.

Diurnal salivary cortisol. Studies demonstrate that prior life adversity can lead to a reduced cortisol response to stress which may result in reduced attenuation of the inflammatory response to stress.[12] Further, our recently published paper reported that chronic stress is associated with hypocorticolism in female informal caregivers.[52] Thus, we have included diurnal salivary cortisol as a moderator in our model.

Childhood maltreatment. Data substantiates an independent link between early adverse life events, such as childhood maltreatment, and elevated levels of proinflammatory cytokines.[53] Further, a recent study showed that a history of adverse childhood experiences nearly doubled the risk of coronary heart disease as compared to those individuals without adverse childhood experiences [54] It is theorized that prior adverse events alter neuroendocrine-immune pathways during critical developmental windows resulting in chronic low grade inflammation during adulthood, predisposing the individual to inflammatory disease.[55] Biological embedding posits that early life adversities and cumulative disadvantages "recalibrate" the physiological response to stress leading to a dysfunctional stress response pattern and risk for disease.[56] Further, evidence demonstrates that when these individuals are faced with acute stress, they respond with an

exaggerated inflammatory response and dysregulated HPA axis activity, furthering their risk for developing inflammatory disease, such as coronary artery disease or stroke.[18] Co-investigators on this application (Janusek and Mathews) recently published results demonstrating childhood adversity to result in more intense and persistent depressive symptoms and elevated plasma IL-6 in women recovering from the stress associated with breast cancer treatment.[5] Thus, childhood maltreatment will be measured as a moderator between psychological well-being and CVD risk.

Combat exposure. A recent study of 5,347 older male Veterans from the Korean and Vietnam era wars found that Veterans reported significantly higher levels of trait anger than non-Veterans.[57] Furthermore, the Life Course Socioeconomic Status, Social Context and Cardiovascular Disease Study[2] reported a greater incidence of CHD and stroke among combat Veterans compare to noncombat Veterans and non-Veterans. However, no literature is available related to combat exposure and risk of inflammatory disease in women Veterans. Thus, combat exposure will be measured as a moderator in the proposed study.

Collectively, evidence supports that psychological stress heightens the risk of CVD and that MBSR improves psychological well-being; yet little is known about the extent to which MBSR may decrease the risk of CVD. Further, research identifies women Veterans to experience greater amounts of prior life adversity than civilian women.[4] Therefore, examining the capacity of MBSR to improve psychological well-being and decrease CVD risk in women Veterans may offer an important contribution to improving the health of our Veterans.

Innovation

Our proposed study is innovative in that it will address the effectiveness of MBSR in women Veterans who are at risk for CVD. To the best of our knowledge, this has not been examined in prior studies. Further, we will consider prior life adversity, social support, health behaviors, and diurnal salivary cortisol as moderating factors influencing the strength of relationships among psychological well-being, proinflammatory cytokines, and cardiovascular risk. These factors are particularly important to consider in women Veterans given their common history of chronic stress across their life course. This was not accomplished in prior studies. Our study will also make other significant contributions:

- Little is known about how stress influences inflammatory risk in women Veterans. A better understanding of stress as a predictor of CVD in women Veterans may help guide development of risk profiles to facilitate early detection.
- Although studies have found that women Veterans experience high levels of prior life adversity such as childhood abuse and sexual trauma[4] and data substantiates an independent link between early adverse life events and elevated levels of proinflammatory cytokines[18], no previous studies have addressed the link among prior life adversity, chronic stress, inflammation, and risk of CVD in women Veterans. Furthermore, no studies have examined a stress reduction intervention, such as MBSR in decreasing inflammation associated with chronic stress and prior life adversity.
- No previous studies have examined endothelial dysfunction in relation to MBSR. Examining endothelial dysfunction in relation to stress reduction may shed light on the mechanism by which MBSR reduces inflammation and CVD.
- The Hines VA is one of 37 VA Women's Health Practice-Based Research (PBRN) sites and the co-Director of the Hines VA PBRN, Dr. Bhoopalam, is a co-investigator for this study. Dr. Bhoopalam has worked closely with the research team regarding the design of this study. We have also obtained the endorsement of Dr. Susan Frayne, the Director of the National PBRN **(see letter of support).** Collaboration with the PBRN will allow us to optimize dissemination of findings throughout the VA and promote implementation at other VA sites.

Preliminary Studies

Three studies have been completed related to stress and inflammation in chronically stressed, vulnerable populations. Although these studies have focused on a different population of women than that of the proposed study, they document the strong relationships among psychological stress and physiological biomarkers. In addition, they attest to Dr. Saban's ability to measure and interpret physiological measures, such as cortisol and cytokines. It is also important to note, that Dr. Saban will be working with a team of

mentors and co-investigators who are experts in conducting randomized clinical trials. Dr. Linda Janusek (co-mentor) and Dr. Herbert Mathews (co-investigator) have examined MBSR in relationship to immune function, quality of life, and coping in women recently diagnosed with breast cancer.[26] In addition, they are currently conducting a randomized clinical trial, funded by the National Cancer Institute (R01 R01 CA125455) evaluating the effect of an MBSR program for women with breast cancer to determine whether MBSR can improve the psychosocial response to cancer diagnosis and treatment, reduce neuroendocrine stress activation, restore immune function, and increase quality of life. Furthermore, Dr. Eileen Collins (co-mentor) is currently the principal investigator for two VA Merit Review Awards (Reducing Dynamic Hyperinflation Through Breathing Retraining VA RRD F6955R, Fall Prevention Training Program for Older Adults VA RRD E4955R), and co-principal investigator on one Merit Review Award (Structured Exercise in Obese Diabetic Patients with Chronic Kidney Disease: A Randomized, Controlled Trial VA RRD F7264R, David Leehey co-PI). Although the focus of these RCTs is different than proposed in this application, similar measures, such as endothelial dysfunction, are used.

Study examining stress and inflammation in female informal caregivers of Veterans with TBI
Funding source: VA Nursing Research Initiative Pilot Award: Investigators: K. Saban (PI), E. Collins (Mentor), L Witek-Janusek (co-Mentor)

Study design and purpose: The purpose of this descriptive, cross-sectional pilot was to evaluate caregiver and recipient characteristics, caregiver psychological and behavioral responses (health promotion and health care utilization), caregiver physiological measures of stress (cortisol, cytokines), caregiver perceived health, and QOL and the relationships among these variables. The sample consists of 49 informal caregivers caring for a veteran who incurred a moderate or severe TBI within the past one to ten years. We recruited informal caregivers via recruitment letters sent to families of Veterans with moderate or severe TBI, recruitment at Hines caregiver support events, recruitment information posted on several social networking sites and caregiver websites, as well as Hines Polytrauma Case Managers. We obtained our sample size of 49 within six months. The majority of participants were recruited via recruitment letters (84%), followed by caregiver websites and Facebook (9.2%), friends (3.1%), and Polytrauma case managers (2.3%).

Key findings: Findings reveal modest QOL levels (M=18.6 $\pm$0.9,range 4.7-28.0), moderate levels of caregiver burden including lack of family support (M=2.7$\pm$0.9, range 1.2-5.0), financial burden (M=3.1$\pm$1.0, range 1.0-5.0), impact on schedule (M=3.6$\pm$0.8, range 1.4-5.0), impact on health (M=2.7$\pm$.80, range 1.0-4.8), and high levels of perceived stress (M=20.5$\pm$8.4, range 0-36). Esteem caregiver burden scores were high, suggesting that caregivers valued their caregiving role (M=4.06$\pm$0.63, range 2.7-5.0). Age, hours of caregiving per week, duration of caregiving role, employment status, completion of college degree, caregiver burden, and perceived stress predicted QOL ($p \leq .001$) and explained 72% of its variance. However, only the caregiver burden subscale of esteem (p=.007) and perceived stress (p=.001) were significant predictors in the regression model. Results suggest that caregivers with low esteem and high perceived stress may be at risk for low QOL. These findings have implications for development of interventions to enhance QOL, in particular a stress reduction program, for informal caregivers of veterans with TBI.

In respect to cortisol, we found that, controlling for BMI and caregiving frequency and duration, cortisol awakening response was inversely associated with perceived stress (r = -.522,p = .007) and with the grief subscale of panic behavior (r = -.489, p = .013). TNF-alpha, a proinflammatory cytokine, was positively associated with grief subscales: detachment (r = .436, p = .030) and blame/anger (r=.480, p=.015). A blunted CAR may reflect “burnout” resulting from the chronic stress and grief experienced by caregivers. This may unleash proinflammatory cytokines and predispose caregivers to future inflammatory-related health risks.

A secondary aim of this study was to gather information regarding caregiver preferences regarding stress reduction interventions. Fifty-six percent of caregivers indicated that they were interested in a participating in a stress reduction and relaxation program. Participants were also asked to select (all that applied) their

preferred modes of delivery for such a program. Preferences were mixed with participants selecting the following: face to face, 44% (n=22), via the internet 38% (n=18), and over the telephone, 22% (n=11). However, 23% of participants who chose face-to-face as one of their preferred modes of delivery also chose the internet as a preferred mode of delivery. Further, we found that informal caregivers lived an average of 10.3 miles from the nearest VA facility with 36.7% of participants living greater than 10 miles from the nearest VA facility. Not surprisingly, participants who lived farthest from the nearest VA facility were more likely to select that they preferred the program to be offered via the telephone or internet.

Thus, our preliminary findings suggest that caregivers are interested in a stress reduction program and offering the program online may be an important option, especially for those caregivers who do not live near a VA facility.

Offering a stress reduction program, such as MBSR, for informal caregivers would be ideal. However, we found that many of these women lived a long distance from their nearest VA facility and preferred an online format. The proposed study, although focusing on a different female population, would allow us to gain knowledge and experience related to conducting a face-to-face MBSR program within the VA. Findings from the proposed study could be used to develop an online MBSR program that would be used to benefit a wide-range of Veterans and their families, including informal caregivers.

Status: Data collection completed in September, 2011 (N=49).

Dissemination of results: Papers are in progress. However, several presentations have been completed:

Saban, K.L., Pape, T.L., Witek Janusek, L., Griffin, J.M., Bryant, F.B. & Collins, E. (March, 2012). Predictors of Quality of Life Among Wives Providing Informal Care to Veterans with Traumatic Brain Injury. Oral presentation, MNRS Annual Meeting, March, 2012, Dearborn, MI.

Saban, K.L., Pape, T.L., Witek Janusek, L., Griffin, J.M., Bryant, F.B. & Collins, E. Stress and Inflammation Among Wives of Veterans with Traumatic Brain Injury: Preliminary Findings of Nursing Research Initiative Study. Invited Speaker, National VA Caregiver Research Special Interest Group, National teleconference, April 18, 2012.

Saban, K.L., Mathews, H.L., Hogan, N.S., Griffin, J., Pape, T.B., Collins, E.G. & Witek Janusek, K. (June, 2012). Grief is Associated with Cortisol Awakening Response and TNF-alpha in Female Partners of Veterans with Traumatic Brain Injury. Poster presentation. Psychoneuroimmunology Research Conference, San Diego, CA.

Study examining the stress and inflammation in female informal caregivers of stroke survivors
Funding sources: Sigma Theta Tau, Chicago Institute of Neurosurgery and Neurological Research Foundation, Ruth Palmer Foundation, and Loyola University Chicago- Investigators: K. Saban (PI), L. Janusek (Co-I), H. Mathews (Co-I), and F. Bryant (Co-I)

Study design and purpose: The purpose of this descriptive, cross-sectional, national, pilot study was to describe the psychological and physiological stress response of 45 female caregivers of stroke survivors three to twelve months following the stroke. This study included the measurement of salivary diurnal cortisol and cytokine levels. All data collection was done via mail.

Key findings: Over half of the sample reported high levels of depressive symptoms as indicated by the Center of Epidemiologic Study for Depression (CES-D) instrument. Findings also revealed that awakening cortisol levels were inversely associated with depression (r=-.457, p=.002), as well as inversely associated with the perceived caregiver burden subscales: lack of family support (r=-.320, p=.037), financial burden (r=-.441, p=.003), and impact on schedule (r=-.511, p<.001). Depression, caregiver burden, age, race, and employment status significantly predicted awakening cortisol levels (p<.001) and explained 53% of its variance (adjusted R^2=.529). However, only the caregiver burden subscale of impact on schedule (p<.001)

and race (p=.039) remained significant variables in the regression model. Results indicate that greater caregiver burden and depression are associated with decreased cortisol awakening levels. This finding is significant because altered patterns of cortisol are associated with higher risk of inflammatory disease, such as cardiovascular disease.

Status: Data collection and analysis completed.

Dissemination of results:

Saban, K.L, Mathews, H., O'Brien, T., Bryant, F. B. & Witek Janusek, L**.** (2012). Depressive Symptoms and Diurnal Salivary Cortisol Patterns Among Female Caregivers of Stroke Survivors. *Biological Research for Nursing.* Epub head of print, April 24, 2012.

Saban, K.L. & Hogan, N.S. (2012). Women Caregivers of Stroke Survivors: Coping and Adapting to a Life That Once Was. *Journal of Neuroscience Nursing.*44 (1) 2-14.

Saban, K.L. (April, 2011). The Psychological and Physical Health of Informal Caregivers of Stroke Survivors, Invited Speaker, Palmer Research Symposium, Loyola University Chicago, Maywood , IL

Saban, K.L**.,** Mathews, H., & Janusek, L. Relationships Among Depression and Caregiver Burden with Salivary Cortisol Patterns in Caregivers of Stroke Survivors.
(June, 2011) Invited Speaker, VA Research Day, Hines, IL
(March, 2011). MNRS, Columbus, OH.

Saban, KL. (February, 2011). Stress and Inflammation in Stroke Survivors and their Families. Invited speaker for the State of the Art Nursing International Stroke Conference, American Heart Association. Los Angeles, CA.

Pilot study examining social determinants of inflammation in African American women with CVD
Funding source: Loyola University Chicago, School of Nursing. K. Saban (PI), H. Devon (Co-I).

Study design and purpose. The aims of this pilot study were to: (1) describe perceived discrimination, subjective social status, perceived stress, and hsp70 in African American (AA) and non-Hispanic White (NHW) women diagnosed with CVD; and (2) determine the extent to which perceived discrimination, subjective social status, and perceived stress predict hsp70 when controlling for age, race, and household income. The sample for this cross-sectional, descriptive pilot study consisted of 10 AA and 21 NHW women admitted to the hospital for elective percutaneous cardiac intervention (PCI) or carotid endarterectomy. Participants completed written questionnaires measuring psychosocial variables and provided a blood sample for analysis of hsp70.

Key Findings: Race, age, household income, perceived stress, everyday discrimination, lifetime discrimination, and subjective social status together significantly predicted hsp70 and explained 32% of its variance. However, only everyday discrimination (p = .009) was a significant independent predictor of hsp70. Results indicate that social conditions, in particular lower subjective social status and greater perceived discrimination, are associated with the inflammatory biomarker, hsp70. Findings support the importance of social mediators in the inflammatory cascade. Although longitudinal studies with larger samples sizes are needed to confirm these results, findings imply that social characteristics, which may be amenable to nursing intervention, play a more important role in predicting inflammatory markers of CVD than race.

Status: Completed, publication in process.

Dissemination of results:

Saban, K.L., Hoppenstead, D., Bryant, F.B & DeVon, H.A. (under revisions. Social Predictors of Inflammatory Biomarkers Among African American and Non-Hispanic White Women with Cardiovascular Disease. Submitted to *Journal of Cardiovascular Nursing.*

Saban, K.L. DeVon, H.A Hoppensteadt-Moorman, D. &. (2012). Social Determinants are Associated with Heat Shock Protein-70 in African American and Non-Hispanic White Women with Cardiovascular Disease. Poster presentation for the International Stroke Conference, American Heart Association, January 31, 2012, New Orleans, LA

Figure 2: Overview of Study Design

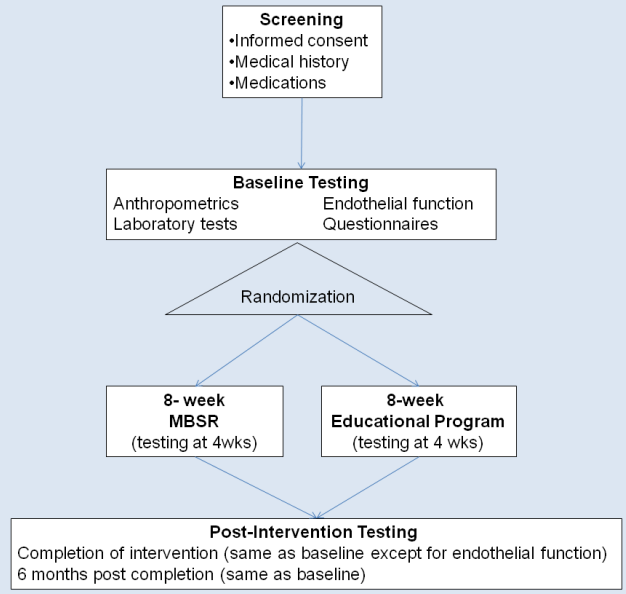
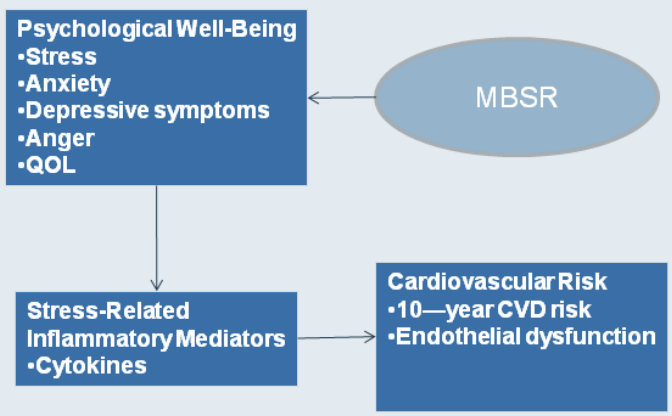


Figure 3: Conceptual Model for Study



Research Design and Methods

Overall Design

A repeated measures randomized clinical trial (RCT) design will be used to examine the extent to which an 8-week MBSR program (intervention) is effective in improving psychological well-being, reducing inflammatory burden, and decreasing cardiovascular risk in women Veterans at risk for cardiovascular disease. **See Figure 2**. The intervention group (MBSR) will be compared to an 8–week educational program (attention-only control). Measures of psychological well-being, inflammatory burden, and cardiovascular risk will be obtained prior to the program, 4 weeks after beginning the program, at completion of the program, and 6 months after completion. These measurement time points will allow us to assess both short term and long term effectiveness of the program and are consistent with frequency of measurement time points of other clinical trials examining MBSR.[14]

Conceptual Model

The conceptual model for the study will be based on allostatic load theory. **See Figure 3.** Evidence supports that MBSR improves psychological well-being and reduces allostatic load by attenuating proinflammatory cytokines.[26] Furthermore, studies demonstrate that psychological well-being influences inflammation and heightened inflammation leads to cardiovascular disease.[58] Prior life adversity, social support, health behaviors (including diet and physical activity), acculturation, and diurnal cortisol will be

evaluated as moderating factors and age, BMI, menstrual status, medications, and SES will be evaluated as covariates.

Aims

Aim 1. Determine the extent to which training in MBSR (1) improves psychological well-being, (2) decreases inflammatory burden, and (3) reduces cardiovascular risk in women Veterans.

H_1: Women Veterans trained in MBSR will exhibit greater improvements in psychological well-being, reduction in proinflammatory cytokines, and reduction in CVD risk compared to women Veterans completing a health education program.

H_2 Reduction in proinflammatory cytokines will mediate the effect of MBSR on cardiovascular risk.

Aim 2. Evaluate protective and risk factors posited to moderate the effect of MBSR on psychological well being, inflammatory burden and cardiovascular risk in women Veterans.

H_1: Prior life adversity (childhood maltreatment and combat exposure), social support, health behaviors, acculturation, and diurnal salivary cortisol will moderate the effect of MBSR on psychological well-being, inflammatory burden, and cardiovascular risk in women Veterans.

Exploratory Aim: (Added October 28, 2013). Determine the feasibility of offering a MBSR program to women Hines VA employees.

Sample size estimation

For hierarchical linear modeling (HLM), an N of 55 for each group (total N=110) provide 80% power to detect a treatment group X time interaction that is small in magnitude (Cohen's d=0.3). Based on prior research using the Perceived Stress Scale to examine MBSR, Cohen's d was 0.89.[59] and 0.41[60] for depressive symptoms using the CES-D. Therefore, we chose a conservatively small effect size. Further, this target N is based on prior research on the effects of sample size on power on HLM.[61] Bickel (2007) also recommends using at least 50 cases per group in HLM.[62] Attrition is a concern due to the longitudinal nature of the study. Comparable studies report attrition rates of 13 to 29%.[14] To compensate for attrition, we will over sample by 25%. Therefore, the total sample size will be 224 with 112 participants in each group. Power Analysis and Sample Size (PASS 11.0; Hintze, 2011) software was used to conduct the power analysis (PASS 11. NCSS, LLC. Kaysville, Utah, USA).

Sample characteristics

The convenience sample will consist of 224 (112 per group) of women Veterans over the age of 18 who are receiving care at the Edward Hines Jr. VA Hospital. Please see **Table 1** for inclusion and exclusion criteria. Information regarding date of last menstrual cycle and menopause stage will be collected on the health history form.

Table 1: Inclusion/Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Age 18 and older Female Veterans Able to write, read, speak English Must have1 of any of the following: • BMI $\geq$ 25 • Total cholesterol $\geq$ 240 • Diabetes mellitus or pre-diabetic • Systolic blood pressure> 120 and/ or diagnosis of hypertension and/or taking antihypertensive medications • Parental history of MI prior to age 60 • Currently smokes or history of smoking	History of myocardial infarction or ischemic heart disease/angina, left ventricular hypertrophy, ischemic stroke Pregnant, planning on becoming pregnant during study period, gave birth in prior 6 weeks, or lactating Major autoimmune disorders requiring the use of immune suppressant medications such as azathioprine, cyclosporine, monoclonal antibodies, and corticosteroids. Current cancer Active infection Current substance abuse History of suicide attempt(s) within past year Major psychosis Unable to participate in a group setting without feeling uncomfortable or being disruptive Already trained in MBSR

A preliminary review of de-identified patients found that in 2011 there were 635 women between the ages of 35 and 70 seen at the Hines VA with any two of the following diagnoses (ICD-9 codes): (1) Hypertension (401.0, 401.1, 401.9, 402, 402.01, 402.10, 402.11, 402.90, 402.91) (2) Diabetes or hyperglycemia (250.00 to 250.80), (3) Overweight or obese (278.00, 278.01, 278.02), (4) Hypercholestermia (272, 272.3, 272.4, 277.7). Excluding ischemic heart disease, cardiovascular disease, left ventricular dystrophy, cancer, PTSD, and tobacco use dependency, 325 women meet eligibility criteria based on 2011 data. If we conservatively estimate that 15% of these women will be interested and eligible in participating in our study, we will be able to enroll around 48 women per year allowing for three cohorts (consisting of an average of 16 participants for each cohort) to complete the interventions per year. Enrolling 48 women per year will allow us to reach our needed sample size of 224 women.

Setting and Recruitment Strategies
Participants will be recruited from the Women's Health Center and Community Based Outpatient Clinics (CBOCs) at Edward Hines, Jr. VA Hospital via clinical staff, newsletters, and electronic sources such as the Hines VA electronic message board. Recruitment flyers, brochures, and posters will be displayed in key areas of the hospital and outpatient clinics. In addition, recruitment letters will be sent to women Veterans enrolled at Edward Hines, Jr VA, Jesse Brown VA and Captain James A. Lovell Federal Health Care Center who meet inclusion criteria based on ICD-9 codes and demographic information. We will adjust the number of letters sent based upon response rate and will track response to various recruitment strategies in order to provide information regarding optimal recruitment strategies for future studies.

Randomization
Stratified block randomization (i.e. randomized permute blocks)[63] will be used to assign qualified participants who meet the criteria for participation to experimental groups. Age (categorized as 18-24, 25-34, 35-45, 46-59, and 60 and above years) will be used as clustering strata in structuring the random assignment of participants to experimental conditions, in order to optimize the distributional equivalence of intervention and control groups with respect to the age categories, ensure comparable sample sizes across conditions, and maximize statistical power.

The biostatistician (Bryant) and research assistant will prepare sealed envelopes for stratified randomization. Age strata will be (18-24), (25-34), (35-45), (46-59), and (60 and above) years. To maintain testing integrity, Dr. Saban will supervise testing and perform flow-mediated dilation tests of subjects and will be blinded to group assignment. Data analysis will be done in a blinded fashion by Drs. Saban, Collins, Janusek, and Mathews.

Variables and Instrumentation
Key variables and instruments are listed in **Table 2**. All instruments are included in **Appendix**. Perceived social support, prior life adversity (childhood maltreatment and military history), and health behaviors will be examined as moderators among psychological well-being and stress biomarkers and age, BMI, medications, socioeconomic status (SES), and diurnal salivary cortisol will be included as covariates in our model. Participants assigned to the intervention group will keep a daily log of the amount of time they spend practicing MBRS. In addition, to detect changes in mindfulness, all participants will complete the Five Facet Mindfulness Questionnaire (FFMQ)[64] at each measurement time point. The FFMQ is a 39 item tool that assesses 5 facets of mindfulness: observing, describing, acting with awareness, non-judging of inner experience, and non-reactivity to inner experience. Items are rated on a 5-point Likert scale. FFMQ has good internal consistency and construct validity with other tools assessing mindfulness.[64] Veteran participants will be administered the PTSD (PCL) checklist at each data collection time point..

Table 2: Key Variables and Instruments

Psychological Well-Being
Perceived current stress. Perceived current stress will be measured using the Perceived Stress Scale-10 (PSS).[65] The PSS-10 is a measure of the degree to which situations in one's life are considered to be stressful It contains

ten questions asking how often one felt stressed or thought a certain way in the past month, with five responses on a Likert-type scale ranging from never, almost never, sometimes, fairly often, to very often. Cronbach's alpha ranges from 0.75-0.90. [65] The PSS will be provided at each measurement time point.

Chronic stress. The Chronic Stress Questionnaire (CSQ) is a 51-item checklist that measures the presence of chronic stress in 8 domains: general stress, financial matters, employment, love and marriage, family and children, social life and recreation, residence, and health of self and close others.[66] As CSQ is a checklist of possible chronic life stressors, it is not amenable for determination of Cronbach's alpha (personal communication, Dr. Linda Janusek with L.Hawkley, PhD; May 26, 2009). The CSQ will be administered at each measurement time point.

State-trait anxiety. The State-Trait Anxiety Inventory (STAI)[67] is a 40- item instrument that includes separate measures of state and trait anxiety. Measurement of state anxiety consists of 20 items using a four-point Likert-type scale (1= not at all to 4=very much so) identifying the degree to which the respondent feels at the present time. The STAI has been used extensively with concurrent validity and test-retest reliability having been established within several studies.[67] The STAI will be administered at each measurement time point.

Depressive symptoms. The Center for Epidemiologic Studies Depression tool (CES-D) [68] will be used to measure level of depressive symptoms at each measurement time point.. The CES-D is a 20-item scale that measures the respondent's level of depressive symptoms using a 4-point Likert-type scale with responses ranging from 0 (none) to 3 (most of the time). A total score is obtained by summing each item; higher numbers indicate the presence of more depressive symptoms. The CES-D has been shown to be a reliable tool for assessing depression in healthy adults (α=.85).[69]

Social isolation. We will use the UCLA Loneliness Scale to assess social isolation.[76] This 20-item scale assesses the respondent subjective level of social isolation using a 4 –point Likert-type scale. Responses range from 1 (never) to 4(always). A total score is calculated by reverse scoring positive items and then summing scores. Higher scores indicate greater degrees of loneliness.

Sleep disturbance. Sleep disturbance will be measured using the Pittsburgh Sleep Quality Index (PSQI)[77], which consists of 19 items assessing various aspects of sleep during the past month, such as time needed to fall asleep and hours of actual sleep. The PSQI differentiates "poor" from "good" sleep using 7 subscales: overall sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, problems with daytime functioning, and medications taken for sleep. Scores from the 7 subscales are summed to form an overall rating of sleep quality, with higher scores reflecting poorer sleep quality. Total PSQI scores range from 0 to 21. A score of 5 or greater indicates poor sleep. Acceptable test-retest reliability and validity for the PSQI have been reported [77].

Anger. The Spielberger Trait Anger Scale[70], is a Likert type scale (ranging from 1 to 4) (57 items) that assesses a person's typical experience will be used to assess trait anger at each measurement time point. Individual responses are summed to yield an overall score. Cronbach's alphas range from .81 to .92.

Positive emotions. The Savoring Beliefs Inventory (SBI)[56] is a 24- item Likert type scale that assesses one's tendency to enjoy pleasant experiences in the moment (savoring the present), pleasurably anticipate them beforehand (savoring the future), and pleasurably recall them afterword (savoring the past). The instrument has been used in previous studies to assess changes in positive emotions associated with meditation. [79] The SBI has been shown to be a reliable tool for assessing savoring in healthy adults (Cronbach's alpha ranging from .87-.92). [79]

Resilience. The Connor-Davidson Resilience Scale 25 (CD-RISC-25) will be used to measure resilience. The CD-RISC-25 is a 25 item questionnaire that examines the extent to which statements representing resilience, such as " I am able to adapt when changes occur" are true for the subject[118]

Quality of life. The Ferrans and Powers Quality of Life Index – Generic Version III (QLI)[71] will be used to assess satisfaction with life. This instrument operationally defines quality of life as the degree of satisfaction with domains that are important to one and is a global measure of QOL. The tool consists of 33 items that measure satisfaction (1=very dissatisfied to 6=very satisfied) and importance (1=very unimportant to 6=very important) in four domains: health and functioning, psychosocial/spiritual, social and economic, and family. In addition, an overall global quality of life score can be calculated. Scores for the each of the four subscales as well as overall quality of life range from 0 to 30 with higher scores reflecting higher levels of perceived quality of life. The QLI demonstrates excellent internal consistency as demonstrated by Cronbach's alphas ranging from .73 to .99 across 48 different studies.[71]

Inflammatory Markers

Cytokines. Circulating IL-6, IL-10, and interferon gamma will be collected at each measurement time point. Heparinized blood (20ml, collected between 9 AM – 12 noon) will be transported immediately to Dr. Janusek's/Mathew's laboratory. Peripheral blood mononuclear cells (PBMC) will be isolated and plasma frozen as we have described previously.[72] Circulating cytokines will be measured under optimal conditions in bulk PBMC culture supernatant fluids.[73] PBMC (1 $X10^6$ cells/ml) will be cultured with and without PMA/PHA (PMA at 20 ng/well; PHA at 0.05%/well) in 24-well plates for 48 h at 37^0C. Aliquots of the culture supernatants will be stored at 80^0C for subsequent cytokine analysis. All cytokines will be measured using quantitative sandwich enzyme immunoassay techniques (Quantikine kits, R&D Systems, Minneapolis, MN). Sensitivities for cytokines are: IL-6 <0.7 pg/ml, IL-10

<2 pg/ml, and IFN-gamma <3 pg/ml. The coefficient of variation ranged between 2.6% and 4.9% for the individually assessed cytokines.
Soluble vascular cell adhesion molecule-1 (sVCAM-1). ELISA will be used to measure circulating levels of VCAM-1 (R&D Systems, Minneapolis). Sensitivity is <0.6 ng/mL. The intra-assay coefficient of variance is 3.5% and the inter-assay coefficient is 7.7% (R&D Systems). sVCAM-1 will be measured at each measurement time point.
Cardiovascular Risk
CHD/Stroke risk. We will use the Clinically Simplified Model B for Global CVD Risk Prediction of the Reynolds Risk Score [74] to measure CHD/stroke risk in women at each measurement time point. Measures for age, HbA1c, smoking status, SBP, high-density lipoprotein cholesterol (hdl), total cholesterol, high sensitivity C-reactive protein (hsCRP), and parental history of myocardial infarction before age 60 will be weighted using the computational formula for 10 year risk of global CVD (myocardial infarction, ischemic stroke, coronary revascularization, and cardiovascular death). The Reynolds Score was validated in a cohort of 24,558 initially healthy women over the age of 45 who were followed for a median of 10.2 years. DeFilippis et al.[75] suggested that the Reynolds Risk Score may provide additional predictive information than the Framingham. In addition, low density lipoprotein (LDL)) will be collected.
Endothelial dysfunction. Endothelial function will be assessed by measurement of flow-mediated vasodilation using Peripheral Arterial Tone (PAT) signaling technology to non-invasively measure arterial tone changes in peripheral arterial beds (Endo-PAT 2000, Itamar Medical, Framingham, MA). The Endo-PAT is available in Dr. Eileen Collin's laboratory and she has graciously agreed to allow it to be used for this pilot study. The PAT Signal is measured from the fingertip by recording finger arterial pulsatile volume changes. Based on PAT Technology, the noninvasive Endo-PAT2000 system comprises a measurement apparatus that supports a pair of modified plethysmographic bio-sensors. The unique feature of the PAT bio-sensors is that they impart a uniform sub-diastolic pressure field to the distal two thirds of the fingers including their tips. Endo-PAT quantifies the endothelium-mediated changes in vascular tone, elicited by a 5-minute occlusion of the brachial artery (using a standard blood pressure cuff). When the cuff is released, the surge of blood flow causes an endothelium-dependent Flow Mediated Dilatation (FMD). The dilatation, manifested as reactive hyperemia, is captured as an increase in the PAT Signal amplitude. A post-occlusion to pre-occlusion ratio is calculated, and comparison is made to the opposite arm to control for concurrent non-endothelial dependent changes in vascular tone. This device has been used in the Framingham Heart Study, Gutenberg Heart Study, and the Jackson Heart Study among others. It has been shown to demonstrate good reproducibility,[76] and improve with various pharmacologic interventions.[77] However, the influence of chronic stress on endothelial function monitored in this fashion is not known and will be addressed in this proposal. Endothelial function will be measured at baseline and 2 weeks after completion of the intervention. Dr. Saban or designee will perform flow-mediated dilatation tests and as stated will be blinded to study group assignment.

Moderating factors

Childhood maltreatment. The short form Childhood Trauma Questionnaire (CTQ-SF)[78] will be used to measure adverse childhood events. The CTQ-SF is a 28-item questionnaire that generates a total score and five subscale scores for childhood adversity: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. The CTQ-SF has demonstrated excellent reliability and validity (intraclass correlation coefficients r=.76-.86).[78] This variable will be assessed at baseline only.

Combat exposure. The commonly-used, widely validated Combat Exposure Scale (CES)[79] will be used to examine military trauma. The CES is a 7-item self-report measure that assesses wartime stressors experienced by combatants. Items are rated on a 5-point frequency (1 = "no" or "never" to 5 = "more than 50 times"), 5-point duration (1 = "never" to 5 = "more than 6 months"), 4-point frequency (1 = "no" to 4 = "more than 12 times") or 4-point degree of loss (1 = "no one" to 4 = "more than 50%") scale. Respondents are asked to respond based on their exposure to various combat situations, such as firing rounds at the enemy and being on dangerous duty. The total CES score (ranging from 0 to 41) is calculated by using a sum of weighted scores, which can be classified into 1 of 5 categories of combat exposure ranging from "light" to "heavy." The CES will be administered at baseline only.

Perceived social support. The Social Provisions Scale (SPS) is a 24-item instrument that measures attachment, social integration, reassurance of worth, reliable alliance, guidance, and opportunity for nurturance.[80] Respondents rate the degree to which their social relationships support their social needs. Cronbach's alpha is reported at 0.84.[80] The SPS will be administered at each measurement time point.

Health behaviors. Items adapted from the Behavioral Risk Factor Surveillance System Survey (BRFSS)[81] will be used to measure alcohol use, sleep quality and health promotion activities, such as annual physical exams. The adapted Kaiser Physical Activity survey[82] will be used to measure physical activity, including aerobic exercise. Questions related to diet habits have been adapted from Patterson, et al.,[83] from the Women's Health Initiative. Information regarding health behaviors will be collected at each measurement time point. Physical Activity Questionnaire (IPAQ) will be used to examine physical activity over past 7 days.[115]

Acculturation. The Multigroup Ethnic Identity Measure (MEIM)[84] will be used to measure acculturation.[84] This scale consists of 15 items which assess the participant's ethnic/cultural sense of identity and affirmation (sense of belonging to one's ethnic/cultural group). An overall score is calculated, ranging between 1 and 4 with higher scores indicating greater ethnic/cultural identity and affirmation. Cronbach's alphas were greater than .80 across a wide range of ethnic groups and ages.[84]

Diurnal salivary cortisol. The neuroendocrine response to stress will be assessed by measuring diurnal salivary cortisol. For salivary measures, using a "saliva collection kit," subjects gently chew (60-90 seconds) on a soft swab that fits into a holder resting in a centrifuge tube. Participants will collect saliva (using salivettes) at awakening, and 30 minutes post-awakening as well as at 1200, 1700, and bedtime (n=5 collections per day). [119,120] Subjects will bring the collected saliva (packaged appropriately) to their appointments (at baseline, mid-way through intervention, at completion of intervention, and 6 months post-intervention) and saliva samples will be immediately taken to Drs. Janusek and Mathew's laboratories and centrifuged (1,000 x G for 7 minutes) and frozen (-80C) for later batch analysis. Samples will be assayed in duplicate using immunoassay kits (Salimetrics, State College, PA). In a previous study, the lower detection limit of the enzyme immunoassay (EIA) was 0.3 nmol/L. The intraassay imprecision (CV) was 5.2% at 3.1 (SD, 0.2) nmol/L (n = 10) and 2.6% at 10.4 (0.3) nmol/L (n = 10). Interassay (total) imprecision (CV) was 11% at 2.8 (0.3) nmol/L (n = 10), 11% at 10.1 (1.1) nmol/L (n = 10), and 6.9% at 25.0 (1.7) nmol/L (n = 10).[85] We will provide telephone reminders during salivary cortisol collection as well as 24/7 phone support.

Covariates

Anthropometric measures. Blood pressure, pulse and height and weight will be obtained using standard equipment at each measurement time point. Body Mass Index (BMI) (kg/m^2) will be calculated

Medications. We will control for use of statins, beta blockers, NSAIDS, ACE inhibitors, and HRT (yes/no). A medication list will be completed at each measurement time point.

Socioeconomic status. Socioeconomic status, such as education level and household income will be collected using a form developed by the investigator as well as the Barratt Simplified Measure of Social Status.[86] The Wealth Index[87] (# of specific assets that participants own among the following: one or more cars, a home or paying mortgage on a home, land, or an investment (stocks, bonds, mutual funds, or retirement investments) will be used to measure financial assets. This information will be collected at baseline only.

Menstrual cycle. Date of first day of last menstrual period will be collected In addition, menopause stage will be categorized as Pre-, Peri-, or Post-menopausal using bleeding patterns from the Stages of Reproductive Aging Workshop.[88] These are: Pre-menopausal (no decrease in predictable bleeding onset in prior 12 months), Peri-menopausal (menstrual period in last 3 months, but less predictable onset in last 12 months) and Post Menopausal (no bleeding in past 12 months).

PROCEDURES

The study will be approved by the VA Hines Institutional Review Board (IRB) and R&D committee. If eligibility criteria are met and the individual expresses interest in participating in the study, the individual will be provided a verbal explanation of the study including the purpose of the study, risks and benefits, provisions of confidentiality, and the voluntary nature of the study. If the participant expresses an interest in participating in the study and no exclusions have been uncovered, an appointment that is convenient for the

potential participant will be made with one of the study personnel (Dr. Saban or the Project Manager or Research Assistant) to provide informed consent. In addition, a copy of the informed consent form will be mailed to the participant to review prior to the appointment. At the appointment, the study will be reviewed again and any questions that the participant has will be addressed. The individual can either sign the informed consent form at this time (she will be provided a copy) or take it home to think about it. If the participant decides to participate in the study, the salivary cortisol collection kit will be given to the participant along with an explanation of the procedure for collecting saliva. Instructions accompanied by a telephone number for support and research staff-follow up phone contacts will guide the standardized, daily self-collection process. In addition, an appointment will be coordinated with the participant to collect data (written questionnaires, blood draw and endothelial dysfunction measure). The appointments will be provided on a weekday morning, as convenient for the participant. A member of the research team will call the participant the night before saliva collection is to begin to review the procedure for collecting saliva. Individuals will collect their saliva samples (upon awakening, 30 minutes after awakening, noon, late afternoon and bedtime) for 2 consecutive weekdays directly preceding their scheduled appointments. The participants will keep their collected saliva samples in their refrigerators and bring them to their appointments.

At the appointment, the participant will be randomized to the intervention (MBSR) or control group (health education). Height and weight will be obtained and blood will be drawn (30ml) to measure cytokines and Reynolds labs (Hba1c, hsCRP, LDL, HDL, total cholesterol) by a certified phlebotomist or registered nurse (project manager). Samples will be taken immediately to the respective laboratories (i.e. cytokines will be taken to Dr. Janusek's/Mathew's lab and Reynolds labs to the Hines VA clinical laboratory). Following a rest period of 10 minutes, blood pressure will be collected using an automated Critikon Dynamap® 8100 non-invasive blood pressure monitor. Three blood pressure readings will be obtained and the average reading will be recorded. Endothelial function will then be measured using peripheral artery tone (PAT) signaling technology (**see details in Table 2**). After the endothelial function measurement, the participant will be provided a quiet place to complete the written questionnaires. During this time, the participant will be provided a light snack (i.e. bagels, juice). At the completion of baseline testing the participant will be given $25.

If for some reason the participant is not able to collect their saliva at home prior to the appointment, the participant will be provided Infectious Agent B compliant packaging which includes an inner tube to place the specimen in and acceptable outer box and ice gel packs for overnight shipping. All study personnel involved in the shipping of biohazardous materials will be certified in "General Awareness and Function Specific Shipping of Hazardous Materials" offered thru the Hines VA Hospital. All data shipped will be identified by a study code number only. Upon completion of saliva collection, the participant will call a provided toll-free UPS number to have the materials picked up from their home and delivered to the investigator overnight.

If the subject agrees (see Informed Consent), blood and saliva specimens will be saved for future research use related to stress and inflammatory disease. Leukocytes will be extracted from blood samples and save to test for global DNA methylation. Specimens will be stored at a secured location within the U.S chosen by the investigator. They will be stored up to 10 years unless used up. After 10 years, unused specimens will be destroyed.

This data collection procedure will be repeated for each data collection time point (baseline, 4 weeks, at completion of 8-week intervention, and 6 months after intervention) with the exception of endothelial function being measured only at baseline and 2 weeks post intervention.

Intervention

The MBSR program will be based on that of Kabat-Zinn, who originally developed MBSR.[7] The program will consist of 8 weekly (2.5 hours per week) group sessions plus one weekend day retreat session. Dr. Chris Chroniak, a clinical psychologist **(see Letter of Support)** from the Insight Center based in Chicago, will lead

the sessions. Dr. Chroniak has been trained and certified in MBSR and has been teaching MBSR for more than 15 years. Dr. Chroniak has been participating in Dr. Linda Witek Janusek's (co-mentor for this proposal) NCI R01 funded study examining MBSR in women with breast cancer for the past 4 years.

The topics of each session are detailed in **Table 3**. **(Additional details are in Appendix**). Cohorts of participants will consist of 7-10 participants each and sessions for the treatment and control group will be provided concurrently (for example, MBSR on Monday evenings and Education Program on Wednesday evenings). Topics for the education program (attention-control) were selected as to not confound the overall objectives of the MBSR program of reducing psychological distress and improving CVD risk. The Project Manager, a master's prepared nurse, will coordinate and teach the health education program. Participants will be provided with$25 cash at the end of each session to defray travel costs.

Participants who miss a session will be contacted promptly and any issues will be addressed as appropriate.

To note, if desired, participants completing the MBSR or Health Education program will be allowed to "cross-over" to the other program after they have completed the study. These participants will be considered to be "off-study" and will not participate in any further data collection.

Table 3. Description of weekly sessions for each program

Session	MBSR Program (Treatment)	Health Education Program (Attention-Control)
1	Introduction to Mindfulness and Formal Practice	Introduction to Health Education Program, VA Benefits, and Communicating with Your Healthcare Provider
2	The Attitudes of Mindfulness and Role Perception	Body Mechanics to Protect your Back
3	The Pleasure and Power of Being Present to Your Life in the Face of an Uncertain Future	Healthy Cooking
4	From Stress Reactivity to Mindful Responding	Photography as a Hobby
5	The Role of Thoughts and Emotions in Health and Illness	My healthy Vet presentation and Health Screenings
6	Embracing the "Enemy" in Interpersonal Conflict	Over –the-Counter Medications: Potential Hazards
7	Exploring Attachments – Identifying Social Support Networks	Safety
8	Continuing and Deepening the Practice	Enhancing your Memory

- Health Education classes may vary due to presenter availability.

Event Summary
Please see **Table 4** for summary of events related to the proposed study. Participants will be randomized after completing baseline measures.

Table 4: Time and Events Schedule

Event	Screen	Baseline	Weeks 1-8	Week 4	Week 8	6 m post-intervention
Consent	•					
Medical history	•			•	•	•
Current meds	•			•	•	•
Anthropometrics		•		•	•	•
Blood collection for lab tests *(cytokines, sVCAM-1, CRP, HbA1C, FBG, HDL, LDL, cholesterol)*		•		•	•	•
Diurnal salivary cortisol X 2 days – collected at home		•		•	•	•
Endothelial function		•			•	
Written questionnaires		•		•	•	•
MBSR program*			•			
Educational program			•			
Weekly log/diary*			•			

* For MBSR participants only

Time Summary
The two groups of participants (MBSR group and health education control group) will complete the same testing procedures at the same time intervals. Time required for participants to complete baseline testing is approximately 2.25 hours. About 1.5 hours is required for week 4 and 6 month post intervention data collection time points and 2 hours at week 8 data collection time point. **(See Table 5)**. In order to enhance adherence to the data collection time points, we will be flexible in scheduling a time that is convenient to the participant. In addition, since participants will be required to provide data in the morning because we are collecting fasting labs, we will provide participants with a light snack (bagels, juice, etc.) while they are completing the written questionnaires. Participants will also be reimbursed for each data collection time point as follows: Baseline - $25; 4 Week - $100; 8 Week - $75; 6 Month - $75

In addition to the time required for data collection, participants will also attend either the 8- week MBSR program or 8-week health education (control) program. These weekly sessions will each be approximately 2.5 hours and will occur at the Hines VA during a weekday, either morning, or evening.. In addition, the MBSR participants will be asked to attend a weekend day Mindfulness retreat during the MBSR intervention period. We will offer subjects who have completed the MBSR 8 week program the opportunity to attend the weekend day retreat sessions in future MBSR sessions. This is standard practice for MBSR programs. Participants will be reimbursed $15 for each weekly session that they attend to defray travel costs (8 sessions X $15 =$120).

Table 5: Summary of time required of participants for data collection

Procedure	Baseline	4 wk	8 wk	6 month
Questionnaires	80 min	65 min	65 min	65 min
Endothelial dysfunction	30 min	0 min	30 min	0
Blood draw	5 min	5 min	5 min	5 min
Anthropometrics	10 min	10 min	10 min	10 min
Saliva Collection (at home)	10 min	10 min	10 min	10 min
Total Time	**2.25 hrs**	**1.5 hours**	**2 hours**	**1.5 hours**

Intervention Fidelity MBSR. Intervention fidelity is ensured by using strategies set by "Best Practices and Recommendations from the NIH Behavior Change Consortium." [89] Strategies address key areas of "best practices" (trained providers, delivery of treatment, receipt and enactment of treatment skills). Importantly,

the intervention will be given by a single experienced MBSR interventionist for all cohorts and will be delivered using standardized course materials. The interventionist, Dr. Chris Chroniak, is a licensed clinical psychologist, trained in MBSR by Kabat-Zinn. He has more than 20 years of experience teaching MBSR to well and ill groups. His approach is consistent with the Kabat-Zinn model. At the start of each MBSR cohort, Dr. Janusek and Dr. Chris Chroniak will review program objectives, content, and delivery approaches to ensure equivalence and prevent "decay" of delivery over time/cohorts. Also, they will have weekly discussions regarding the class progress and any issues that threaten the study's internal validity. The intervention provides each group with a fixed number, length, and frequency of MBSR sessions, consistent with Kabat-Zinn. A standardized session-by-session workbook based on the Kabat-Zinn program is used by the interventionist and participants. Each session has practice exercises and weekly readings assigned from Kabat-Zinn's book, *Full Catastrophe Living*.[7] Home practice is done with the Kabat-Zinn CDs/tapes. The Kabat-Zinn book, CD/tapes, and program workbook are provided. Strategies to monitor subject receipt and enactment of treatment include weekly review of subject self-monitoring logs that document weekly practice. Dr. Chroniak will assess the transfer of MBSR skills by asking questions and discussing material with participants and monitoring skill development during in-class practice. Subjects complete a brief post-MBSR evaluation form to provide feedback regarding use of MBSR skills during appropriate life situations. See **Appendix** for MBSR program outline and Post-MBSR evaluation form.

Intervention Integrity. Multiple mechanisms will be used to ensure integrity of the MBSR intervention and Control Condition. These mechanisms satisfy Dane & Schneider's 5 dimensions of intervention integrity: adherence (extent to which program objectives are met), quality of delivery (interventionist effectiveness), exposure ("dosage"), participant responsiveness (engagement), and program differentiation.[90] Adherence to program objectives will be monitored using multiple informants and methods to collect integrity data. Interventionists will complete a self-evaluation checklist after each weekly session indicating achievement of session objectives. Exposure will be monitored by recording attendance at sessions and by asking subjects to complete a checklist indicating their receipt of session objectives at the end of each session. Participant responsiveness to MBSR will be monitored using weekly logs in which subjects record their reactions to each session and the frequency and duration of their MBSR home practice. Logs will be reviewed weekly. To detect changes in mindfulness, participants will complete the Five Facet Mindfulness Questionnaire (FFMQ) at each data collection time point. Program differentiation will be maintained by ensuring that best practices are followed, while contraindicated or irrelevant elements are excluded. Participants enrolled in both groups will be requested not to enroll in any other stress reduction or mind-body programs during the study. This will be monitored at each data collection (See Health Assessment Survey in the **Appendix**). Program uniformity and best practice across cohorts is ensured by using the same expert MBSR interventionist and the same educator for the control condition (with "scripted' classes). Dr. Saban will conduct training sessions for all research personnel prior to study implementation and the importance of adhering to the research design will be emphasized. Intervention integrity data will be discussed at each research team meeting.

Fidelity of the Control Condition will be per method of Stanton[91] and similar to that described for the intervention. Dr. Saban will train the Control Condition instructor in the study protocol prior to study initiation. Session content will be reviewed and approved (PowerPoint slides, videos & demonstrations) prior to study initiation (and between cohorts) to ensure appropriate content and best practice. The content will be "set" and delivered consistently across cohorts. Subject receipt of the Control Condition will be monitored by attendance and subject rating of educators' achievement of session objectives (See **Appendix).** The Control Group instructor will not include content on stress reduction or methods (yoga, meditation, etc.) taught in MBSR. Control instructors will be mentored on ways to handle questions or topics raised by control subjects that relate to stress/symptom management. Strategies to redirect class focus will be demonstrated (role playing) and discussed. Classes will be provided in the identical setting as MBSR, at the identical time but on a different weekday to avoid crossover (diffusion) of effects. Control Condition women will be surveyed about health services and/or health practices used outside of and within traditional medicine.

PLANNED ANALYSES

Analysis of Aim 1.Determine the extent to which training in MBSR (1) improves psychological well being, (2) decreases inflammatory burden, and (3) reduces cardiovascular risk in women Veterans

Means, medians, proportions, and standard deviations will be computed for each time point for each study variable. Differences between the groups will be examined using tests for continuous variables, chi-square tests for categorical variables, and non-parametric Mann-Whitney U tests for ordinal and non-normal variables. Temporal changes within the groups will be analyzed using hierarchical linear modeling (HLM). Evidence suggests that hierarchical linear modeling is a more robust statistical analysis method than repeated-measures ANOVA in that it has less strict assumptions, has more flexible data requirements to deal with missing data, and stresses individual change over group differences.[92] This last point is important because, for our study, HLM will allow us to directly test the question of whether (and by how much) the rate of reduction in perceived stress, anxiety, and depressive symptoms within individuals depends on their level of symptoms at baseline. Repeated measure ANOVA would not permit such a test. Latent growth curve (LGC) analysis via structural equation modeling (SEM) will also be used to examine correlates of individual differences (such as age, SES) in rates of change within and across experimental groups. Finally, feasibility of the protocol will be assessed based on recruitment rate, attrition, and reported perceived benefits of the study.

Additional multiple regression analyses will test competing mediational models of the underlying processes through which the program has its effects (e.g., participation decreases levels of IL-6, which in turn improve cardiovascular risk), using both cross-sectional as well as longitudinal techniques.[93]

One advantage of HLM analysis compared to other statistical techniques is that it allows participants whose data are missing at one time point to be included in other time points for which they have valid data. For the LGC analyses, we will use full information maximum likelihood estimation to avoid loss of power associated with missing data.[94]

Analysis of Aim 2. Evaluate protective and risk factors posited to moderate the effect of MBSR on psychological well being, inflammatory burden, and cardiovascular risk in women Veterans.

Similar to the analysis for Aim 1, the main effect of each potential moderator variable will be analyzed in relation to well-being variables, inflammatory measures, and cardiovascular risk variables. Moderators will then be tested by adding a multiplicative-product interaction term for the main independent variable (i.e., CVD risk) and the moderating variable to the models as described above.[95] Significance of the interaction term will indicate if the particular variable moderates the relationship between the main independent variable of interest (e.g. CVD risk) and the dependent psychological well-being and inflammatory burden variables (i.e., if the variable changes the magnitude or direction of associations).

Participant Retention Plan

As recommended,[96] multiple mechanisms will be used to maximize subject retention and decrease missing data. 1) Project identity will be established to increase subject commitment to and identification with the study. This includes: using a study logo for business cards, letters, workbooks, and flyers; 2) Subject cash incentives for each data collection time point point (Baseline - $25; 4 Week - $100; 8 Week - $75; 6 Month - $75) plus$15 to defray travel costs for each of the 8 sessions of the program ($15 X 8 = $120) for a total of $395 for completing the program; 3) Strategies to increase compliance with study appointments include: reminder phone calls prior to appointments, on-going contact with subjects between assessments by sending hand-written cards restating study goals and progress, and accommodating subject's time constraints by scheduling appointments for data collection at times that are convenient for the subject; and 4) Training of nurse project manager/research assistant in subject retention strategies and in the importance of subject follow-up; well-trained staffs are critical for subject retention. The study procedural manual will include retention plans and participant tracking databases. Retention progress and issues will be addressed at each research team meeting As noted in the Research Plan, subjects who miss appointments or classes will be promptly called to understand the underlying reason and to discuss and implement plans (as appropriate) to retain the subject or to reschedule the missed appointment.

Potential Problems and Alternatives/Limitations of Study

There are several limitations of the proposed study. First, given the longitudinal nature of this study and the significant commitment necessary to participate in the study, attrition is an inherent potential issue. Janusek et al. [26] found attrition rates of 15% using a similar design in a previous study of women in the midst of breast cancer treatment. For the proposed study, we will over-sample by 25% to accommodate potential attrition. In addition, we will take several steps to minimize attrition including collecting phone contact information and best time of day to call for participants and reviewing and updating this information at each data collection time point. We will also provide periodic updates via meetings and newsletters to staff (physicians, nurses, etc) in the Hines Women Center and CBOCs to remind them of the study and the importance of their support.

Second, the saliva collection requires the participant to collect saliva at 5 specific time points during the day. Given busy schedules, participants may forget to collect their saliva. We will provide easier to understand instructions and telephone support. We have experienced high adherence to collecting saliva samples in previous studies.[52] Third, although we will collect information regarding diet and exercise behaviors, we will not be able to completely control for the effects of diet and exercise on psychological well-being and cardiovascular disease risk. In addition, there may be other confounding variables, such as marital discord, that we will not measure in this study. Yet, these life stressors will be identified by our general measure of stress (Perceived Stress Scale).

Dissemination and/ or Implementation Plan

Several products are expected as a result of this study:(1) At least three published papers describing the findings of the study; (2) Dissemination of results at both national and local conferences, including the Annual VA HSR&D Meeting and VA Women's Health Research Conference, (3) Development and submission of a VA IIR examining MBSR in women Veterans at multiple sites and with other inflammatory risks. **Please see Table 6 for details**. In addition, findings will be exported throughout the VHA thru the Women's Practice-Based Research Network (PBRN). The intended audiences for the research are other VA researchers, nurses, psychologists, social workers, and physicians as well as women Veterans. Estimated costs for dissemination of results are primarily related to travel to scientific conferences to present findings.

If the findings of the proposed study demonstrate that MBSR is effective in improving psychological well-being and reducing cardiovascular risk, we will explore implementing the program at the Hines VA as well as other VA sites. One potential challenge will be training and certifying nurses and mental health providers in MBSR. However, several clinicians, including nurse practitioners, at the Hines VA have already expressed an interest in becoming certified MBSR trainers. The Center for Mindfulness in Medicine, Health, Care, and Society based at the University of Massachusetts Medical School (www.umassmed.edu) offers several programs a year to train professionals in teaching MBSR and obtaining MBSR Teacher Certification. In addition, the Insight Center for Stress Management and Integrative Psychotherapy (Chicago, IL) offers classes for clinicians interested in teaching MBSR.

We would also like to explore developing the MBSR program **online** in order to benefit Veterans and their families (including informal caregivers) who are unable to attend face-to-face sessions. In addition to the data we gathered from the NRI pilot study of informal caregivers of Veterans with traumatic brain injury related to their preferences for a stress-reduction program (method of delivery, schedule), we are also currently conducting a pilot study (Locally-Initiated Pilot) of women Veterans at the Hines VA in which we are including questions about their preferences for a stress-reduction program. We are specifically asking them about their preferences related to online programs. Findings from these pilot studies as well as the proposed study will be used to support the development of a proposal to examine an online MBSR program within the VA.