ACUPUNCTURE FOR SYMPTOM MANAGEMENT IN A MENOPAUSAL POPULATION: AN EXPLORATION OF MECHANISM AND EXPERIENCE

by

Cheryl LeAnn Wright

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DEDICATION

To my husband, Andrew Harbison, for his honest critiques,

tempered with humor, love and home-cooked meals.
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ABSTRACT

This early phase study examined the effects of a series of ten standard-protocol acupuncture treatments over four weeks in 12 healthy, but symptomatic, menopausal women. In addition to measuring two biologic markers that may shed light on the physiologic impact of acupuncture, symptom experience data were collected. The two markers included heart rate variability to evaluate effects of acupuncture on the autonomic nervous system as it relates specifically to the heart, and interleukin 6, a proinflammatory cytokine, to capture physiologic impact of acupuncture on the immune-inflammatory response.

Women who exhibited significant menopausal symptoms, that included vasomotor symptoms (hot flashes and sweats), fatigue, mood disorders, sleep disturbances, urogenital symptoms, and pain as measured by a minimum score of 22 out of a possible 44 points on the Menopause Rating Scale (MRS), underwent 10 sessions of a standard acupuncture point protocol intervention over a four-week period. Daily symptoms were evaluated for 28 days using the MRS. Immediately after the intervention was complete, one final open-ended question was asked of each participant to explore subjective experiences not captured by the validated instrument. Physiologic measurements included a 30-minute continuous measure of electrocardiogram data before and during acupuncture, as well as pre and post acupuncture measures of serum interleukin 6 at the first and final acupuncture treatment. Each set of measures were taken at baseline, immediately before and after each acupuncture treatment throughout the four-week/10- treatment intervention. Heart rate variability was assessed using spectral
analysis, and interleukin 6 changes were measured using enzyme linked immunoabsorbant assay. Analysis included linear regression, Wilcoxon Signed Ranks test and Spearman correlation coefficients.

After the acupuncture intervention, menopausal symptoms improved significantly. Various elements of heart rate variability did not show consistent or predictable intra or inter-subject changes, however several components of HRV, interleukin 6 and symptoms were found to have significant correlations with each other.

The study was deemed feasible. Menopause symptoms improved. Heart rate variability showed no consistent changes or trends. Interleukin 6 decreased in seven subjects, between the first and tenth acupuncture treatment, but did not reach statistical significance.

This small project suggests that heart rate variability, when measured at each acupuncture treatment, does not change in a consistent or predictable manner. Rather, acupuncture may provide a modulating influence that depends on the body’s functional state at each treatment.
CHAPTER ONE: INTRODUCTION

Purpose of Study

The purpose of this study was to examine the feasibility for a larger study to assess the effectiveness of acupuncture for managing menopausal symptoms, and to gain new knowledge of the physiology of acupuncture. This new knowledge was achieved by correlating menopausal symptom experiences with the effects of acupuncture and two biomarkers: heart rate variability to establish cardiac autonomic nervous system reactions to acupuncture, and interleukin 6, a proinflammatory cytokine to explore the immune inflammatory system response to acupuncture.

Specific Aims and Hypotheses

Specific Aim 1: To determine the feasibility and preliminary indication of effectiveness necessary for a larger study to describe the effects of acupuncture on specific menopausal symptoms: vasomotor symptoms (hot flashes/sweats), mood disturbances, sleep disturbances, pain and urogenital symptoms in an otherwise healthy group of women.

Hypothesis 1: It will be feasible with indications of effectiveness to conduct a larger study with at least 70% of participants (Matson, 2000; Polit, D. F. and Beck, C. 2004) completing all acupuncture treatments and measurements.

Exploratory Aim 2: To describe the effect of acupuncture on the cardiac autonomic nervous system through the use of heart rate variability.

Hypothesis 2: Acupuncture will increase heart rate variability, defined by measures such as the ratio of parasympathetic to sympathetic power in spectral analysis,
as well as low frequency and high frequency measures in both absolute power and normalized units. When compared to baseline measures, increased heart rate variability will demonstrate decreased low frequency to high frequency ratio, decreased values of low frequency and increased values of high frequency measures.

Exploratory Aim 3: To describe the effect of acupuncture on the inflammatory immune response as measured by serum levels of interleukin 6.

Hypothesis 3: Acupuncture will influence the inflammatory immune response reflected by decreased levels of serum interleukin 6 after acupuncture intervention.

Exploratory Aim 4: To explore the relationship between heart rate variability, interleukin 6 and menopausal symptoms before and during the acupuncture intervention.

Hypothesis 4: There will be a significant relationship between heart rate variability, interleukin 6, menopausal symptoms and acupuncture intervention. Those who show improvement on one measure will tend to show improvement on the other.

Definitions of Terms

*Acupuncture:* The insertion of filiform needles into skin and muscle at predetermined anatomic locations in concordance with standard traditional Chinese medicine texts (Deadman and Al-Khafaji 1998).

*Autoregressive Model:* In Heart Rate Variability, a mathematical model for evaluating heart rate variability using spectral analysis, a smoother model than the fast-Fourier transforms.

*Heart rate variability:* The variance in cardiac beat-to-beat interval as measured on an electrocardiogram and interpreted with a software program. Decreased heart rate
variability indicates a preponderance of cardiac sympathetic tone and is associated with the menopausal state as well as poor health outcomes in those with existing cardiac disease (Malik 1996; Fukusaki 2000; Mercuro 2000).

*High Frequency Heart Rate Variability*: A measurement of heart rate variability using spectral analysis that is within the frequency range of 0.15-0.4 hertz.

*Interleukin 6*: A pro-inflammatory cytokine that has been correlated with low levels of serum estrogen, aging and symptoms of fatigue, depression, increased perception of pain and disordered sleep (Bower, Ganz et al. 2002; Brooks-Asplund, Tupper et al. 2002; Hogan, Morrow et al. 2003; Andrei, Fraguas et al. 2007).

*Frequency Domain Methods*: In Heart Rate Variability, a spectral method for analyzing an electrocardiogram recording. “Power spectral density analysis provides the basic information of how power (variance) distributes as a function of frequency. Independent of the method used, only an estimate of the true PSD of the signal can be obtained by proper mathematical algorithms” Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, (Malik 1996).

*Low Frequency Heart Rate Variability*: A measurement of heart rate variability using spectral analysis, defined as frequency range of 0.04-.15 hertz.

*Menopause*: The normal cessation of menstruation for 12 months not due to pregnancy or pathology; this is a retrospective diagnosis (Sherwood 2004; Kasper 2005).

*Menopausal symptoms*: Symptoms experienced by women transitioning through perimenopause and menopause. They most often include vasomotor symptoms, sweating and hot flashes, sleep disturbances, mood disturbances, fatigue, altered libido and/or
vaginal dryness with painful intercourse, and new onset joint pains (Sherwood 2004; Kasper 2005; NAMS 2005). In this project, eleven symptoms were measured using the menopausal rating scale (MRS), hot flashes (vasomotor instability), heart palpitations, sleep quality, mood, irritability, anxiety, fatigue, sexual dysfunction, bladder dysfunction, vaginal dryness and joint or muscle pain. An additional question was added to measure the amount of distress these symptoms caused each woman.

Non-parametric Model: In Heart Rate Variability, the mathematical model that uses a fast-Fourier transform to analyze signals. The model is not as smooth as the autoregressive model.

Normalized Units: In Heart Rate Variability, “units of measure that represent the relative value of each power component in proportion to the total power minus the very low frequency (VLF) component. The representation of low frequency (LF) and high frequency (HF) in normalized units emphasizes the controlled and balanced behavior of the two branches of the autonomic nervous system. Moreover, the normalization tends to minimize the effect of the changes in total power on the values of LF and HF components” Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, (Malik 1996).

Parametric Model of Heart Rate Variability- See Autoregressive Model

Perimenopause: The transition time when menstruation becomes irregular and midlife women experience symptoms related to the menopausal transition to the final menstrual period (Sherwood 2004; Kasper 2005).
Premenopause: The fertile years between menarche, the onset of menses and the perimenopause (Sherwood 2004; Kasper 2005).

RMSSD: In Heart Rate Variability, a statistical Time Domain measure of Heart Rate Variability, the square root of the mean squared differences of successive normal QRS complex-to-normal QRS complex (N-N), also known as (R-R) intervals. Recommended for estimating short-term components of heart rate variability.

Spectral Analysis: In Heart Rate Variability, a measure of the cyclic fluctuations of R-R intervals. “Two peaks are seen in 5-minute interval power spectra, a high frequency peak between 0.15 and 0.40 hertz (Hz), and a low frequency peak between 0.04 and .15 Hz.” (Kleiger, Stein et al. 2005)

Symptom severity: How severe or strong the menopausal symptom is rated, and distress, the degree of bother or distress a woman rates the symptom, will be measured on the Menopause Rating Scale (MRS) with a 4-point scale where 0 indicates ‘no symptoms’ and 4 ‘very severe symptoms.’ Distress will be evaluated by one final question, “How distressing or bothersome were your symptoms today?” with the response indicated on a 1-10 scale with 1 being no distress and 10 indicating the worst distress imaginable.

Time Domain: In Heart Rate Variability according to Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, “either the heart rate at any point in time or the intervals between successive normal complexes are determined. In a continuous ECG record, each QRS complex is detected, and the so-called normal-to-normal (NN) intervals (that is, all intervals between adjacent QRS
complexes resulting from sinus node depolarization) or the instantaneous heart rate is determined. Simple time domain variables that can be calculated include the mean NN interval, the mean heart rate, the difference between the longest and shortest NN interval, the difference between night and day heart rate, and so forth. Other time domain measurements that can be used are variations in instantaneous heart rate secondary to respiration, tilt, Valsalva maneuver, or phenylephrine infusion. These differences can be described as either differences in heart rate or cycle length” (Malik 1996).

Significance of Study

This study was the first study to explore the physiologic response of heart rate variability to ten therapeutic acupuncture treatments intended to treat a specific set of symptoms. Two prior studies examined the response of heart rate variability to a series of acupuncture interventions for migraine headache (Backer, M, Grossman, P., Schneider, J., Michalsen, A., Knoblauch, N., Tan, L., Niggemeyer, C., Linde, K., Melchart, D. and Dobos, G.J. 2008 ) and anxiety (Agelink, M. W., Sanner, D., Eich, H., Pach, J., Bertling, R., Lemmer, W., Klieser, E. and Lehmann, E. 2003), however, while subjects received a therapeutic intervention, for ten to twelve consecutive sessions, heart rate variability was only measured on the first and final acupuncture treatment sessions. This prevented the previous investigators from seeing the large amounts of variability encountered at each intervention that were seen in this study.

Additionally, several prior studies of acupuncture for relief of menopausal vasomotor instability report consistent improvement of this particular symptom, and those results were reproduced in this study. Importantly, however, ten other common
Menopause symptoms were captured, including a perception of global distress, all of which improved with statistical significance.

Furthermore, serum levels of interleukin 6 did change in the immediate pre-post acupuncture environment at both the first and tenth (final) acupuncture sessions, usually increasing slightly, but some decreased. Interestingly, though not reaching statistical significance, interleukin 6 decreased in 70% of the sample between the post-first level and pre-tenth level.

Finally, upon examination of the relationships between the three variables, symptoms, heart rate variability and serum interleukin 6, there were numerous significant Spearman’s correlation coefficients, which suggests a possibility that symptoms, heart rate variability and interleukin 6 may have dependent relationships.

**Background**

**Menopause**

Menopause is a normal physiologic event that every woman who lives long enough will experience. As the aging process unfolds, ovarian senescence leads to declines of serum estrogen levels and subsequent symptom experience (Kasper 2005; Sherwood; Sherman et al. 2001). Menopausal symptom experience varies widely from mild to severe, and while many women transition through menopause with manageable symptoms, others experience more severe symptoms that include hot flashes and sweats (vasomotor instability) (Dormire and Reame 2003; Freedman 2001; North American Menopausal Society [NAMS] 2004; Shapiro 2001; Soules, Sherman et al. 2001), new onset joint pain (Richette, Corvol et al. 2003; Tsai and Liu 1992;), vaginal dryness, sleep
and mood disturbances (Keenan, Mark et al. 2003; Moe 2004; Shapiro 2001; Soules, Sherman et al. 2001). While about 70% of women will seek symptom relief, some women will experience very mild symptoms and may not see the need to seek symptom relief from a healthcare provider (Kasper 2005).

Currently, menopausal women have an array of options for symptom management including hormone therapy, estrogen and/or progesterone (Cohen, Soares et al. 2005; Ettinger 2005; Harman, Naftolin et al. 2005; Kasper et al. 2005), off label use of pharmaceutical agents such as antihypertensives or antidepressants (Albertazzi, Bottazzi et al. 2003; Barton, Loprinzi et al. 2003; Cohen, Soares et al. 2005; Freedman 2005; Loprinzi, Stearns et al. 2005), lifestyle changes, such as diet, exercise, alcohol and tobacco abstinence, and complementary and alternative therapies, such as herbs, meditation, or acupuncture (Bair, Gold et al. 2005; Carpenter and Neal 2005; Keenan, Mark et al. 2003). For some women, such as estrogen receptor positive breast cancer survivors, estrogen therapy may be an absolute contraindication, and alternate pharmaceutical agents such as selective serotonin reuptake inhibitors (Barton, Loprinzi et al. 2003; Chlebowski, Kim et al. 2003), α-1 adrenergic agents, (Freedman 2005; Loprinzi, NAMS 2004; Stearns et al. 2005; Siddiqui 2005) or anticonvulsants (Albertazzi, Bottazzi et al. 2003; Chlebowski, Kim et al. 2003; Loprinzi, Stearns et al. 2005; NAMS 2004) may be used. Unfortunately, a body of evidence suggests these pharmaceutical agents may only provide marginal symptom relief yet carry significant side effects (National Institutes of Health [NIH] 2005).
In the past 10 years, new information from two large studies, the Heart and Estrogen/Progestin Replacement Study (Grady 2002; Herrington, Fong et al. 1998; Hulley, Grady et al. 1998;) and the Women’s Health Initiative (McDonough 2002; Rossouw, Anderson et al. 2002) have led women to consider the inherent safety of hormone therapy. Both of these large, multi-center studies suggest risks of exogenous hormone therapy may outweigh benefits, specifically increased risks of breast cancer, stroke, heart disease or thrombosis (Ettinger 2005; Harman, Naftolin et al. 2005; Hendrix, Wassertheil-Smoller et al. 2006; Hsia, Criqui et al. 2006; McDonough 2002; Rossouw, Anderson et al. 2002; Rapp, Espeland et al. 2003).

For women who are not able to, or choose not to take hormones or pharmaceutical agents, alternative therapies may offer symptom relief with far fewer side effects (Santoro, Clarkson et al. 2004). Alternative therapies used by menopausal women may include herbs such as black cohosh (Low Dog 2005; Ness; Rice and Whitehead 2006), traditional Chinese herbs (Guthrie 2005) dietary soy (Low Dog 2005; Ness, Aronow et al. 2006; Speroff 2005), exercise (Aiello, Yasui et al. 2004; Freedman 2005; Lee, Lin et al. 2000; Lindh-Astrand, Nedstrand et al. 2004), mediation or paced respiration (Freedman 2005; NAMS 2004) and acupuncture (Borud, Alraek et al. 2007; British Acupuncture Council 1999; Cohen, Rousseau et al. 2003; NAMS 2004; Porzio 2002; Vincent, Barton et al. 2007; Wyon, Wijma et al. 2004). However, to date, studies examining effectiveness of complementary and alternative therapies for menopausal symptom relief have been small and yielded contradictory findings, which raise concerns regarding the effectiveness of these therapies (Ernst 2003, 2006).
**Acupuncture for Menopausal Symptoms**

Acupuncture as an alternative intervention for menopausal symptom management may be useful as a non-pharmacological and relatively safe intervention (Lewith, White et al. 2006; Macpherson, Scullion et al. 2004; NAMS 2004; Vas, J. E., Perea-Milla et al. 2006). Various studies of acupuncture intervention for menopausal symptoms have shown some promise for managing menopausal symptoms (Cumins and Brunt 2000; Johnstone, Polston et al. 2002; Kronenberg, Mindes et al. 2005; Lee, Lin et al. 2000; NAMS 2004; Nedstrand, Wijma et al. 2005; Porzio 2002; Tukmachi 2000), and thus, acupuncture continues to deserve careful consideration and further research.

**Significance of Menopause Symptoms**

Based on the 2000 United States Census, women aged 45-59 years, the age group most likely to be transitioning through menopause (Kasper, 2005; Sherwood, 2006) represents 20.6% of the population or roughly thirty-one million women (United States Census, 2000). In 2005, the NIH, state-of-the-science panel convened to examine menopausal symptom experience, management and defined areas in need of greater research. The group consisted of representatives from the National Institute of Aging, Office of Medical Applications of Research, the National Center for Complementary and Alternative Medicine, the NIH Office of Research on Women’s Health as well as representatives from other NIH institutes. They identified four symptoms consistently and specifically associated with the menopausal transition: vasomotor instability, vaginal dryness/pain with intercourse, sleep disturbances and mood disorder/depression. In addition, two symptoms not consistently or conclusively linked to menopause, but also
addressed were sexual dysfunction and quality of life, and two final symptoms not believed to be specifically associated with menopause, but that the group felt was worthy of more study included joint and muscle pains, weight gain/metabolic changes (Santoro, 2006).

The resulting executive summary revealed roughly 80% of women transitioning though menopause experience vasomotor symptoms and about 70% of these women seek some type of intervention for relief. Sleep disturbance occurred in about 40% of women transitioning through menopause (Santoro, 2006). Mood disturbance, primarily depression has not been conclusively linked to the menopausal transition; yet, the group identified two studies that suggest a link between major depressive disorder and the menopausal transition (Cohen, Soares et al. 2006; Freeman, Sammel et al. 2006). The executive summary identified several areas where further research is indicated: developing new methods for treating menopausal symptoms, attempting to increase the biologic and physiologic understanding of symptom etiology, and continuing to examine traditional and alternative, behavioral and non-pharmacologic therapies. Traditional Chinese medicine was specifically included in these recommendations (Santoro, 2006).

*Significance of Acupuncture Research to the World and the United States*

Acupuncture, a non-pharmaceutical intervention, is the piercing of skin and muscle with fine, filiform needles at specific, traditionally defined anatomic locations, and is one of the better-known modalities in the traditional Chinese medicine armamentarium. Acupuncture is about two thousand years old (Unschuld, 1985) and has been used for a wide variety of ailments. Acupuncture use has spread from the Far East

The WHO (2003) has recognized that there are many disease processes and symptoms that might be managed with ethnic medical traditions, including acupuncture, and has called for more research into these areas, specifically acupuncture (WHO 1995). Furthermore, many developing countries currently lack access to technically complex medical interventions, but may have easier access to a simpler technology such as acupuncture, where all that is required is a bottle of 70% isopropyl alcohol, an absorbent material such as cotton balls, and a box of sterile needles. Thus, acupuncture has potential to be a valuable intervention for symptom relief in many populations.

In addition to the WHO, the NIH (Acupuncture 1998) has also called for researchers to design studies that will shed light on physiologic mechanisms that can be attributed to the acupuncture needle. More specifically, the NIH has called for researchers to begin exploring options such as acupuncture for menopausal symptom management (NIH 2004, 2005; Sherman 2005). The state-of-the-science in acupuncture research is emerging and in need of enthusiastic, creative researchers. Historically, the majority of acupuncture research has focused on the effectiveness of acupuncture for specific complaints mainly through small, underpowered pragmatic trials, case reports or
retrospective case series reports (MacPherson 2008, pp 23-29). Research design has proved to be a particularly thorny issue due to the nature of the intervention; a valid placebo has been difficult to establish, and while blinding the subject is difficult, blinding the practitioner is all but impossible. Thus, many attempts to carry out blinded, placebo-controlled trials have demonstrated lack-luster results. Among the many challenges that confront acupuncture researchers is finding a valid means to determine what if any specific effect occurs as the result of acupuncture. The commonly accepted ‘gold standard’ research design, double blind, placebo controlled experimental design works very well for pharmaceutical agents, but has not shown promising results in complementary therapies research. Proponents of complementary and alternative research do not deny the need for rigorous trials, including control groups, but suggest that perhaps researchers need innovative, creative designs tailored to ask complex questions of this complex phenomenon.

Furthermore, there is a clear need to understand if there are any physiologic changes specific to an acupuncture intervention. Other aspects that require more understanding include the recipient’s experience through subjective report and qualitative inquiry (Paterson & Britton, 2008). Finally, because we do know that not all people or laboratory animals respond in the same way to acupuncture, researchers need to understand which symptoms or disorders, and which populations benefit from acupuncture and which do not (MacPherson 2008).
Significance of Acupuncture for Symptom Management to Nursing

The holistic ideas that underpin traditional Chinese medical theory are appealing to many healthcare providers, including nurses (Chang 2001; Chen 1998; Sherman, Cherkin et al. 2005; Flowers 2006; Lund, Lundeberg et al. 2006; Sherwin 1992), and this interest is reflected in nursing research (Cohen, Rousseau et al. 2003). Acupuncture for symptom management resonates with many nurses, including some who have left the profession to become acupuncturists or providers of other forms of complementary and alternative therapy. However, many nurses would prefer to practice a holistic, effective therapy in conjunction with daily nursing activities rather than leave the profession (Andrews 2003; Fowler and Newton 2006; Sherwin 1992).

Additionally, interest in complementary and alternative therapies is rapidly growing in the United States (Eisenberg 2002; Lee, Lin et al. 2000; Tindle, Davies et al. 2005), and select therapies are already being utilized in hospitals and clinics in the United States, and around the world (Mitzdorf, Beck et al. 1999). Of all health care personnel, nurses have the most frequent and intimate encounters with patients and many nursing actions revolve around comfort and symptom management. Acupuncture is a very simple, easy to learn and inexpensive to administer method to relieve symptoms, therefore, if effectiveness for symptom management can be demonstrated, acupuncture could be another means for the nurse to manage symptoms in a quick, minimally invasive manner, with few if any side effects.

Finally, nurses are a significant source of patient information. In this position, nurses are uniquely able to offer evidenced-based, effective therapies to patients, thus the
findings of this study will provide nurses with information specific to acupuncture for menopausal symptom management, and nurses will be positioned to educate and advise women on safe and effective means to manage menopausal symptoms.

Summary

For many women, menopausal symptoms result in considerable symptom burden. Some of these symptoms may be ameliorated with pharmaceutical agents, but an ideal therapy would avoid the additional cost and potential drug interactions and side effects associated with pharmacological intervention. Fourteen prior studies suggest acupuncture for menopausal hot flash symptoms may ameliorate these vasomotor symptoms. This study was unique because it explored the range of menopausal symptoms. In addition, we measured two physiologic biomarkers in an attempt to begin to understand underlying physiologic mechanisms. Acupuncture intervention for menopausal symptom management may provide a more effective, as well as cost effective means to manage symptoms, rather than pharmacologic interventions. In response to the World Health Organization and National Institutes of Health calls to action, the examination of the influence of acupuncture on the cardiac autonomic nervous system and the inflammatory immune biomarker correlates may shed light on the physiologic mechanism by which acupuncture eases symptoms.
CHAPTER TWO: CONCEPTUAL ORIENTATION, THEORETICAL MODELS AND LITERATURE REVIEW

Introduction

The conceptual orientation will review the theoretical framework and models, menopause physiology and symptoms—specifically, vasomotor symptoms (sweats and hot flashes), mood disturbances, sleep disturbances, vaginal dryness, and pain. Additionally, there will be a review of estrogen’s influence on the autonomic nervous system as well as the immune-inflammatory system. Heart rate variability will be discussed, including the technique for measurement, data interpretation and significance in relation to the autonomic nervous system, as well as the theoretical impact of the autonomic nervous system on the immune-inflammatory response. Throughout the narrative, specific symptoms will be linked to declining serum levels of estrogen, disordered autonomic nervous system and mild inflammation as measured by increased levels of interleukin 6. Finally, this section will review current understanding of the physiology of acupuncture, which will include what is known from prior research of acupuncture for menopausal symptom management, the impact of acupuncture on both the autonomic nervous system as well as the immune-inflammatory response.

Theoretical Framework

The theoretical framework is based on physiologic theory. Figure 1, the first model, illustrates the physiological effects of low serum estrogen on the autonomic nervous system and the immune inflammatory system resulting in symptom experience. Figure 2, the second model, illustrates how acupuncture is hypothesized to affect
physiology in order to influence the autonomic nervous system, immune inflammatory system, and improve symptom experience.

FIGURE 1. Model 1 - Physiology of Estrogen

*Model One, Estrogen Physiology and Symptoms*

The estrogen model illustrates the physiologic changes that are hypothesized to occur as women move into perimenopause and estrogen levels begin to fluctuate more dramatically (Simpson and Dowssett 2002; Chlebowski, Kim et al. 2003) from about 300 picograms (pg) per milliliter (ml) into a range of 5-38 pg/ml (McLendon Clinical Laboratories, 2006).
Model One, Discussion

The two pathways shown in the model represent the focus for this study, the response of low serum estrogens (triangle apex) on the autonomic nervous system (right base of the triangle) and the immune inflammatory system (left base). The immune inflammatory response will be measured by serum interleukin 6, due to theoretical support for this phenomenon (Brooks-Asplund, Tupper et al. 2002; Knupfer and Preiss 2007; Papanicolaou, Wilder et al. 1998; Rachon, Suchecka-Rachon et al. 2006; Simpson 2003). The autonomic nervous system, measured by heart rate variability, is also based on prior work examining heart rate variability in healthy adult acupuncture subjects, as well as in a menopausal population (Cowan 1995; Malik 1996; Eckberg 1997; Farag 2003; Fukusaki 2000; Haker, Egkvist et al. 2000; Knardahl, Elam et al. 1998; Mercuro 2000; Saleh 2007).

Symptoms are hypothesized to emerge as serum levels of estrogen fluctuate and decline following either the left or right pathway in the model. The left pathway illustrates symptoms that result from low levels of serum estrogen and subsequent subclinical inflammation manifested by mild elevation of proinflammatory cytokines, pain, mood disturbances, sleep disorders, low energy and fatigue (Bower, Ganz et al. 2002; Hogan, Morrow et al. 2003; Maes, Libbrecht et al. 1999). The right pathway illustrates proposed symptoms that result from a preponderance of sympathetic tone, namely, vasomotor instability and anxiety. Many symptoms likely feedback and reinforce each other, this is represented by dual-direction arrows. The moderators and mediators (not measured in this project) located in the boxes above and to each side of the model,
acknowledge that symptoms will be influenced by extraneous factors and, while not measured, should be acknowledged.

Model 2: Acupuncture Physiology

**Filiform Needle penetrates Skin & Muscle**

- Peripheral nerves stimulated, impulse travels to Dorsal Horn Of Spinal Cord (*bioactive molecules released*)
- Impulse Continues to specific areas of CNS

**Immune-Inflammatory Response Dampened**

*Moderators & Mediators: Patient-Provider relationship, Attitudes towards Acupuncture, Environment, reframing symptoms, Ritualistic nature of treatment other non-specific Effects: placebo,

*Endogenous opioids, neurotransmitters* & Autonomic Nervous System Influenced

- Parasympathetic cardiac modulation Influenced via Vagal stimulation Measured by increased HRV
- ↓Vasomotor Symptoms (hot flashes/sweats) intensity & frequency
- ↓Serum Interleukin 6
- Fatigue, mood, low energy, sleep Disturbances & pain improve

↓Anxiety

FIGURE 2. Model 2 - Acupuncture Physiology
Model Two, Acupuncture Physiology

As in model 1, there are moderators and mediators, which will not be measured in this project, but are acknowledged. These include non-specific effects that are also aspects of a placebo response, and include the ritualistic nature of an acupuncture treatment, the patient’s re-conceptualization of symptoms within a traditional Chinese medicine context, the nature of the patient-provider relationship, patient expectations, treatment environment, and other subtle aspects of treatment associated with complementary and alternative medicine (Ader 2000b; Allan 2006; Bausell, Lao et al. 2005; Cahana 2007; Caspi and Bootzin 2002; Bootzin and Bailey 2005; Dhond, Kettner et al. 2007).

Model Two, Discussion

Model 2 illustrates the insertion of a filiform needle, which penetrates skin and muscle tissue, the noxious stimulation of the acupuncture needle stimulates the peripheral pathway that originates in the small, myelinated sensory afferent type II and/or III nerve fibers (Pomeranz as cited in Stux and Hammerschlag, 2001, p. 4). The impulse travels from peripheral nerve to the dorsal horn of the spinal cord where it acts locally as well as ascends one of six potential pathways including the anterolateral and spinothalamic tracts (Pomeranz, as cited in Stux and Hammerschlag, 2001). Specific areas of the brain are activated or down-regulated in a network fashion (Bai and 2007; Cho, Hwang et al. 2006; Dhond, Kettner, et al. 2007; Fang, Krings et al. 2004; Fukunaga et al. 2005; Hui, Liu et al. 2000; Hui, Liu et al. 2005; Jeun, Kim et al. 2005; Kong, Ma et al. 2002; Liu, Feldman et al. 2004; Nakagoshi, Napadow, Makris et al. 2005; Parrish, Schaeffer et al. 2005) to
influence a multitude of bioactive molecules including endogenous opioids and neurotransmitters (Cheng and Pomeranz 1981; Pomeranz and Chiu 1976) in addition to influencing the parasympathetic nervous system (Cho et al. as cited in Stux and Hammerschlag 2001).

This project did not measure the influence of endogenous opioids or neurotransmitters. Thus, the model will acknowledge this process, but focus on the influences of the cardiac autonomic nervous system and immune inflammatory response.

Following the right side of the model, parasympathetic activation results in relaxation of the vasculature. This may decrease vasomotor instability and improve anxiety. The left side of the model suggests that increased parasympathetic tone will dampen the immune inflammatory system (Borikova 2000; Czura and Tracey 2005; Pavlov 2003; Tracey 2007), measured as serum levels of interleukin 6. As serum interleukin 6 declines, symptoms associated with increased proinflammatory cytokines will begin to moderate. These symptoms include fatigue, low energy, depressed mood, disordered sleep and pain.

Model Assumptions

There are several assumptions that need to be acknowledged in each of these models. First, acupuncture is able to exert a specific physiologic influence as opposed to the common belief that it mainly exerts a non-specific or placebo effect (Ernst 2003, 2007). Second, there is a high likelihood that peripheral nerve stimulation does affect the autonomic nervous system, CNS (Hui, K.K, Liu, J. et al. 2005), endogenous opioids (Pomeranz and Cheng 1981) and/or the immune system in humans (Cabyoglu, M. T., Ergene, N. and Tan, U. 2006). Next, acupuncture is able to influence the immune
inflammatory response (Jeong, H.J., Hong, S.H., et al. 2003; Jeong, H.J., Kim, B. S., et al. 2003) and that this phenomenon will be reflected in measures of serum cytokines, specifically interleukin 6. Additionally, the entire mechanism of symptom relief may be due directly to the effect of acupuncture on endogenous opioids and/or neurotransmitters (Cheng, R.S. and Pomeranz, B. 1979). Finally, it has not been clearly established whether or not mild elevation of interleukin 6 will be significant enough to lead to fatigue, pain, mood disturbances and sleep disturbances.

Variables Not Included in the Model

As mentioned in the models section, environmental stressors are known to aggravate menopausal symptoms; however, these were not measured. Additionally, centrally activated opioids and neurotransmitters, both viable explanations for symptom relief, were not measured due to the invasiveness of sample collection, which requires cerebral spinal fluid. Finally, more of a design limitation, but important to address is placebo/expectancy effect was not measured, and a control group was not used.

Review of the Literature

The Menopausal Transition

Menopause is a normal consequence of aging, and is usually a natural transition. However, it can also be induced abruptly by surgical removal of the ovaries or chemotherapy-induced ovarian failure. Primary symptoms include hot flashes and sweats (vasomotor instability), mood disturbances usually manifesting as depression, irritability, sleep disturbances, vaginal dryness and painful sexual intercourse. Symptoms associated with aging, but not clearly linked with menopause include joint and muscle pains and
weight gain or metabolic changes. While additional longitudinal research is needed (Santoro, 2006), women do not always suffer with severe symptoms, nor do they necessarily hold a negative view of this transition (Deeks 2004; Kasper 2005; Sherwood 2004). Some women, however, may suffer with more severe symptoms, especially if ovarian function ceases abruptly (Carpenter 2002; Kasper, 2005; Sherwood, 2006).

**Menopause Physiology and Symptoms**

The physiology of menopause is thought to be well understood, however there remains some disagreement on the exact mechanisms that lead to the cessation of ovarian function (Sherwood, 2004, p. 781). The physiology that leads to a woman’s menopausal symptom experience is less clearly understood, but in addition to hormonal fluctuations, lifestyle factors such as cigarette smoking, obesity and other psychological stressors are thought to be significant moderators and mediators of symptom experience (Cohen 2005; Greendale 2005; Kasper 2005; Sherwood 2004). Freedman (2005), a prominent researcher in the field of menopause, postulates symptoms are related to the relatively rapid drop in serum estrogens as perimenopause progresses to cessation of menses. In the presence of low serum estrogen, the central nervous system, which has a high concentration of estrogen receptors (ERα and ERβ), may produce irregular pulses of norepinephrine, which may in turn increase sympathetic activity, and result in vasomotor instability experienced as a hot flash, with or without sweats.

Central nervous system (CNS) origination of the hot flash was explored by Freeman (2006) using functional magnetic resonance with blood oxygen-level dependent imaging (fMRI with BOLD technique). He examined twelve women experiencing a hot
flash and concluded that the structure most activated was the insula, rather than the hypothalamus as he expected. This finding more broadly defined the area in the CNS involved temperature perceptions during the hot flash. Craig, et al (2000) also identified the insula, in an animal model, as a major CNS structure involved in temperature perception. Other external factors highly correlated with vasomotor instability include exposure to elevated environmental heat as well as increased anxiety (Freedman, 2005; 2001). While the exact mechanism that precipitates vasomotor instability remains unclear, it has become evident that in addition to the increased centrally mediated noradrenergic activity, there is a physiologically reduced thermoneutral zone. This zone is an inter-threshold or null zone that allows humans to maintain a core body temperature between sweating and shivering. Research indicates that menopausal women perceive environmental temperatures differently than premenopausal women. Menopausal women experience a narrowed temperature-related comfort zone so that they feel hot at lower environmental temperatures and cold at higher temperatures (Freedman, 2005).

Pharmaceutical studies may shed some light on vasomotor instability physiology as they have demonstrated that selective serotonin reuptake inhibitors may also decrease the frequency and intensity of vasomotor symptoms (Barton, Loprinzi et al. 2003; Gainford 2005; McPhail and Smith 2000; Moe 2004; NIH 2005; Rice 2005). While this would suggest a role for serotonin in vasomotor instability, selective serotonin reuptake inhibitors may effect change simply by decreasing anxiety, a known aggravating factor in precipitating vasomotor instability (Freedman, 2005). In another class of pharmaceuticals, one agent that can decrease vasomotor symptoms is clonidine, an α-
adrenergic agent used to reduce hypertension through vasodilatation by reducing central nervous system (CNS) sympathetic outflow (Loprinzi, et al. 2005) suggesting another physiologic model in which stabilizing and/or decreasing sympathetic nervous system stimuli may decrease vasomotor instability.

Thus, there are two possible means by which acupuncture may be able to reduce menopausal vasomotor instability via the neurophysiologic model. Either, influencing centrally produced norepinephrine and/or serotonin, or reducing CNS sympathetic outflow. Each may be a viable mechanism by which acupuncture improves the vasomotor instability seen during the menopausal transition.

Other menopausal symptoms such as increased perception of pain or new onset joint pains may be the direct result of lower serum estrogens in the CNS. Estrogens may function to increase pain thresholds, as a pain modulator via its relationship with endogenous opioid production and central nervous system estrogen receptors (Eckersell, Popper et al. 1998; Smith, Stohler et al. 2006). Thus, acupuncture may provide relief from joint pains by increasing pain thresholds via direct action on the endogenous opioid production in the CNS, via the peripheral nervous system (Andersson and Lundeberg 1995; Pomeranz and Chiu 1976; Pomeranz 1978; Pomeranz 1996; White 2006). The remaining symptoms of interest in this study, low energy, fatigue, mood and sleep disturbances, are hypothesized to be the result of elevated proinflammatory cytokines such as tumor necrosis factor alpha and interleukin 6 (Johnson 2006; Motivala, Sarfatti et al. 2005). Both, aging and menopause, which leads to significant decreases of serum
estrogens, can lead to a chronic, low-grade inflammation as measured by serum interleukin 6 (Crandall, Petersen et al. 2004; Simpson, et al 2003; Moe 2004).

**Immune-Inflammatory Response: Brief Cytokine Overview**

Cytokines are short-lived, short acting, relatively small, usually glycosolated proteins that, while produced by many different cell types, are mainly produced by lymphocytes and macrophages (in the past they were also referred to as lymphokines and monokines, respectively). Cytokines are also produced in endothelium, epithelium, and connective tissue and tumor tissue. While over fifty different cytokines have been isolated, details of mechanisms and functions are far from being conclusively identified (Thomson and Lotz 2003).

Cytokines function as messenger molecules in the immune system, hence the moniker ‘interleukins’ which implies their role maintaining communication between and among leukocytes. Additionally, cytokines are known to play a significant role in both acute and chronic inflammatory states-- either promoting or inhibiting the inflammatory response, and many cytokines have a wide range of effects with overlapping functions, thus they are classified by function rather than origin (Decker 2006). Cytokines are often produced in a cascade fashion, as a cytokine targets a cell, which leads to additional single or multiple cytokine release. Furthermore, cytokines may act in a synergistic or antagonistic fashion with each other. Cytokines are able to alter physiology in one of three ways: they act on the same cell that produced them (autocrine); they affect other cells close by (paracrine) or they can affect many cells or distant target cells systemically (endocrine) (Decker 2006; Kumar, et al., 2005; Thompson and Lotz, 2003).
In addition to the dramatic elevations of cytokines observed in response to infection or tissue trauma, investigators have recently correlated subclinical elevations of cytokines with symptoms often associated with chronic disease as opposed to acute illness. These symptoms include arthralgias, myalgias, fatigue, sleep and mood disturbances (Bondeson, Wainwright et al. 2006; Bower et al. 2007; Bower, Ganz et al. 2002; Cutolo, Capellino et al. 2006; Gaab, Rohleder et al. 2005; Hogan, Morrow et al. 2003; Hulejova, Baresova et al. 2007; Lekander, Elofsson et al. 2004; Silvestri, Pulsatelli et al. 2006; Wright, Strike et al. 2005; Yang, Xie et al. 2007) symptoms, which are also seen in post-menopausal women.

**Interleukin 6**

Interleukin 6 (IL6) is a pleiotrophic, proinflammatory cytokine, which also demonstrates some anti-inflammatory properties (Papanicolaou et al, 1998) that originates from monocytes, macrophages, T-Helper 2 cells and stromal cells. IL6 activates B cells, specifically by differentiation into plasma cells; it activates plasma cells yielding antibody secretion, and participates in stem cell differentiation. Additionally, IL6 has various roles in tumor growth promotion and acute phase responses (Decker 2006; Karczewska, et al. 2000; Purohit, et al. 2005).

Other factors that affect serum levels of IL6 include upregulated gene expression initiated by inflammatory stimuli such as infection, as well as the presence of other cytokines such as interleukin-1 involved in stromal remodeling which occurs during wound healing and/or tumor growth. Other cytokines that enhance the expression of IL6 include tumor necrosis factor alpha (TNF-α), platelet-derived growth factor, and
interferon beta (Kishimoto, 1989). Additionally, agents that increase intracellular cyclic adenosine monophosphate (AMP) also enhance accumulation of IL6 messenger ribonucleic acid (mRNA). Factors that diminish expression of IL6 include glucocorticoids (Kishimoto, 1989), androgens (Papanicolaou, et al. 1998) and estrogens (Simpson 2003).

Interleukin 6 In Healthy Premenopausal Women

In a normal, healthy population, serum IL6 ranges from 0.5 picograms (pg) per milliliter (ml) to 14.6 pg/ml with a mean value of 6.3 pg/ml (Robac, et al., 1999). There are no significant gender differences reported in the literature. In one prospective trial that examined normal IL6 values, Ho and colleagues observed thirty-six young women, ages 16.3-22.7 years (mean age 19.5 years) who were currently free of infectious processes and not taking medications. They found that while levels of IL6 did not correlate with age (in this young, narrow age range), neither did cigarette smoking, alcohol use, time of day (phlebotomy before or after noon), or day of menstrual cycle. There was, however, a positive, significant correlation with higher body mass index (BMI) and elevated levels of serum IL6. In this particular study, the women’s weights had remained stable, so fluctuating weight was not a factor, none-the-less, the authors felt that in populations where weight may be fluctuating, such as perimenopause, a single sample for serum levels of IL6 may be confounded. The authors also conclude that cytokines may fluctuate more than reflected in this young population, and that a single measurement of serum IL6 may not be representative of an individual’s exposure to this
cytokine over time (Ho, et al. 2004). For these reasons, multiple IL6 levels was drawn on each subject in this study to examine serum fluctuation.

In a separate study of 265 subjects, 91 male and 174 female, an individual’s perception of health status was predictive of serum levels of proinflammatory cytokines, specifically IL6 (Lekander, M., Elofsson, S., et al., 2004) indicating that the symptoms associated with elevated proinflammatory cytokines may be relatively easy for an individual to perceive. Additionally, exposure to stress in a healthy population has been shown to elevate serum levels of proinflammatory cytokines (O'Connor, K. A., Johnson, J. D., et al., 2003), and there are genetic as well as other environmental variations that may effect IL6 at any given time (Pantsulaia, I., S. Trofimov, et al., 2002). Some of these influences include elevations of catecholamines, which stimulate IL6 production and glucocorticoids, estrogens, and androgens, which suppress IL6 (Papanicolaou, et al. 1998).

IL6 also plays a role in normal physiological events such as vigorous exercise (Keller et al 2005a; 2005b). Researchers have demonstrated that human skeletal muscle upregulates IL6 messenger ribonucleicacid (mRNA) in response to exercise as well as inflammatory stimuli (Bruunsgaard 2005; Keller 2005b; Nielson, et al. 1996; Ostrowski et al. 1999; Ostrowski et al. 1998). The observation that contracting muscle fibers produce IL6 indicates an endocrine function of this proinflammatory cytokine that is hypothesized to influence substrate mobilization in the liver and adipose tissues (DeRossi, et al., 2000; Gallucci et al, 1998). IL6 is also thought to enhance lipid
oxidation via activation of adenosine monophosphate activated kinase (AMPK) (Kelly et al. 2004).

The importance of normal physiologic variations of serum IL6 cannot be overstated, thus when investigators use serum levels of IL6 as an outcome measure or biomarker for disease progression or intervention effects, these changes should be interpreted with caution, as there are many normal physiological factors that may influence serum IL6 levels.

*Interleukin 6 in Menopausal Women*

In healthy, reproductive aged women, serum IL6 will fluctuate with physiological influences mentioned above, and these factors will also apply to menopausal women. In addition, several other factors will influence IL6 in this population. As women transition through menopause, changes will occur that are unique to declining levels of serum estrogens. Estrogens and androgens are known to suppress serum IL6 (Papanicolaou, et al. 1998) possibly through the cyclic AMP second messenger signaling system and/or regulation of CYP19 expression (Simpson 2003). As ovarian estradiol production wanes with a subsequent decrease in levels of serum estrogens, estrogens remain concentrated in local tissues such as adipose, breast, osteoblasts, chondrocytes, brain, vascular endothelium and aortic smooth muscle, mainly due to the enzyme aromatase (Simpson 2003). However, as serum estrogens decline, serum IL6 (and other proinflammatory cytokines) tends to become slightly elevated. While this is not a dramatic increase, e.g. from 6.31 pg/ml to 7.90 pg/ml (technically within normal range), this mild elevation of proinflammatory cytokines is thought be a significant influence in the development of
osteoporosis in this population (Oestergaard, et al 2006; Racho 2005; Weitzman and Pacifici 2006).

In addition to menopause, it is prudent to mention the impact that central adiposity has on IL6. The aging process leads to physiologic changes that include decreased muscle mass, decreased basal metabolic rate and a tendency towards adiposity (Edwards, 2005). Central or truncal deposition of adipose has been correlated with a significant elevation of serum IL6 as well as other proinflammatory cytokines, which fortunately has been shown to be reversible with a combination of decreased caloric intake and aerobic exercise (Bruunsgaard 2005; Niklas, et al. 2004). Additional factors associated with aging, especially in a population over 65, includes a decline of the immune system associated with advanced age, which for reasons that are not clear, leads to mild elevations of proinflammatory cytokines, specifically TNFα and IL6 (Krabbe, et al. 2004). Thus, in menopausal women several factors including declining serum levels of estrogen as well as the aging process may contribute to an elevated serum IL6.

Although there are several factors that contribute to elevated IL6 measurements in menopausal women, these will not be measured, but will be included in future work.

*The Relationship Between the Nervous System and the Immune Inflammatory System*

Recent studies have examined the influence of the nervous system on the immune system and the immune system’s impact on the CNS, which suggests another possible mechanism of action for acupuncture. Proinflammatory cytokines, such as interleukin 6 are thought to signal or communicate with the CNS to generate the subjective feelings associated with illness such as fatigue, myalgias, and lowered pain thresholds. While
these symptoms are not solely the result of peripherally produced cytokines, they are likely the result of a signaling system that results in the separate generation of glial cytokines. This process may be a significant contributing factor in the underlying physiology that results in chronic pain syndromes (Watkins and Maier 2005).

Acupuncture has been shown to improve some chronic pain syndromes such as fibromyalgia (Assefi, Sherman et al. 2005; Harris, Tian et al. 2005; Maes, Libbrecht et al. 1999; Martin, Sletten et al. 2006; Singh, Wu et al. 2006; Zheng 2005), and while the mechanism may be opioid induced, a cytokine-neural relationship may be a potential mechanism (Cabyoglu, et al., 2006).

A second potential mechanism of neural-immune interaction involves the vagus nerve, which directly and indirectly influences both pro and anti-inflammatory cytokines (Czura and Tracey 2005; Pavlov 2005; Tracey 2002, 2007; Watkins and Maier 2005). Electrical stimulation (as occurs with electroacupuncture) of the vagus nerve has been shown to release acetylcholine. Macrophages express nicotinic receptors on their cell surfaces, which respond to the acetylcholine release by suppressing TNFα release, and this could moderate an inflammatory reaction (Libert 2003). Other researchers have demonstrated a possible role for acetylcholine as a possible mechanism for the effects of acupuncture as an antipyretic, possibly through hypothalamic suppression of proinflammatory cytokines (Son, Park et al. 2002).

A third hypothesized mechanism, based on neuroimaging data, suggests that the common signaling molecule between the CNS and immune system may be an opioid
peptide, which is released, both locally in the peripheral nerve as well as centrally from the mid-brain (Gollub, Hui et al. 1999).

Therefore, acupuncture may affect the immune system via the nervous system. The immune effects of acupuncture have been demonstrated in four small studies. These include two studies involving asthma, which demonstrated significantly decreased serum levels of eosinophils, interleukin 10 and interleukin 6, and increased serum levels of lymphocyte subpopulations as well as interleukin 8 (Jeong, Kim et al. 2002; Joos, Schott et al. 2000). Another study that examined acupuncture for allergy symptoms showed decreased serum levels of interleukin 10 in patients with chronic allergic rhinitis (Petti, Liguori et al. 2002), and finally a study that investigated headache, which demonstrated decreased serum levels of tumor necrosis factor alpha (TNFα) in subjects who experienced decreased cephalgia in response to acupuncture (Jeong, Hong et al. 2003).

The mechanism by which acupuncture may affect the immune system is uncertain, but several reasonable hypotheses exist. Bonta (2002) hypothesizes that β-endorphin may influence cytokines, though he does not elaborate on how this may occur. Zijlstra (2003) theorizes that acupuncture causes nerve impulses to travel in a retrograde fashion, which leads to a release of calcitonin gene-related peptide, substance P and β-endorphin. In turn, these substances lead to mast cell activation, macrophage release of inflammatory mediators with a cumulative effect of release of TNFα and nitric oxide (NO). In response to the proinflammatory cytokine release, there is a subsequent release of anti-inflammatory cytokines such as interleukin10 and/or interleukin 4. Zijlstra also theorizes the response to acupuncture may be local, stimulating cytokine release in
response to microtrauma with resulting inflammation and vasodilation (Zijlstra, van den Berg-de Lange et al. 2003).

In summary, by accessing the peripheral nervous system via acupuncture, the immune system may be stimulated and thus influence the cytokine signaling system, which may result in lower serum interleukin 6 levels. The final impact of reduced serum interleukin 6 via acupuncture may be improved symptoms such as reduced pain and improved energy levels. The question is, “Are we able to elucidate which mechanism of acupuncture effects change, and/or is the cytokine IL6 influenced by acupuncture?” By measuring serum levels of IL6 before and after acupuncture and correlating symptom response, we may gain more insight into the mechanism of acupuncture by validating that an IL6 response does occur, and assess simultaneous directionality of symptom experience.

Heart Rate Variability

*Heart Rate Variability and the Autonomic Nervous System*

The definition of heart rate variability (HRV) is the ventricular beat-to-beat variation represented as the electrical activity seen on an oscilloscope (digital or otherwise) in the form of a QRS complex. The marker point to count the R-to-R interval is generally measured at the peak of the R wave. This oscillatory phenomenon occurs in the interval between consecutive heartbeats, in addition to the oscillations between consecutive instantaneous heart rates (Malik 1996). Respiratory oscillations are the major influence of parasympathetic influence on heart rate via the central nervous system and vagus nerve. However, the precise physiology that HRV is measuring is far from simple.
As pointed out by Malik and Camm (1993) both high and low frequency components of HRV are influenced by both vagal and sympathetic activity, and at physiologic levels, each frequency can, and probably does, respond to both vagal and sympathetic influences. Thus, HRV must be recognized as measuring the *degree* of autonomic *modulations* rather than measuring an absolute level of cardiac sympathetic or parasympathetic tone (Malik and Camm 1993).

While the heart has intrinsic pacing tissue such as the sinoatrial (SA) and atrioventricular (AV) nodes, the heart rate is primarily determined by autonomic nervous system (ANS) influences on the SA node. The SA node is innervated by both the sympathetic nervous system (thoracic nerves 1-4) and the parasympathetic nervous system (vagus nerve), and these two forces maintain a constant tension to regulate cardiac homeostasis. Sympathetic influences, associated with the fight or flight response, lead to an increased heart rate, decreased beat-to-beat variability, and other features such as increased cardiac output. Parasympathetic influences are vagally mediated through release of acetylcholine, result in a slower heart rate, and are strongly influenced by respiratory oscillations also known as the respiratory sinus arrhythmia. Respiratory sinus arrhythmia refers to the respiratory cycle, on inspiration the heart rate speeds up, and on expiration, the heart rate slows down. The origination of this correspondence is thought to be in the nucleus ambiguous, located within the medulla oblongata, where expiration is thought to increase parasympathetic stimulus via the vagus nerve. On inspiration, inhibitory signals are produced with no vagal nerve stimulation, and thus, a relatively increased heart rate occurs (Neff, et al. 2003).
A healthy person at rest will usually have a predominance of cardiac parasympathetic tone, with a slower heart rate and increased beat-to-beat variability (Chess, G.F., Tam, R.M.K. and Calareus, F.R. 1975 as cited in the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Thus, as a researcher attempts to establish a sympathetic or parasympathetic preponderance of cardiac tone in humans, heart rate variability has good physiologic theory. Reliability of heart rate variability measurements are high and reproducible as long as similar times are used for comparison, such as 5-minute recordings compared to other 5-minute recordings versus compared to an 8 or 24-hour recording (Cowan 1993 as cited in Cowan 1995).

*The History of Heart Rate Variability*

Heart rate variability first came to the attention of researchers in 1965 when they noticed that fetal distress was preceded by decreased beat-to-beat variability before heart rate deceleration and bradycardia occurred (Hon and Lee, cited in Malik 1996). Since then, heart rate variability has been used to assess diabetics for cardiac autonomic neuropathy (Ewing, Martin, Young, Clarke, cited in Malik 1996), and post-myocardial infarction mortality rates (Wolf, Varigos, Hunt, Sloman, cited in Malik 1996). As technology advances have led to smaller electrocardiogram units, digital data recording and increasingly rapid and complex computation ability, heart rate variability may be able to provide researchers and clinicians with valuable information regarding the autonomic nervous system’s relationship to overall cardiac health status. Unfortunately, one common misconception is that heart rate variability can serve as a proxy for overall
vagal tone, but this is a mistaken assumption (Stein 2005). HRV only reflects influences on cardiac autonomic function.

*Heart Rate Variability Data Collection and Interpretation*

Heart rate variability is calculated from a continuous electrocardiogram recording for a pre-defined period. As mentioned above, the measurement is generally from the peak of the QRS complex, or R-R interval, which clearly defines the oscillation measured as beat-to-beat versus instantaneous change in heart rate. Length of recording depends on the research question, if a phenomenon of interest includes a circadian rhythm question such as overall cardiac health status post myocardial infarction, then a 24-hour recording maybe necessary. If, on the other hand, the research question is specific to an isolated response to an intervention of interest, then recordings as short as 5 to 20 minutes are acceptable (Malik 1996).

Heart rate variability is analyzed in one of three ways: time domain, frequency domain and non-parametric measures. However, the time and frequency domains are most commonly used, and non-linear dynamics such as integral complexity measures have proved disappointing in heart rate variability analysis (Malik 1996). Time domain analysis is the simplest analysis to perform, and is not only used more frequently for 24-hour samples, but many of the time domain statistics are more accurate in 24-hour samples. The time domain analysis requires an initial determination of QRS or R-R interval, which is then re-named as normal-to-normal or N-N intervals. Time domain analyses offer mean N-N interval, mean heart rate, the difference between the shortest and longest N-N interval and a few other measures (Malik 1996). Statistical
interpretations include the standard deviation of the N-N interval or the SDNN, which is usually recorded in a 24-hour period and captures circadian-type cycles that reflect variability (Malik 1996; Cowan 1995). Other statistical analyses include the SDANN, standard deviation of the average N-N interval, similar to SDNN, but analyzed in 5 minute intervals, and the 24-hour SD, which is the standard deviation of the set of all heart periods, is easy to use on most 24 hour data sets and yields valuable information (Cowan 1995). Finally, the root mean square successive difference (RMSSD) is an index of beat-to-beat variation in R-R intervals, and may be used reliably in samples as short as 5 minutes (Cowan 1995). Additional time domain measures include the NN50 and pNN50 each which measure successive N-N intervals greater than 50 milliseconds and the proportion derived from dividing NN50 by the total number of NN intervals, respectively (Malik 1996). RMSSD, NN50 and pNN50 all have a high correlation with each other and are considered a reflection of the high-frequency bandwidth of power spectral analysis, a measure of cardiac parasympathetic tone (Kleiger, et al. 1991 as cited in Cowan 1995), however the RMSSD is a preferred method due to statistical properties (Malik 1996). Time domain analyses of 24-hour data can also be converted into geometric patterns by identifying specific sample density distribution of N-N intervals and creating triangular, linear, elliptical Lorenz plots. The advantage of this type of analysis is the N-N quality can be less than ideal, while the disadvantage is the large amount of data needed (Malik 1996).

Frequency domain analysis, the preferred method in recordings less than 24 hours (Malik 1996), more specifically identifies the preponderance of cardiac sympathetic or
parasympathetic activity (Cowan, 1995). Also referred to a power spectral analysis, it “provides estimates of the power spectrum density of heart periods within finite frequency bandwidths” (Cowan, 1995 p. 35). It breaks down the heart rate signal into frequencies and assigns a numerical value according to their intensity or power. This ‘power spectrum analysis’ isolates parasympathetic from sympathetic signals. Frequency ranges of 0.15-0.40 hertz are considered high frequency and represents an index of parasympathetic neural activity (Akselrod, et al. 1981; Ecksberg et al. 1980; Katona & Jih, 1975 as cited in Cowan 1995). Low frequencies in the range of 0.01-0.04 are considered reflective of sympathetic stimulation as it can be altered with sympathetic blockade (Malliani, Pagani, Lombardi & Cerutti, 1991; Pagani, et al. 1986 as cited in Cowan, 1995).

Analysis of frequency domain data is accomplished with computer software that incorporates complex mathematical calculations, such as fast Fourier transformations (FFT) and autoregressive (AR), data smoothing, techniques. With increasing computation power, the fast Fourier transform has become more efficient and easy to use, however, it still has some disadvantages in the form of artifact created by the calculations, but these are easily identified visually, and eliminated from the data set. For this reason, FFT should not be used for small data sets with less than 5 minutes of data points (Malik 1996). This point also underscores the importance of visually inspecting each raw signal (QRS complex) with the data point mark in place to ensure accurate scoring by the computer program.
Heart Rate Variability and Menopause

Several studies indicate that reproductive-aged women, with higher serum estrogen levels, tend to have a sympathovagal balance with a preponderance of parasympathetic effects, which result in an increased heart rate variability, as well as a decreased incidence of cardiac arrhythmia (Fukusaki 2000; Mercuro 2000; Saleh 2007). This is thought to be due to the significant number of estrogen receptors in the central nervous system, from the spinal cord to the insular cortex, and thus estrogens may play a central role in the regulation of autonomic tone (Saleh 2007). Menopause is associated with cardiovascular changes that have been linked to increased sympathetic activity as well as decreased HRV. In one small study of 28 women without significant underlying cardiac disease undergoing oophorectomy or hysterectomy with ovaries retained, the women who underwent oophorectomy demonstrated significant imbalance of the autonomic nervous system with a reduction in parasympathetic mediated indices of HRV and a shift to sympathetic hyperactivity. The authors point out that these results may not generalize to the slower withdrawal of estrogen that occurs with natural menopause (Mercuro 2000). However, results from a later study of 52 women, 34 of whom were menopausal, suggest that even in a normotensive, non-smoking, naturally occurring menopausal women, there is significant disruption of the autonomic function with a physiologic balance of sympathetic activity as measured by HRV (Farag 2003). Whether the preponderance of sympathetic tone in this population is due to low serum levels of estrogens or the normal course of aging has not been clearly determined (Kajantie and Phillips 2006).
Heart Rate Variability and Acupuncture

Several studies suggest that acupuncture may affect HRV by increasing the high frequency component, theoretically a decrease in cardiac sympathetic tone (Carpenter 2006; Haker, Egekvist et al. 2000; Li, et al. 2005; Middlekauf 2004;). However, other studies indicate that acupuncture influences HRV by increasing the low frequency component, reflecting an increase in cardiac sympathetic tone. From a common-sense perspective, it is logical that initially, acupuncture would increase sympathetic influences on the heart because acupuncture can be a painful or uncomfortable experience. On the other hand, after the needles have been inserted, many people report a feeling of profound relaxation, so this initial sympathetic response could or should yield a parasympathetic response, and be seen as an increase in the high frequency band of HRV, which Li et al (2005) reports.

A Mechanism by Which Acupuncture May Influence Heart Rate Variability

One proposed mechanism, by which acupuncture may modulate cardiac autonomic activity, as reflected in HRV, involves needle stimulation of peripheral nerve fibers, specifically C-fiber and \( \gamma \) afferent size nerve fibers. One research design that may be able to link the physiology of acupuncture to the immune-inflammatory response would be to establish that acupuncture significantly influences the parasympathetic nervous system. An accepted measure of autonomic system activation is heart rate variability (HRV) (Cowan 1995; Malik 1996; Eckberg 1997). By simultaneously measuring proinflammatory cytokines, specifically interleukin 6, before during and after acupuncture, while concurrently measuring HRV, a researcher may be able to establish a
significant correlation between acupuncture, HRV (as a measure of parasympathetic response), serum levels of IL 6 and symptoms.

Physiology of Acupuncture

Neurophysiologic Theory of Acupuncture

The primary and most widely accepted physiologic mechanism attributed to the specific effect of an acupuncture needle, hypothesized for humans and demonstrated in a rat model, is the neurologic model, which was conceived and developed by Pomeranz and colleagues in the late 1970s and 1980s (Cheng and Pomeranz 1979; Cheng and Pomeranz 1981; Cheng and Pomeranz 1981; Pomeranz 1978;). The noxious stimulation of the acupuncture needle is thought to stimulate the small, myelinated sensory afferent type II and/or III nerve fibers located in the skin and muscle tissue. The impulse travels from these peripheral nerves to the dorsal horn of the spinal cord where it acts locally as well as ascends one of six potential pathways including the anterolateral tract and spinothalamic tracts (Pomeranz, as cited in Stux and Hammerschlag (Eds.), 2001). In the spinal cord, interneurons facilitate the release of enkephalin and/or dynorphin from endorphinergic cells, which may result in segmental pain relief. Other substances thought to be released in the spinal cord level include potent peptides such as cholecystokinin, somatostatin, neurotensin, bombesin, calcitonine gene-related peptide, angiotensin, substance P, and vasoactive intestinal peptide (Pomeranz, as cited in Stux & Hammerschlag, (Eds.), 2001, p. 4). In the rat model, as the impulse traveled up the spinal cord, through the midbrain, raphae nuclei, tegmental nuclei and locus ceruleus, endogenous opioids as well as neurotransmitters--serotonin, norepinephrine and
dopamine, were released or inhibited (Cheng and Pomeranz 1981; Pomeranz and Chiu 1976; Pomeranz 1978; Cheng and Pomeranz 1979; Pomeranz and Warma 1988; Pomeranz 1996).

Recent neuroimaging of healthy human subjects undergoing acupuncture using fMRI with BOLD technique and positron emission tomography (PET), have bolstered these early research findings and hypotheses by demonstrating signal changes in CNS structures thought to represent regions associated with endogenous opioid and neurotransmitter production. These signal changes have been interpreted to represent increased or decreased neural activity, and have been positively correlated with acupuncture needle insertion (Biella, Sotgiu et al. 2001; Cho, Chung et al. 1998; Gareus, Lacour et al. 2002; Hui, Liu et al. 2005; Kong, Ma et al. 2002; Pariente, White et al. 2005; Wayne, Krebs et al. 2005; Wu, Sheen et al. 2002; Yan, Li et al. 2005).

Together, these findings support the hypotheses that acupuncture affects the nervous system, both peripherally and centrally, and is likely to induce a cascade of physiologic changes involving norepinephrine, serotonin, dopamine and endogenous opioids. These biochemical substances are thought to influence both pain and menopausal symptoms (Cabyoglu, Ergene et al. 2006; Cheng and Pomeranz 1981; Han, Tang et al. 1980; Ma 2004; White 2006).

*The Autonomic Nervous System and Acupuncture*

Acupuncture has also been hypothesized to directly affect the autonomic nervous system. In two separate, but small studies, researchers have linked acupuncture in healthy subjects to increased heart rate variability and increased cardiac parasympathetic
modulation (Carpenter 2006; Haker 2000;). These findings are significant for two reasons. First, menopausal symptoms, specifically vasomotor symptoms, have a significant physiologic correlation to increased sympathetic nervous system activation (Freedman, 2005). Additionally, vasomotor instability tends to decrease with the administration of central alpha 2-adrenergic agonist pharmaceutical agents such as clonidine, which decreases CNS sympathetic outflow by inhibiting the production of norepinephrine and results in relaxed arterial vasculature (Hardman, 1996, p. 217). Finally, vasomotor instability increases with heightened states of anxiety, a sympathetic nervous system response to stimuli. Additionally, stimulation of the parasympathetic vagus nerve has been shown to have a direct effect on the inflammatory immune response by decreasing serum levels of proinflammatory cytokines (Tracey, 2007).

The neurophysiology model of acupuncture provides a logical means by which acupuncture may relieve pain, but many have wondered by what physiologic mechanism acupuncture could alleviate other maladies such as asthma or allergy symptoms. Three small studies suggest that acupuncture is able to influence allergic rhinitis (Petti, et al. 2002), allergic asthma (Joos, et al. 2000) and proinflammatory cytokines (Petti, et al. 2002; Joos, et al. 2000; Jeong, et al. 2003), however, no mechanism was postulated.

**Acupuncture for Menopausal Vasomotor Symptom Relief**

1998; Porizo, et al., 2002; Sandberg, et al. 2002; Tukamachi, et al. 2000; Vincent, et al. 2007; Wyon, et al., 2004; Wyon, et al., 1995). Of these fourteen studies, sample sizes were small ranging from 30 to 160 subjects. These small sample sizes illustrate the common criticism that most acupuncture studies are too small to generalize findings to the larger population, however, the high cost of these studies and lack of funding, is a major limitation to larger, population-based studies. Another frequent criticism of acupuncture is poor study design and this is seen in all fourteen studies as only eight had control arms. However, it is important to keep in mind that when exploring new phenomenon, alternative research designs that include smaller sample sizes allow for exploration and feasibility of mechanism as well as effectiveness, and thus, are a necessary stage in the scientific endeavor.

Further weaknesses of these fourteen studies included lack of objective or physiological measures. Only three measured biomarkers, which included urinary excretion of the neuropeptide- calcitonine gene-related peptide (CGRP), and in this study CGRP decreased in both superficial needle insertion (SNI)—the placebo control group and electroacupuncture (EA) group (Wyon, et al. 1995). The remaining two examined serum follicular stimulating hormone (FSH), lutenizing hormone (LH), estradiol, progesterone and prolactin in which Ping, et al. (1998) showed a decrease in serum LH & FSH, but no change in estrogen and Dong, et al. (2001) showed no changes in any of these serum hormones.

Interestingly, in all studies where subjects received any type of acupuncture including placebo needle intervention in the form of superficial needle insertion or non-
specific acupuncture (which was not designed to be therapeutic for the condition treated) or verum (‘real’ or therapeutic for the condition under study) acupuncture, vasomotor symptoms improved. Studies, which used control groups with any type of placebo needling, have not been able to show a statistical difference between the verum and placebo acupuncture arms (Cohen, et al., 2003; Vincent, et al., 2007; Wyon, et al., Sandberg, et al., 2002). These findings have led critics to regard the effects of acupuncture as simply placebo or having non-specific effects, and have reviewers questioning the validity of specificity of acupuncture points, as well as the validity of a specific physiologic mechanism of action for traditional acupuncture in theory and practice. Researchers must consider the placebo or non-specific response when examining any intervention; for instance, one study compared electro-acupuncture and relaxation for the relief of menopausal hot flashes, and both interventions demonstrated a significant decrease in symptoms with no statistical difference between groups (Nedstrand, et al., 2005).

Summary of the Literature

Review of the related literature confirms that menopause is a common event, and that menopausal symptoms are problematic for many women. These symptoms include vasomotor symptoms (hot flashes and sweats), in addition to sleep and mood disturbance, low energy/fatigue, joint and muscle pain and vaginal dryness. Menopausal symptoms are most often attributed to varying levels of serum and tissue levels of estrogens, which may in turn be directly affecting the inflammatory immune response leading to increased levels of proinflammatory cytokines, specifically interleukin 6. There may also be an
intrinsic relationship between estrogens, estrogen receptors and endogenous opioids and a woman’s perception of pain. This feasibility study sought to understand how women who experience menopausal symptoms, experience an acupuncture intervention, and how those symptoms respond. While prior work suggests they will have a favorable improvement in vasomotor symptoms, fatigue, low energy, depressed mood and sleep disturbances have not been examined as often.

In addition, there are significant gaps in the literature regarding an acupuncture mechanism of action that may explain why menopausal women experience symptom reduction—specifically vasomotor symptoms (hot flashes) in response to acupuncture. Several small studies examined the effects of acupuncture on heart rate variability, but these reported conflicting results. Additionally, several small studies examined the effects of the menopausal and perimenopausal state on heart rate variability, also with inconsistent results, however a lower estrogen state did tend to correlate with lower serum levels of interleukin 6. This study sought to determine the feasibility of investigating the physiologic impact of an acupuncture intervention on the autonomic nervous system, as well as the inflammatory immune response, and to correlate physiologic change with symptom experience. In light of the dearth of understanding regarding the physiologic impact of acupuncture, this pilot study could elucidate important information specific to the acupuncture effects on the autonomic nervous system as well as the inflammatory immune response in a menopausal sample.
CHAPTER THREE: METHODS

Review of Purpose

The purpose of this study was to examine the feasibility for a larger study to assess the effectiveness of acupuncture for managing menopausal symptoms, and to gain new knowledge of the physiology of acupuncture.

Specific Aims and Hypotheses

Specific Aim 1: To determine the feasibility and preliminary indication of effectiveness necessary for a larger study to describe the effects of acupuncture on specific menopausal symptoms: vasomotor symptoms (hot flashes/sweats), mood disturbances, sleep disturbances, pain and urogenital symptoms in an otherwise healthy group of women.

Hypothesis 1: It will be feasible with indications of effectiveness to conduct a larger study with at least 70% of participants (Matson, 2000; Polit, D. F. and Beck, C. 2004) completing all acupuncture treatments and measurements.

Exploratory Aim 2: To describe the effect of acupuncture on the cardiac autonomic nervous system through the use of heart rate variability.

Hypothesis 2: Acupuncture will increase heart rate variability, defined by measures such as the ratio of parasympathetic to sympathetic power in spectral analysis, as well as low frequency and high frequency measures in both absolute power and normalized units. When compared to baseline measures, increased heart rate variability will demonstrate decreased low frequency to high frequency ratio, decreased values of low frequency and increased values of high frequency measures.
Exploratory Aim 3: To describe the effect of acupuncture on the inflammatory immune response as measured by serum levels of interleukin 6.

Hypothesis 3: Acupuncture will influence the inflammatory immune response reflected by decreased levels of serum interleukin 6 after acupuncture intervention.

Exploratory Aim 4: To explore the relationship between heart rate variability, interleukin 6 and menopausal symptoms before and during the acupuncture intervention.

Hypothesis 4: There will be a significant relationship between heart rate variability, interleukin 6, menopausal symptoms and acupuncture intervention. Those who show improvement on one measure will tend to show improvement on the other.

Research Design

The research design was an early phase, exploratory, longitudinal study. There was no control group as each woman acted as her own control, in other words each subject’s change (or lack thereof) was compared to her baseline. The sample size was 12 women; the smaller sample size was due to the nature of a feasibility study (Polit, D. F. and Beck, C. 2004) with many data collection points, in order to answer questions about data collection, timing of potential effects and proper methods of analysis. A longitudinal design was chosen because it fit well with the underpinning theoretical model of physiologic change over time (Polit, D. F. and Beck, C. 2004), in addition to providing a research design with higher internal validity in an area of research (complementary and alternative medicine, specifically acupuncture) where strong design has historically been lacking (Ernst 1997, 2003, 2006).
The strengths of this design include strong internal validity due to a highly homogenous population with frequent sampling and subsequent large number of data points. Additionally, there was less chance of subject drop out due to the potentially demoralizing process of not being randomized into the treatment group. Moreover, this design offered increased chances of seeing a change over time, as opposed to gathering data at a single point in time. This is especially true given the purpose and specific aims, which are to see physiologic change in the autonomic nervous system and immune system as measured by serum interleukin 6, as well as evolution of symptom experience. It was more reasonable to expect these events to occur in the context of change over time. Finally, the use of a standardized TCM acupuncture protocol, results should be replicable.

On the other hand, the strength of the acupuncture protocol may compromise external validity, as this is not representative of how TCM is practiced in community clinics. Acupuncture is only one of many therapeutic modalities, and many times an herbal formula or lifestyle changes and modifications are prescribed. Additionally, external validity will be somewhat weaker due to the small sample size and homogeneity. Lack of a control group prevented comparison analyses, however, feasibility was the primary aim, and based on results from this study, a larger trial may be conducted using a randomized cross-over design to facilitate comparison with a ‘no treatment’ group.

Human Subjects

Human subjects’ approval was obtained through the University of Arizona Institutional Review Board (IRB). The Data Monitoring and Safety Board (DSMB)
consisted of two college of nursing faculty members, Drs. K. Moore and C. Michaels, who were not otherwise associated with the study. The DSMB board met every thirty days to review data collection procedures, subject enrolment, dropout rates and adverse events. Reports were submitted to the University of Arizona IRB after each meeting. There were no adverse events or subject dropout. Consent forms and IRB forms are in appendix A.

Study Sample

Sampling

A convenience sample was used (Polit, D. F. and Beck, C. 2004), and subjects were recruited using radio and newspaper advertisements (appendix B). Inclusion criteria included postmenopausal, at least 12 months, but no more than 45 months, without spontaneous menstrual bleeding through natural menopausal transition (Sherwood 2004), moderate to severe menopausal symptoms as defined by a minimum score of 22 (score range 0-44) on the Menopause Rating Scale (Heinemann, L.A. 2007), and be able and willing to provide informed consent. Exclusion criteria included any pathophysiology that would adversely influence the biological markers, which included any inflammatory process, suppression of inflammation (Kishimoto, T., 1989; Pantsulaia, I., Trofimov, S., Kobyliansky, E., Livshits, G.2002) or irregular heart rhythms (Malik 1996). Specific exclusion criteria included surgical menopause (Farag, N. H., Bardwell, W.A., Nelesen, R. A, et al. 2003), autoimmune disease, diabetes, significant infection in the past three months, steroids or chronic nonsteroidal anti-inflammatory therapy, hormone replacement therapy, atrial fibrillation, supraventricular tachycardia, frequent premature
ventricular contractions. Finally, anyone who had used acupuncture for menopausal symptom relief in the past 12 months for menopausal symptoms or in the past six months for any reason experienced acupuncture in the past six months for any reason, or who were needle phobic were excluded (Molsberger, A. F., Streitberger, K., Kraemer, J., Brittinger, C. S., Witte, S., Boewing, G. and Haake, M. 2006). The subjects did not receive compensation.

Sites and Settings

The acupuncture and sample collection took place at one of two acupuncture clinic sites, the Asian Institute of Medical Studies located at 3131 N. Country Club Rd, or Ancient Ways Acupuncture Clinic, located at 3340 N. Country Club Rd., Tucson AZ.

Study Schema

See Table 1 (below) for study schema.

**TABLE 1. Study Schema**

<table>
<thead>
<tr>
<th>Study Schema</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening &amp; Informed consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menopause Rating Scale</td>
<td>Daily</td>
<td>Daily</td>
<td>Daily</td>
<td>Daily</td>
<td></td>
</tr>
<tr>
<td>Acupuncture: 3X per week for Weeks 1 &amp; 2; 2X per week for Weeks 4 &amp; 5.</td>
<td>#1-#2-#3</td>
<td>#4-#5-#6</td>
<td>#7-#8</td>
<td>#9-#10</td>
<td></td>
</tr>
<tr>
<td>Medication log to note any changes in medication regimens</td>
<td>Daily</td>
<td>Daily</td>
<td>Daily</td>
<td>Daily</td>
<td></td>
</tr>
<tr>
<td>Serum collection for interleukin 6</td>
<td>Baseline before &amp; after the first acupuncture</td>
<td>Before/ during each acupuncture session</td>
<td>Before/ during each acupuncture session</td>
<td>Final treatment, # 10, before &amp; after acupuncture</td>
<td></td>
</tr>
<tr>
<td>Heart rate signal collection-40 minutes total at each session</td>
<td>Baseline, before &amp; during each acupuncture</td>
<td>Before/ during each acupuncture session</td>
<td>Before/ during each acupuncture session</td>
<td>Before/during each acupuncture session</td>
<td></td>
</tr>
<tr>
<td>Data Management occurred as data was accrued.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each subject’s data was analyzed at the time of collection.
Measurements

There were five measurements in this study: feasibility, menopausal symptoms and distress, serum interleukin 6, heart rate variability, and the relationships among these variables to each other. Each measurement was collected using validated, sensitive instruments. Additionally, initial screening was performed using the same instrument that was used to capture menopausal symptoms during the study, the Menopausal Rating Scale. Demographic data were collected using 14 items from the Women’s Health Questionnaire that captured annual income, marital status, menstrual history, height, weight, body mass index, current medications-prescription and over-the-counter, occupation, formal education, spouse’s occupation, who lives at home and insurance status (Berg, 1999; Lee et al., 1994; Mitchell, Woods & Lentz, 1991).

Specific Aim 1, Feasibility Assessment and Rationale

Feasibility studies are an essential element in the research process because full-scale research projects are costly, complex and time-consuming. Smaller scale studies, known as feasibility studies, allow the researcher to evaluate aspects of a plan of research and evaluate the project’s prospects for success (Matson 2000). Feasibility studies are not meant to be a forum for new ideas or concepts, which should already be identified, but rather, the feasibility study should be a tool to aid the researcher in decision making specific to the conduction of the larger study being considered (Matson 2000). The a priori definition of feasibility success was set at 70% completion of all acupuncture treatments and measurements based on literature review of usual study drop out rates, which range from 25%-40% (Polit, D. F. and Beck, C. 2004).
Menopausal Symptoms and Distress-The Menopausal Rating Scale

The Menopause Rating Scale (MRS) was developed specifically to evaluate women’s severity and frequency of menopause symptoms, and to compare symptom experience before and after therapeutic intervention (Heinemann, L.A.J. 2007). The questionnaire consisted of 11 items, which measured three distinct dimensions, psychological, urogenital, and somato-vegetative, each on a five-point scale ranging from 0-4 with possible scores ranging from zero, indicating no symptoms, to four, which indicates severe symptoms. One additional question was added to capture the experience of distress the menopausal symptoms generated, “How distressing or bothersome were your symptoms today?” A visual 10-point scale from one through ten was provided, and used to quantify the global distress experienced due to menopausal symptoms.

The MRS was validated in 9907 women across eleven countries, which included 1440 American women (Heinemann 2007). Cronbach’s alpha for the total score, in 1440 United States women was 0.88 (Heinemann 2007), test-retest reliability in U.S. women was 0.96. Sensitivity was reported to be 70.8% and specificity is 73.5%. Discriminant validity was established using the Kupperman Index, a physician administered scale for measuring menopausal symptoms, with a Kendall’s tau-b coefficient of 0.75 (95% CI 0.71-0.80) and a Pearson’s correlation coefficient r= 0.91 (95% CI 0.89-0.93) suggesting the MRS measures phenomenon similar to that found in the Kupperman Index (Heinemann 2007). A copy of the instrument is in appendix C.
Symptom Data Collection

After informed consent was obtained, and the baseline scores collected, a booklet
with 28 copies of the Menopausal Rating Scale Questionnaire, designed to be self-
administered, was given to each subject. The subjects were instructed to complete the
MRS each day, beginning after the first acupuncture treatment and to continue until all 28
forms were filled out, representing 28 days. The completed forms were returned on clinic
visits or by mail.

Symptom Data Management

Questionnaire data were collected weekly and manually entered into the database
as it was collected. Data were reviewed and cleaned weekly using two checks, outliers by
inspecting for frequency distribution, and wild codes, or codes that were not defined
(Polit and Beck 2004). There were no missing data, but had there been missing data, the
plan was to carry the last value forward or average the prior and subsequent values if the
missing information was between two valid data points.

Symptom Data Analysis

The symptom data were examined both at the individual and group level, and
correlations were examined for effect size and trend significance that might indicate
avenues for future research. A priori statistical analysis was to have been multiple
regression; however, that was an over-optimistic plan for this small project. In regression
models, the smaller the sample size, the greater the risk of seeing a strong effect in
random data. For a reliable regression model, at least 10-15 cases per predictor would be
necessary. In this study, with symptoms, heart rate variability and interleukin 6 as
outcome variables, a sample size of at least 80 would be needed in order to see a medium effect (Field 2005). As this project was feasibility and exploration, linear regression models were used instead to look for and characterize trends. The results presented in tables 4a and 4b were derived using a simple linear regression model, where $R^2$ provided the researcher with an estimation of the strength of the relationship between the actual data and the regression model (Field 2005). $R^2$ does not measure causality, but it does inform how well the data fit the linear regression model, or in other words, how well the slope represents the actual symptom trend (Field 2005). To assess statistical significance of improvement, a Wilcoxon Signed-Rank Test was performed using the baseline and final symptom score. This test was selected because the data were non-parametric, and a binomial outcome could be described (Field 2005). In this instance, whether the baseline scores decreased or increased. Results are summarized in tables 4a and 4b.

In this project, the variable relationship was between time (number of treatments) and each specific symptom. The intervention, acupuncture, occurs through time, so changes in symptoms may be simply related to time or to the intervention. If the subject did not initially report the symptom, the slope and the $R^2$ were reported as zero. If the subject did report the symptom, the symptom score slopes could have been one of three values, negative for symptom improvement, positive for worsening symptoms or zero for no change in symptoms. To differentiate between those subjects who did not initially report the symptom and those with no change in the symptom, the reported $R^2$ informs the reader whether or not the symptom was reported and scored.
**Exploratory Aim 2, Effects of Acupuncture on Interleukin 6**

The inflammatory immune response was measured using the pro-inflammatory cytokine interleukin 6 and enzyme-linked immunoabsorbant assays, which are commercially available assays purchased from Pierce Endogen, Thermo Scientific (http:www.piercenet.com).

Enzyme linked immunoabsorbant assays are sensitive methods used to detect specific cytokines, enzymes, viruses, bacteria, or other biologic molecules of interest. The assay depends on a reaction of antibody-antigen, and an enzymatic substrate that allows a reaction to occur which produces shades of color that are read using optical computer sensors. The depth or darkness of the color corresponds to the amount of bioconjugation or levels of biomolecules of interest (Crowther, J.R. 1995).

The ELISAs were performed in triplicate by the University of Maryland Core Cytokine laboratory, which reported sensitivity and specificity measure physiologically relevant levels of serum cytokines at 1.5 to 100 pg/ml with a sensitivity < 1 pg/ml. Regarding specificity, commercially available kits report no cross reactivity.

**Interleukin 6 Collection Procedure**

Interleukin 6 was obtained from serum samples via peripheral phlebotomy by this researcher. The first two samples were drawn at baseline (before acupuncture) and immediately after the first acupuncture treatment. The final two samples were drawn immediately before and after the tenth and final acupuncture treatment. To minimize phlebotomy, an indwelling 23 gauge butterfly needle with attached tubing was inserted into the appropriate vein (usually brachiocephalic), and secured with Micropore ® tape.
Needle patency was maintained with a five-milliliter normal saline flush after the first sample. Standard sterile technique and universal precautions were observed.

Each venous sampling required seven milliliters of blood, which was collected in a preservative free, red-top vacuum tube. The sample was allowed to clot for 10 minutes in a refrigerated environment at 45º F. After the sample was clotted, it was centrifuged at 3000 revolutions per minute. After the serum was separated, the serum was aspirated and placed in preservative-free micro-tubes, and kept refrigerated at 45 º Fahrenheit until it could be transported to the University of Arizona College of Nursing (consistently within 2 hours of collection) where it was frozen at -20 degrees Fahrenheit. Maximum sample storage time was 4 months. Once all samples were collected, the samples were sent, frozen on dry ice to the University of Maryland Core Cytokine laboratory for interleukin 6 analysis using enzyme-linked immunoassay methods. Confirmation from the receiving laboratory that the samples arrived frozen was obtained. Upon receipt of the interleukin 6 results, they were entered into the database for analysis.

Interleukin 6 Data Analysis

The interleukin 6 data were analyzed by descriptive statistics including range with a 95% confidence interval and mean. A Kolmogorov-Smirnov test was performed to confirm that the interleukin 6 data were normally distributed. While not extremely meaningful on such a small sample, for exploratory purposes, three one-tailed paired t-tests were performed: the first and tenth pre-acupuncture and post-acupuncture levels, and the difference between the first post-acupuncture and the tenth pre-acupuncture level.
**Exploratory Aim 3, Effects of Acupuncture on Heart Rate Variability**

*Electrocardiogram Equipment and Heart Rate Variability Analysis Software*

The electrocardiogram was digitally recorded using a Cadwell Easy Ambulatory 2 system, which had been tested for electrical safety and certificated by Intertek Testing Services NA, incorporated. The certificate of test was dated September 24, 2003, does not have an expiration date, and was deemed safe for use in humans. Sensitivity and specificity of the electrocardiogram is dependent on lead placement and artifact that may be due to movement, ambient electrical interference or biological parameters such as adiposity. Artifact was minimized by instructing subjects to move as little as possible during the recording phase, adjusting leads as necessary and reducing unnecessary use of electrical equipment in the treatment room. Final disposition of sensitivity and specificity resided with the researcher who visualized the electrocardiogram tracing, evaluated the quality of the signal and made adjustments as needed. The researcher has twelve years experience reading and interpreting electrocardiograms.

A software program written by Mikel Aickin, PhD was used to convert the electrocardiogram file into an inter-beat-interval file using Stata statistical software version 10. Heart rate variability results were obtained using software downloaded from the Department of Physics at the University of Kuopio, in Kuopio, Finland at www.bsamig.uku.fi.

*Electrocardiogram Collection Procedure*

Each subject was seen at the same time of day, plus or minus two hours to avoid confounding circadian rhythm changes (Malik 1996), and the treatment room was kept at
an ambient temperature between 68-72 degrees Fahrenheit to prevent temperature-associated discomfort that may affect heart rate variability (Malik 1996).

Each subject was placed in a comfortable, supine position on a massage table, and three skin sensor electrode leads were placed on the chest wall. Placement was consistent for all subjects as follows to capture a standard lead two QRS configuration: the left lead was placed immediately lateral to the left sternal border at the second intercostal space, the right lead was also placed at the second intercostal space two to three inches lateral to the left lead, and the ground lead was placed at the fifth intercostal space at the mammary line. No postural changes or movement occurred during thirty minutes of electrocardiogram collection.

Electrocardiogram Conversion and Heart Rate Variability Data Management

Electrocardiogram (ECG) data in the form of a digitally compressed file was collected using the Cadwell Easy Ambulatory 2 EEG/ECG Recorder software program installed on a Dell Inspiron 1200 computer. The sampling rate was 200 hertz. In the Cadwell proprietary software file format, the ECG signal was downloaded and stored on the computer’s hard disk drive. Next, the ECG signal was converted into a European Data Format (EDF) file, and the EDF file was then transferred to a second computer, a Dell Inspiron 6400. The EDF file was then converted into an ASCII file using a free-ware program written by Marco Roessen, and may be found at http://www.edfplus.info

Each electrocardiogram recording was visually inspected for abnormal QRS wave morphology, dysrhythmias, movement artifact, and to ensure that the R-wave was correctly marked by the Stata software program that would permit accurate detection of
the R-R interval. Ten minutes of artifact and ectopic beat-free segments were accepted for analysis. From each 10-minute segment, the last 5 minutes were used for HRV analysis.

After visual inspection, the ASCII files were converted into inter-beat-interval (ibi) files, which were also maintained in an ASCII file format. The ibi files were then converted into heart rate variability reports using the Kuopio HRV software. The Kuopio HRV software used a fast-Fourier transform to analyze the frequency components. Early in the project, analysis occurred at least weekly, under the guidance of Mikel Aickin, Ph.D., the heart rate variability technical consultant for this project. After about one month of guided analysis, independent analysis by this researcher occurred after each data collection session.

Heart Rate Variability Data Analysis

Three time points for assessing heart rate variability were selected: the resting phase, which was collected before commencement of each acupuncture session, after five minutes of acupuncture and the final five minutes of acupuncture, which represented twenty minutes of acupuncture needle retention, with needle stimulation at the half-way point.

Five measures or components of frequency domain using spectral analysis and an autoregressive model were used based on literature review (Malik 1996). These components included the low frequency to high frequency ratio (LF:HF), low frequency (LF MS²) and high frequency (HF MS²), each measured in absolute power or milliseconds squared, and finally, both low (LF NU) and high frequency (HF NU), each
measured in normalized units. The normalized unit is a mathematical subtraction of the
very low frequency component of heart rate variability, and is commonly used in
conjunction with absolute power to report heart rate variability results (Malik 1996).
Each of these five components of spectral analysis of heart rate variability measures were
assessed during each phase of the acupuncture treatment, resting, after five minutes with
acupuncture needles retained, and the final 5 minutes. As with symptom scores, each
measure of heart rate variability was assessed for change over time by calculating slopes
using linear regression.

The hypothesized direction of change for each phase was as follows: the ratio was
to decrease, seen as a negative slope, which would represent either less sympathetic
cardiac modulation or greater parasympathetic cardiac modulation. The low frequency,
measured both as absolute power and in normalized units, was to decrease, again seen as
a negative slope, and by decreasing, would represent less sympathetic cardiac
modulation. Finally, the high frequency, measured both as absolute power and
normalized units, was to increase, seen as a positive slope, and by increasing, would
represent increased parasympathetic cardiac modulation. Visual inspection of each of
these phases of heart rate variability data on line graphs revealed large amounts of
variability, which was reflected in very low R² values. Group means with 95%
confidence intervals and standard deviations are presented, along with results of the
Kolmogorov-Smirnov test for normal distribution.
Exploratory Aim 4, Exploration of Relationships Between Symptoms, Heart Rate Variability and Interleukin 6

Data Analysis for Evaluating the Relationships Between Symptoms, Heart Rate Variability and Interleukin 6

Relationships between symptoms, interleukin 6 and heart rate variability, in temporal slope form, were analyzed using Spearman’s correlation coefficient. A separate correlation, also using Spearman’s correlation, was performed between absolute values of heart rate variability components at the time of the interleukin 6 sampling and interleukin 6, which are presented in table 8. As the data were non-parametric, the Spearman’s was the preferred test for assessing correlation. In this test, the data is ranked, and then Pearson’s equation is applied (Field 2005). Tables 14 and 15 present a summary of significant slope correlations, and the findings are reiterated in narrative. Significance was set at .05 or 0.01.

Descriptive Qualitative Collection and Analysis Procedure

Each subject was asked a final question at the completion of the study, in order to capture each subject’s experience of acupuncture, separate from the symptom experience. The subject was given a tape recorder, and before the researcher left the room, she asked the subject to answer the following, “Is there anything else that you would like to share about your acupuncture experience?” The subject then spoke their answer into the tape recorder. At a later date the researcher transcribed the reply, verbatim. The transcript was analyzed using a descriptive qualitative analysis described by Spradley (1979). Primary
themes and emic codes were identified, which were merged into domains, and the domains into overarching taxonomies. Full transcripts are in appendix D.

Traditional Chinese Medicine

Traditional Chinese Medicine Theoretical Rationale

For future replication and internal validity, a standard acupuncture point protocol was used. This researcher, a licensed acupuncturist with five years of experience, administered all acupuncture. Acupuncture method used was a standard traditional Chinese medicine (TCM), with point locations and needle insertion depths as published in *A Manual of Acupuncture* (Deadman and Al Khafaji, 1998).

The needles were placed at pre-selected acupoints per the TCM-driven protocol for this study. The protocol and point selection was determined based on the most common traditional Chinese medicine patterns seen with the biomedical condition and symptoms congruent with ‘menopause’. These patterns included Liver Qi Stagnation, Spleen Deficiency, Kidney and Liver Yin Deficiency with Liver Yang Rising, Kidney Yang Deficiency, Kidney Yin and Kidney Yang deficiency, Kidney and Heart Not Harmonized, Phlegm and Damp Accumulating, Empty Heat Harassing the Heart and finally, Blood Stasis (Flaws 2005; Maciocia 1998; McClean and Lyttleton 1998; Scheid 2006).

Traditional Chinese Medicine Acupuncture Procedure

Each subject received ten treatments, three times a week for two weeks and twice a week for two weeks. Acupuncture treatments were administered with the patient in a supine position. Standard clean needle technique was maintained. Seirin® stainless steel,
single use, sterile, disposable needles were used. Needle length and gauge was determined by body habitus and point selection, and ranged from a length of 30 mm to 75 mm, and a gauge of 0.16 mm to 0.30 mm (MacPherson, White et al., 2002).

**Traditional Chinese Medicine Acupuncture Protocol**

Each acupuncture point chosen for the protocol would address at least one or more of these identified patterns of disharmony. After needle insertion, each needle was stimulated with gentle rotation, thrusting and lifting technique until the subject reported a heavy or dull aching sensation (the ‘de qi’ sensation). The needles remained in place for 10 minutes and were then stimulated again until the ‘de qi’ sensation was reported. The needles were removed after 20 minutes.

**TABLE 2. Traditional Chinese Medicine Acupuncture Protocol**

<table>
<thead>
<tr>
<th>Point Name: Conventional English &amp; (Chinese: Pin Yin)</th>
<th>Anatomic Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese Ear model: Sympathetic Nervous System point Heart point</td>
<td>Both located in the fossa of the pinna.</td>
</tr>
<tr>
<td>Heart 7 (Shenmen)</td>
<td>Located on the flexor aspect of the forearm, at the lateral wrist joint, on the radial side of flexor carpi ulnaris at the proximal juncture of the pisiform bone</td>
</tr>
<tr>
<td>Pericardium 6 (Neiguan)</td>
<td>Located on the flexor aspect of the forearm, about 2 inches proximal to the wrist crease, between the Palmaris longus and flexor carpi radialis tendons</td>
</tr>
<tr>
<td>Stomach 36 (Zusanli)</td>
<td>About 3 inches below the knee, on the lateral aspect of the leg, about 1 inch lateral to the tibial crest in the tibialis anterior muscle</td>
</tr>
<tr>
<td>Liver 3 (Taichong)</td>
<td>On the dorsum of the foot, about ½ inch distal to the junction of the first and second metatarsals</td>
</tr>
<tr>
<td>Kidney 6 (Zhaohai)</td>
<td>Located on the medial ankle, between the posterior tibialis tendon, anteriorly, and the flexor digitorum longus tendon posteriorly</td>
</tr>
<tr>
<td>Spleen 6 (Sanyinjiao)</td>
<td>On the medial aspect of the lower leg, about 3 inches superior to the prominence of the medial malleolus, posterior to the medial tibial crest</td>
</tr>
<tr>
<td>Du 20 (Baihui)</td>
<td>On the vertex of the scalp, midline, about 5 inches posterior to the anterior hairline</td>
</tr>
</tbody>
</table>
CHAPTER FOUR: RESULTS

Results of Data Analysis

Sample Description

The results of data analysis are presented in this chapter. The findings are discussed in five sections: 1) Specific Aim 1, feasibility; 1a) The effect of acupuncture on menopausal symptoms 2) Exploratory Aim 2, the effects of acupuncture on interleukin 6; 3) Exploratory Aim 3, the effects of acupuncture on heart rate variability; 4) Exploratory Aim 4, the relationships between symptoms (as temporal slopes), heart rate variability and interleukin 6; 4a) the relationships between absolute values of heart rate variability and interleukin 6; 5) Qualitative inquiry into the experience of acupuncture.

TABLE 3. Sample Description - Demographic Data

<table>
<thead>
<tr>
<th>Age at Enrollment</th>
<th>Mean- 53.2 Years</th>
<th>Minimum- 48 years</th>
<th>Maximum- 62 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year of Last Menstrual Period</td>
<td>7</td>
<td>2007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1991</td>
<td></td>
</tr>
<tr>
<td>Height in Inches</td>
<td>Mean- 64.83</td>
<td>Minimum- 62</td>
<td>Maximum- 69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight in Pounds</td>
<td>Mean- 154.67</td>
<td>Minimum- 108</td>
<td>Maximum- 185</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated Body Mass Index</td>
<td>Mean- 25.88</td>
<td>Minimum- 19.9</td>
<td>Maximum- 32.9</td>
</tr>
<tr>
<td>Income</td>
<td>Mean- $25,000</td>
<td>Minimum- $7,500</td>
<td>Maximum- $50,000</td>
</tr>
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<td>Education Level</td>
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<td>College Graduate</td>
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<tr>
<td></td>
<td>4</td>
<td>Graduate Degree</td>
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### TABLE 3. Sample Description - Demographic Data - Continued

<table>
<thead>
<tr>
<th>n</th>
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</tr>
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<tbody>
<tr>
<td>4</td>
<td>Part Time</td>
</tr>
<tr>
<td>6</td>
<td>Full Time</td>
</tr>
<tr>
<td>1</td>
<td>Volunteer</td>
</tr>
<tr>
<td>1</td>
<td>Laid Off</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>n</th>
<th>Who the subject lives with</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>Mother</td>
</tr>
<tr>
<td>2</td>
<td>Children</td>
</tr>
<tr>
<td>3</td>
<td>Alone</td>
</tr>
<tr>
<td>5</td>
<td>Spouse</td>
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</table>

<table>
<thead>
<tr>
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<td>2</td>
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### Specific Aim 1, Feasibility

The primary aim of this project was to determine feasibility as measured by compliance rates, drop out rates, refusal of phlebotomy or failure to recruit the
predetermined sample size in the predetermined period of four months. By these a priori feasibility measures, the project was deemed feasible with a total of 71 women screened and 12 subjects recruited from September through December 2008. Compliance with treatment schedule was 100 percent, and there were no refusals for phlebotomy, acupuncture or ECG collection requirements e.g. lying very still during the recording phase. All data sought were obtained, and there were no missing data points. The Menopausal Rating Scales were all completed daily within the twenty-eight day framework. The interleukin 6 data, performed in triplicate using enzyme linked immunoabsorbant assays (ELISAs), electrocardiograms and subsequent heart rate variability were within expected ranges and interpretable.

Specific Aim 1a, Effects of Acupuncture on Menopausal Symptoms

TABLE 4A. Effects of Acupuncture on Menopausal Symptoms

<table>
<thead>
<tr>
<th>ID</th>
<th>Hot Flashes</th>
<th>Palpitations</th>
<th>Sleep Quality</th>
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### TABLE 4A. Effects of Acupuncture on Menopausal Symptoms - Continued

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<th>ID</th>
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<th>Vaginal Dryness</th>
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<th>Global Distress</th>
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<th>Fatigue</th>
<th>Sexual Dysfunction</th>
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<td>Slope R²</td>
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### TABLE 4B. Menopausal Rating Scales - Raw Score Statistical Improvement

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<th>Symptom</th>
<th>Wilcoxon Ranked Test</th>
<th>Sign Test</th>
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<td>Hot Flashes</td>
<td>-2.96, p=.002</td>
<td>p=.001</td>
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<tr>
<td>Palpitations</td>
<td>-2.27, p=.023</td>
<td>p=.031</td>
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<tr>
<td>Sleep</td>
<td>-3.12, p=.002</td>
<td>p=.000</td>
</tr>
<tr>
<td>Depressed Mood</td>
<td>-3.11, p=.002</td>
<td>p=.000</td>
</tr>
<tr>
<td>Irritability</td>
<td>-2.97, p=.003</td>
<td>p=.001</td>
</tr>
<tr>
<td>Anxiety</td>
<td>-3.00, p=.003</td>
<td>p=.001</td>
</tr>
<tr>
<td>Fatigue</td>
<td>-3.13, p=.002</td>
<td>p=.000</td>
</tr>
<tr>
<td>Sexual Dysfunction</td>
<td>-2.75, p=.006</td>
<td>p=.012</td>
</tr>
<tr>
<td>Bladder Dysfunction</td>
<td>-2.39, p=.017</td>
<td>p=.016</td>
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<tr>
<td>Vaginal Dryness</td>
<td>-2.70, p=.017</td>
<td>p=.004</td>
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<tr>
<td>Musculoskeletal Pain</td>
<td>-2.32, p=.020</td>
<td>p=.039</td>
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<tr>
<td>Global Distress</td>
<td>-2.81, p=.005</td>
<td>p=.002</td>
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**Individual Subject System Slope Graphs**

FIGURES 3-14. Hot Flashes – Individual Subject Graphs
Figure 3. Hot Flashes – Subject 101 Graph

Figure 4. Hot Flashes – Subject 102 Graph

Figure 5. Hot Flashes – Subject 103 Graph
Figure 6. Hot Flashes – Subject 104 Graph

Figure 7. Hot Flashes – Subject 105 Graph

Figure 8. Hot Flashes – Subject 106 Graph
**Figure 9. Hot Flashes – Subject 107 Graph**

\[
y = -0.0099x + 2.3547 \\
R^2 = 0.0414
\]

**Figure 10. Hot Flashes – Subject 108 Graph**

\[
y = -0.0222x + 1.2291 \\
R^2 = 0.0534
\]

**Figure 11. Hot Flashes – Subject 109 Graph**

\[
y = -0.0527x + 1.2389 \\
R^2 = 0.3284
\]
Figure 12. Hot Flashes – Subject 110 Graph

Figure 13. Hot Flashes – Subject 111 Graph

Figure 14. Hot Flashes – Subject 112 Graph
FIGURES 15-19. Heart Palpitations – Individual Subject Graphs

Figure 15. Heart Palpitations – Subject 101 Graph

Figure 16. Heart Palpitations – Subject 104 Graph

Figure 17. Heart Palpitations – Subject 105 Graph
Figure 18. Heart Palpitations – Subject 108 Graph

Figure 19. Heart Palpitations – Subject 110 Graph

FIGURES 20-31. Sleep Disorders – Individual Subject Graphs
101 Sleep Disorders

\[ y = -0.0803x + 2.5493 \]

\[ R^2 = 0.5331 \]

Figure 20. Sleep Disorders – Subject 101 Graph

102 Sleep Disorder

\[ y = -0.0512x + 1.803 \]

\[ R^2 = 0.1839 \]

Figure 21. Sleep Disorders – Subject 102 Graph

103 Sleep Disorders

\[ y = -0.0227x + 2.0296 \]

\[ R^2 = 0.1021 \]

Figure 22. Sleep Disorders – Subject 103 Graph
Figure 23. Sleep Disorders – Subject 104 Graph

Figure 24. Sleep Disorders – Subject 105 Graph

Figure 25. Sleep Disorders – Subject 106 Graph
Figure 26. Sleep Disorders – Subject 107 Graph

Figure 27. Sleep Disorders – Subject 108 Graph

Figure 28. Sleep Disorders – Subject 109 Graph
99

Figure 29. Sleep Disorders – Subject 110 Graph

Figure 30. Sleep Disorders – Subject 111 Graph

Figure 31. Sleep Disorders – Subject 112 Graph

FIGURES 32-43. Depression – Individual Subject Graphs
Figure 32. Depression – Subject 101 Graph

Figure 33. Depression – Subject 102 Graph

Figure 34. Depression – Subject 103 Graph
Figure 35. Depression – Subject 104 Graph

Figure 36. Depression – Subject 105 Graph

Figure 37. Depression – Subject 106 Graph
Figure 38. Depression – Subject 107 Graph

Figure 39. Depression – Subject 108 Graph

Figure 40. Depression – Subject 109 Graph
Figure 41. Depression – Subject 110 Graph

Figure 42. Depression – Subject 111 Graph

Figure 43. Depression – Subject 112 Graph

FIGURES 44-54. Irritability – Individual Subject Graphs
101 Irritability

\[ y = -0.0463x + 1.4877 \]
\[ R^2 = 0.2097 \]

Figure 44. Irritability – Subject 101 Graph

102 Irritability

\[ y = -0.0926x + 2.3547 \]
\[ R^2 = 0.6011 \]

Figure 45. Irritability – Subject 102 Graph

103 Irritability

\[ y = -0.0266x + 1.5025 \]
\[ R^2 = 0.3063 \]

Figure 46. Irritability – Subject 103 Graph
Figure 47. Irritability – Subject 104 Graph

Figure 48. Irritability – Subject 105 Graph

Figure 49. Irritability – Subject 106 Graph
Figure 50. Irritability – Subject 108 Graph

Figure 51. Irritability – Subject 109 Graph

Figure 52. Irritability – Subject 110 Graph
Figure 53. Irritability – Subject 111 Graph

Figure 54. Irritability – Subject 112 Graph

FIGURES 55-61. Anxiety – Individual Subject Graphs
Figure 55. Anxiety – Subject 101 Graph

Figure 56. Anxiety – Subject 102 Graph

Figure 57. Anxiety – Subject 103 Graph
104 Anxiety

\[ y = -0.0788x + 3.2512 \]

\[ R^2 = 0.5285 \]

Figure 58. Anxiety – Subject 104 Graph

105 Anxiety

\[ y = -0.0557x + 2.3522 \]

\[ R^2 = 0.3648 \]

Figure 59. Anxiety – Subject 105 Graph

108 Anxiety

\[ y = -0.034x + 1.6823 \]

\[ R^2 = 0.1453 \]

Figure 60. Anxiety – Subject 108 Graph
Figure 61. Anxiety – Subject 111 Graph

FIGURES 62-73. Fatigue – Individual Subject Graphs
101 Fatigue

\[ y = -0.0335x + 1.33 \]

\[ R^2 = 0.1611 \]

Figure 62. Fatigue – Subject 101 Graph

102 Fatigue

\[ y = -0.0655x + 2.5345 \]

\[ R^2 = 0.5074 \]

Figure 63. Fatigue – Subject 102 Graph

103 Fatigue

\[ y = -0.0236x + 1.4926 \]

\[ R^2 = 0.1524 \]

Figure 64. Fatigue – Subject 103 Graph
Fatigue

**104 Fatigue**

\[ y = -0.0754x + 3.1995 \]

\[ R^2 = 0.8319 \]

Figure 65. Fatigue – Subject 104 Graph

**105 Fatigue**

\[ y = -0.0291x + 1.8153 \]

\[ R^2 = 0.1584 \]

Figure 66. Fatigue – Subject 105 Graph

**106 Fatigue**

\[ y = -0.0286x + 1.3941 \]

\[ R^2 = 0.1107 \]

Figure 67. Fatigue – Subject 106 Graph
Figure 68. Fatigue – Subject 107 Graph

Figure 69. Fatigue – Subject 108 Graph

Figure 70. Fatigue – Subject 109 Graph
110 Fatigue

\[ y = -0.0916x + 2.9261 \]
\[ R^2 = 0.3205 \]

Figure 71. Fatigue – Subject 110 Graph

111 Fatigue

\[ y = -0.0581x + 1.4926 \]
\[ R^2 = 0.3643 \]

Figure 72. Fatigue – Subject 111 Graph

112 Fatigue

\[ y = -0.0468x + 1.1158 \]
\[ R^2 = 0.261 \]

Figure 73. Fatigue – Subject 112 Graph
FIGURES 74-84. Sexual Dysfunction – Individual Subject Graphs
Figure 74. Sexual Dysfunction – Subject 102 Graph

Figure 75. Sexual Dysfunction – Subject 103 Graph

Figure 76. Sexual Dysfunction – Subject 104 Graph
Figure 77. Sexual Dysfunction – Subject 105 Graph

Figure 78. Sexual Dysfunction – Subject 106 Graph

Figure 79. Sexual Dysfunction – Subject 107 Graph
Figure 80. Sexual Dysfunction – Subject 108 Graph

Figure 81. Sexual Dysfunction – Subject 109 Graph

Figure 82. Sexual Dysfunction – Subject 110 Graph
Figure 83. Sexual Dysfunction – Subject 111 Graph

Figure 84. Sexual Dysfunction – Subject 112 Graph

FIGURES 85-92. Bladder Dysfunction – Individual Subject Graphs
101 Bladder Dysfunction

\[ y = -0.0118x + 1.2808 \]
\[ R^2 = 0.0424 \]

Figure 85. Bladder Dysfunction – Subject 101 Graph

103 Bladder Dysfunction

\[ y = -0.0291x + 1.2635 \]
\[ R^2 = 0.4144 \]

Figure 86. Bladder Dysfunction – Subject 103 Graph

105 Bladder Dysfunction

\[ y = -0.0493x + 2.0837 \]
\[ R^2 = 0.2976 \]

Figure 87. Bladder Dysfunction – Subject 105 Graph
Figure 88. Bladder Dysfunction – Subject 106 Graph

Figure 89. Bladder Dysfunction – Subject 107 Graph

Figure 90. Bladder Dysfunction – Subject 108 Graph
Figure 91. Bladder Dysfunction – Subject 109 Graph

Figure 92. Bladder Dysfunction – Subject 110 Graph

FIGURES 93-101. Vaginal Dryness – Individual Subject Graphs
Figure 93. Vaginal Dryness – Subject 103 Graph

Figure 94. Vaginal Dryness – Subject 104 Graph

Figure 95. Vaginal Dryness – Subject 105 Graph
y = -0.0266x + 2.6059
$R^2 = 0.1126$

Figure 96. Vaginal Dryness – Subject 106 Graph

y = -0.0069x + 1.1379
$R^2 = 0.1$

Figure 97. Vaginal Dryness – Subject 107 Graph

y = -0.036x + 1.7808
$R^2 = 0.3591$

Figure 98. Vaginal Dryness – Subject 108 Graph
109 Vaginal Dryness

\[ y = -0.0414x + 0.8621 \]

\[ R^2 = 0.3073 \]

Figure 99. Vaginal Dryness – Subject 109 Graph

111 Vaginal Dryness

\[ y = -0.0517x + 1.1897 \]

\[ R^2 = 0.4167 \]

Figure 100. Vaginal Dryness – Subject 111 Graph

112 Vaginal Dryness

\[ y = -0.0438x + 1.83 \]

\[ R^2 = 0.3215 \]

Figure 101. Vaginal Dryness – Subject 112 Graph
FIGURES 102-112. Pain – Individual Subject Graphs (Joint or Muscle Pain)
Figure 102. Pain (Joint or Muscle) – Subject 101 Graph

Figure 103. Pain (Joint or Muscle) – Subject 102 Graph

Figure 104. Pain (Joint or Muscle) – Subject 103 Graph
Figure 105. Pain (Joint or Muscle) – Subject 104 Graph

Figure 106. Pain (Joint or Muscle) – Subject 105 Graph

Figure 107. Pain (Joint or Muscle) – Subject 106 Graph
Figure 108. Pain (Joint or Muscle) – Subject 107 Graph

Figure 109. Pain (Joint or Muscle) – Subject 108 Graph

Figure 110. Pain (Joint or Muscle) – Subject 109 Graph
Figure 111. Pain (Joint or Muscle) – Subject 110 Graph

Figure 112. Pain (Joint or Muscle) – Subject 111 Graph

FIGURES 113-124. Symptom Distress – Individual Subject Graphs
Figure 113. Symptom Distress – Subject 101 Graph

Figure 114. Symptom Distress – Subject 102 Graph

Figure 115. Symptom Distress – Subject 103 Graph
Figure 119. Symptom Distress – Subject 107 Graph

Figure 120. Symptom Distress – Subject 108 Graph

Figure 121. Symptom Distress – Subject 109 Graph
Figure 122. Symptom Distress – Subject 110 Graph

Figure 123. Symptom Distress – Subject 111 Graph

Figure 124. Symptom Distress – Subject 112 Graph
Descriptions of Menopausal Symptom Data

The first symptom examined was hot flashes. One hundred percent of the subjects reported this symptom, 100% of the slopes were negative, suggesting symptom improvement. 50% of the cases had an R² greater than or equal to .30, and 50% of the cases had an R² less than .30. As a group, the mean slope value= -.05 with a 95% confidence interval (CI) = -.07 - -.29, SD= .03. On the raw scores, the Wilcoxon Signed-Rank Test= -2.96, p=.003. 2-tailed Sign Test, p=.001.

The second symptom was heart palpitations. Eight subjects reported the symptom, six slopes were negative, one slope was positive, and one slope was flat. Of the negative slopes, four R² values were greater than or equal to .30, and two R² values were less than .30. The positive and flat slopes had R² values less than or equal to .13 suggesting very poor model fits. This suggests that of those with symptoms, 50% of the subjects may have experienced improvement, while 25% did not, and 25% may have had no improvement or been slightly worse. Mean slope value= -.02, 95% CI= -.04 - -.02, SD= .03. On the raw scores, the Wilcoxon Signed-Rank Test= -2.27, p=.023. 2-tailed Sign Test, p=.031.

The third symptom was sleep disorder. Eleven subjects reported the symptom, ten slopes were negative, one slope was positive. The R² for the positive slope was .03, a very poor model fit. Of the negative slopes, five R² values were greater than or equal to .30, and five R² values were less than .30. This suggests that of those who were symptomatic, 45% may have had improved sleep, while 45% did not, and 9% were worse, albeit with a very poor model fit. Mean slope value= -.04, 95% CI= -.06 - -.02,
SD=.03. On the raw scores, the Wilcoxon Signed-Rank Test= -3.12, \( p=.002 \). 2-tailed Sign Test, \( p=.000 \).

The fourth symptom was depressed mood. Eleven women reported the symptom, and of these, all slopes were negative. Five \( R^2 \) values were greater than or equal to \(.30\), and six \( R^2 \) values were less than \(.30\). This suggests that of those symptomatic, 45% may have had improved mood, while 55% percent did not. Mean slope value= -.04, 95% CI= -.06-.03, SD=.03. On the raw scores, the Wilcoxon Signed-Rank Test= -3.11, \( p=.002 \). 2-tailed Sign Test, \( p=.000 \).

The fifth symptom was irritability. One hundred percent of the subjects reported this symptom. Eleven slopes were negative, and one was positive. The positive slope had an \( R^2 \) value of \(.07\). Of the negative slopes, five \( R^2 \) values were greater than or equal to \(.30\), while seven \( R^2 \) values were less than \(.30\). These results suggest that 42% may have had improvement while 58% did not, and 9% may have deteriorated, but a poor model fit does not strongly support this. Mean slope value= -.04, 95% CI= -.06-.02, SD=.03. On the raw scores, the Wilcoxon Signed-Rank Test= -2.97, \( p=.003 \). 2-tailed Sign Test, \( p=.001 \).

The sixth symptom was anxiety. Ten subjects reported this symptom, nine slopes were negative, and one slope was flat. Of the negative slopes, four \( R^2 \) values were greater than or equal to \(.30\), and five \( R^2 \) values were less than \(.30\). This suggests that, of those who were symptomatic, 40% may have experienced improvement, while 50% did not, and 10% had no change. Mean slope value= -.03, 95% CI= -.05-.01, SD=.03. On the raw scores, the Wilcoxon Signed-Rank Test= -3.00, \( p=.003 \). 2-tailed Sign Test, \( p=.001 \).
The seventh symptom was fatigue. Eleven subjects reported the symptom, and all slopes were negative. Of the negative slopes, five $R^2$ values were greater than or equal to .30, while six $R^2$ values were less than .30. This suggests that 45% of the subjects may have had improvement in fatigue, while 55% did not. Mean slope value= -.04, 95% CI= -.06- -.03, SD= .03. On the raw scores, the Wilcoxon Signed-Rank Test= -3.13, $p=.002$. 2-tailed Sign Test, $p=.000$.

The eighth symptom was sexual dysfunction that included decreased libido. Eleven subjects reported this symptom, and nine slopes were negative, two were positive. Of the negative slopes, five $R^2$ values were greater than or equal to .30, and four $R^2$ values were less than .30. $R^2$ values for the positive slopes were both less than or equal to .10. These results suggest that 45% of those who were symptomatic may have experienced improvement, while 36% did not seem to improve, and eighteen percent, albeit with a very poor model fit, had more symptoms. Mean slope value= -.03, 95% CI= -.05- -.01, SD= .03. On the raw scores, the Wilcoxon Signed-Rank Test= -2.75, $p=.006$. 2-tailed Sign Test, $p=.012$.

The ninth symptom was bladder dysfunction in the form of incontinence, urgency or increased frequency. Six subjects reported this symptom. Of these six, all the slopes were negative. Four $R^2$ values were greater than or equal to .30, and two $R^2$ values were less than .30. This suggests that of those who were symptomatic, 67% may have experienced relief, while 33% did not. Mean slope value = -.02, 95% CI= -.04- -.003, SD= .03. On the raw scores, the Wilcoxon Signed-Rank Test= -2.39, $p=.017$. 2-tailed Sign Test, $p=.016$. 
The tenth symptom was vaginal dryness. Ten subjects reported this symptom, and all slopes were negative. Five R² values were greater than or equal to .30, and five R² values were less than .30. This suggests that 50% of women may have experienced improvement, while 50% of women did not. Mean slope value= -.03, 95% CI= -.04- -.01, SD= .02. On the raw scores, the Wilcoxon Signed-Rank Test= -2.70, p=.007. 2-tailed Sign Test, p=.004.

The final symptom from the MRS was musculoskeletal pain. Ten subjects reported this symptom. Of these ten, nine had negative slopes and one had a positive slope. Of the nine negative slopes, Four R² values greater than or equal to .30. Five R² values were less than .30. The one positive slope had an R² value of .39, a good model fit, suggesting more pain in this subject. Of those subjects reporting less pain, 44% may have had less pain, while 56% percent may not have reached significance. Mean slope value= -.03, 95% CI= -.04- -.01, SD= .03. On the raw scores, the Wilcoxon Signed-Rank Test = -2.32, p = .02. 2-tailed Sign Test, p = .039.

Global distress was measured with a final 10-point scale asking the subject to rate their over all levels of symptom distress with ‘1’ being minimal or no distress at all, and ‘10’ being the worst distress imaginable. All twelve subjects recorded distress levels, and eleven slopes were negative. Of the negative slopes, four R² values greater than or equal to .30. Seven R² values less than .30. The one positive slope had very low R² value of .02, suggesting a very poor model fit. These results suggest that distress decreased in 33% of the subjects, while 58% percent did not have distress relief, and 8% experienced more distress, albeit with a very poor model fit. Mean slope value= -.08, 95% CI= -.11 - -.05,
SD= .05. On the raw scores, the Wilcoxon Signed Ranks Test= -2.81, \( p = .005 \); 2-tailed Sign Test, \( p = .002 \).

Using the linear regression model to assess significance is not standard statistical protocol, although the eye can see the downwards trend of the symptoms. Thus, the Wilcoxon Signed Ranks Test was implemented. By this statistic, all symptoms improved significantly. Finally, a Kolmogorov-Smirnov test suggests that despite the small sample size, all data were normally distributed except heart palpitations, sleep disorders and bladder dysfunction (see Figures 1-122 for individual graphs and slope details).

**Exploratory Aim 2, Effects of Acupuncture on Interleukin 6**

The changes in the levels of serum interleukin 6 are seen in table 5. Individual graphs are presented in figures 123-134 at the end of the section. In the first set of levels drawn, the first value represents the baseline levels of serum interleukin 6 and the second value represents post- acupuncture levels of serum interleukin 6. Normal serum interleukin 6 levels ranges from 0.5 picograms (pg) per milliliter (ml) to 14.6 pg/ml with a mean of 6.3 pg/ml (Robac, et al., 1999). However, as serum estrogens decline, serum IL6 (and other proinflammatory cytokines) tend to become slightly elevated, and so may be closer to 7.90 pg/ml (Racho, et al. 2005).

In this sample, the mean baseline for serum interleukin 6 was 7.4 pcg/ml with a 95% confidence interval of 4.1 pcg/ml-10.7 pcg/ml, and a standard deviation of 5.2 pcg/ml. There was one very significant outlier of 18.29 (subject 112). However, this did not significantly change the statistics, which without this outlier were: mean 6.4 pcg/ml with a 95% confidence interval of 3.66- 9.15 pcg/ml and a standard deviation of 4.1
Immediately after the acupuncture, values for the mean serum IL6 level were 7.9 pcg/ml with a 95% confidence level of 4.3 pcg/ml-11.47 pcg/ml, and a standard deviation of 5.6 pcg/ml. Again, after removing the significant outlier of 21.51 pcg/ml (subject 112), the mean changed to 6.67 pcg/ml with a 95% confidence level of 4.1 pcg/ml to 9.22 pcg/ml, and a standard deviation of 3.8 pcg/ml.

**TABLE 5. Changes in the Levels of Serum Interleukin 6**

<table>
<thead>
<tr>
<th>ID</th>
<th>Interleukin 6 Baseline</th>
<th>IL 6</th>
<th>Interleukin 6 Pre Acupuncture</th>
<th>IL 6 Pre-Treatment Minus Post Treatment</th>
<th>Interleukin 6 Post Treatment Before Acupuncture</th>
<th>IL 6 Post-Treatment Minus Pre Treatment</th>
<th>Interleukin 6 Post Treatment Minus Pre Treatment</th>
<th>Interleukin 6 Post Treatment Minus Pre Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>8.50</td>
<td>7.80</td>
<td>-.70</td>
<td>4.93</td>
<td>8.36</td>
<td>3.43</td>
<td>-2.87</td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>4.49</td>
<td>4.90</td>
<td>.41</td>
<td>5.05</td>
<td>6.20</td>
<td>1.15</td>
<td>.15</td>
<td></td>
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<tr>
<td>103</td>
<td>6.75</td>
<td>6.66</td>
<td>-.09</td>
<td>6.53</td>
<td>8.44</td>
<td>1.91</td>
<td>-.13</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>1.92</td>
<td>1.90</td>
<td>-.02</td>
<td>2.38</td>
<td>3.11</td>
<td>.73</td>
<td>.48</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>1.33</td>
<td>1.88</td>
<td>.55</td>
<td>1.35</td>
<td>1.43</td>
<td>.08</td>
<td>-.53</td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>6.96</td>
<td>5.92</td>
<td>-1.04</td>
<td>2.93</td>
<td>3.38</td>
<td>.45</td>
<td>2.99</td>
<td></td>
</tr>
<tr>
<td>107</td>
<td>4.69</td>
<td>11.09</td>
<td>6.40</td>
<td>1.54</td>
<td>2.17</td>
<td>.63</td>
<td>-9.55</td>
<td></td>
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<td>108</td>
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<td>9.00</td>
<td>1.68</td>
<td>13.50</td>
<td>11.45</td>
<td>-2.05</td>
<td>4.50</td>
<td></td>
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<td>2.13</td>
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<td>2.55</td>
<td>.23</td>
<td>.19</td>
<td></td>
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<tr>
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<td>8.90</td>
<td>-.46</td>
<td>5.81</td>
<td>6.69</td>
<td>.88</td>
<td>-3.09</td>
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<tr>
<td>111</td>
<td>12.77</td>
<td>13.23</td>
<td>.46</td>
<td>10.82</td>
<td>8.94</td>
<td>-1.88</td>
<td>-2.41</td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>18.29</td>
<td>21.51</td>
<td>3.22</td>
<td>2.21</td>
<td>3.75</td>
<td>1.54</td>
<td>-19.30</td>
<td></td>
</tr>
</tbody>
</table>

In the first pre-acupuncture (baseline)/post acupuncture interleukin 6 levels, interleukin 6 did not change significantly from baseline, (M=7.91, SE=1.62) compared to after, (M=7.4, SE=1.50), t(11)=-.679, p=.255, r=.20). Removing the outlier did not change this lack of significance, baseline (M=6.67, SE=1.15) compared to after acupuncture (M=6.41, SE=1.23), t(11)=-.339, p=.371, r=.10).

A second paired t-test was performed on the change between levels after the first acupuncture treatment and before the tenth treatment. These results approached
significance. After the first acupuncture treatment, \( (M=7.91, SE=1.62) \) compared to before the tenth treatment \( (M=4.95, SE=1.10) \), \( t(11)=1.68, p=.061, r=.20 \).

A third paired t-test was performed on pre/post tenth treatment. Again, most likely due to the small sample size, a significant difference was not seen, pre-tenth treatment levels \( (M=4.95, SE=1.10) \) and post-tenth treatment levels, \( (M=5.54, SE=.936) \), \( t(11)=-1.37, p=0.09, r=.15 \). In summary, no significant changes were seen in the group; however, some individuals did show some striking changes between the first post-acupuncture level and before the tenth acupuncture treatment.

**TABLE 6. Summary of Changes in the Levels of Serum Interleukin 6**

<table>
<thead>
<tr>
<th>Serum Interleukin 6</th>
<th>Mean (picograms/ml)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>7.40</td>
<td>4.10-10.70</td>
</tr>
<tr>
<td>After First Acupuncture</td>
<td>4.95</td>
<td>2.52-7.38</td>
</tr>
<tr>
<td>*Pre-Post Treatment 1</td>
<td>0.51</td>
<td>-1.15-2.17</td>
</tr>
<tr>
<td>Pre 10(^{th}) Treatment</td>
<td>7.91</td>
<td>4.35-11.47</td>
</tr>
<tr>
<td>Post 10(^{th}) Treatment</td>
<td>5.54</td>
<td>3.48-7.60</td>
</tr>
<tr>
<td>Pre-Post 10(^{th}) Difference</td>
<td>0.59</td>
<td>-0.36-1.54</td>
</tr>
</tbody>
</table>

* Most frequent and significant correlations with absolute values of HRV components

**FIGURES 125-136. IL 6 (Interleukin 6 Slopes) Pre/Post Treatment – Individual Subject Graphs**
Figure 125. IL 6 Pre/Post Treatments 1 & 10 – Subject 101 Graph

Figure 126. IL 6 Pre/Post Treatments 1 & 10 – Subject 102 Graph

Figure 127. IL 6 Pre/Post Treatments 1 & 10 – Subject 103 Graph
Figure 128. IL 6 Pre/Post Treatments 1 & 10 – Subject 104 Graph

Figure 129. IL 6 Pre/Post Treatments 1 & 10 – Subject 105 Graph

Figure 130. IL 6 Pre/Post Treatments 1 & 10 – Subject 106 Graph
Figure 131. IL 6 Pre/Post Treatments 1 & 10 – Subject 107 Graph

Figure 132. IL 6 Pre/Post Treatments 1 & 10 – Subject 108 Graph

Figure 133. IL 6 Pre/Post Treatments 1 & 10 – Subject 109 Graph
Figure 134. IL 6 Pre/Post Treatments 1 & 10 – Subject 110 Graph

Figure 135. IL 6 Pre/Post Treatments 1 & 10 – Subject 111 Graph

Figure 136. IL 6 Pre/Post Treatments 1 & 10 – Subject 112 Graph
Exploratory Aim 3, Effects of Acupuncture on Heart Rate Variability

TABLE 7. The Low Frequency to High Frequency Ratio

<table>
<thead>
<tr>
<th>ID</th>
<th>Low Frequency to High Frequency Ratio Resting Phase</th>
<th>Low Frequency to High Frequency Ratio First 5 Minutes</th>
<th>Low Frequency to High Frequency Ratio Final 5 Minutes</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Slope</td>
<td>R²</td>
<td>Slope</td>
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<td>-.78</td>
</tr>
<tr>
<td>103</td>
<td>-.01</td>
<td>.00</td>
<td>-.02</td>
</tr>
<tr>
<td>104</td>
<td>.02</td>
<td>.00</td>
<td>.04</td>
</tr>
<tr>
<td>105</td>
<td>-.23</td>
<td>.08</td>
<td>-.28</td>
</tr>
<tr>
<td>106</td>
<td>.15</td>
<td>.10</td>
<td>.22</td>
</tr>
<tr>
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<td>.12</td>
<td>.01</td>
<td>-.47</td>
</tr>
<tr>
<td>108</td>
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<td>-.12</td>
</tr>
<tr>
<td>109</td>
<td>.02</td>
<td>.02</td>
<td>-.01</td>
</tr>
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<td>110</td>
<td>.06</td>
<td>.17</td>
<td>.04</td>
</tr>
<tr>
<td>111</td>
<td>.18</td>
<td>.03</td>
<td>.01</td>
</tr>
<tr>
<td>112</td>
<td>-.02</td>
<td>.00</td>
<td>-.13</td>
</tr>
</tbody>
</table>

The hypothesized direction of change for each phase was as follows: the ratio was to decrease, seen as a negative slope, which would represent either less sympathetic cardiac modulation or greater parasympathetic cardiac modulation. The low frequency, measured both as absolute power and in normalized units, was to decrease, again seen as a negative slope, and by decreasing, would represent less sympathetic cardiac modulation. Finally, the high frequency, measured both as absolute power and normalized units, was to increase, seen as a positive slope, and by increasing, would represent increased parasympathetic cardiac modulation. Visual inspection of each of these phases of heart rate variability data on line graphs revealed large amounts of variability, which was reflected in very low R² values. Group means with 95% confidence intervals and standard deviations are presented, along with results of the
Kolmogorov-Smirnov test for normal distribution. The high degree of variability may be seen in figures 135-146 at the end of the section.

In the first set of ratios representing the resting phase, only three of the subjects, or 25%, had a decreased slope. Of these, the highest $R^2$ value was only .08, indicating a very poor model fit. The highest $R^2$ was .17, scarcely a better model fit, but interestingly, it was for an increased ratio, which the remainder of the sample experienced. Eleven of the twelve $R^2$ values were less than or equal to .10, confirming visual inspection, which revealed large amounts of variability, and no clear patterns. The data were normally distributed, and the group mean slope was 0.9, 95% CI = .03-.05, SD = .13.

In the second set of ratios, representing acupuncture for five minutes, seven subjects had negative slopes, representing lower ratios, and of these, only one had an $R^2$ .32, and the next closest $R^2$ value was .25, however, six $R^2$ values were less than or equal to .12, indicating a very poor model fit. The one flat slope had an $R^2$ of zero, and the remaining four positive slopes had $R^2$ values less than or equal to .10. Again, these results illustrate large amounts of variability without identifiable patterns. The data were not normally distributed, and the group mean slope was 0.13, 95% CI = .30-.05, SD = .27.

The third set of ratios represents subjects who have received twenty minutes of acupuncture. Of these, four subjects had decreased ratios, with two $R^2$ values of .36 and .26. The remaining two decreased ratios had $R^2$ values less than or equal to .15. Eight subjects demonstrated positive slopes, or increased ratios, with the largest $R^2$ value at .35, a fair model fit, and the remaining seven $r$-squared values, suggesting poor model fits at less than or equal to .16. These values illustrate the large amount of inter and intra-
individual variability with lack of trends. The data were not normally distributed, and the group mean slope was 0.0024, 95% CI = -.30-.31, SD = .48.

FIGURES 137-148. AR Model Low (LF) to High Frequency (HF) Ratio – Individual Subject Graphs.
Figure 137. AR Model LF:HF Ratio – Subject 101 Graph

Figure 138. AR Model LF:HF Ratio – Subject 102 Graph

Figure 139. AR Model LF:HF Ratio – Subject 103 Graph
Figure 140. AR Model LF:HF Ratio – Subject 104 Graph

Figure 141. AR Model LF:HF Ratio – Subject 105 Graph

Figure 142. AR Model LF:HF Ratio – Subject 106 Graph
Figure 143. AR Model LF:HF Ratio – Subject 107 Graph

Figure 144. AR Model LF:HF Ratio – Subject 108 Graph

Figure 145. AR Model LF:HF Ratio – Subject 109 Graph
Figure 146. AR Model LF:HF Ratio – Subject 110 Graph

Figure 147. AR Model LF:HF Ratio – Subject 111 Graph

Figure 148. AR Model LF:HF Ratio – Subject 112 Graph
The second heart rate variability component was low frequency, measured in milliseconds squared, also referred to as ‘absolute power.’ It differs from normalized units in that this measure includes very low frequency in the calculation (Malik 1996). Physiologically, the low frequency component of heart rate variability represents both sympathetic and parasympathetic cardiac modulation, unlike the high frequency, which reflects predominantly parasympathetic modulation. In spite of this, low frequency is generally interpreted as a reflection of sympathetic cardiac modulation. Lower values for this component represent less sympathetic nervous system cardiac modulation (Stein 1993).

In the first phase, the resting state, 50% of the subjects demonstrated decreased low frequency heart rate variability. Of these negative slopes, the two highest $R^2$ values were .21 and .18, neither of which suggest a good fit for the models. The remaining $R^2$ values...
values for the negative slopes were less than .10. The other 50% of the sample experienced an increased slope in the low frequency component of heart rate variability, suggesting increased sympathetic cardiac modulation. Of these positive slopes, three $R^2$ values were .48, .28 and .18, with .48 suggesting a good model fit, and the remainder poor model fits. The remaining $R^2$ values are less than .10. In essence, only one $R^2$ value suggested a significant model fit. The data was not normally distributed, and the group’s mean slope was 9.45, 95% CI = -7.69- 26.59, SD = 27.

In the second phase, acupuncture for five minutes, seven subjects had negative slopes, with $R^2$ values less than or equal to .14. The remaining five subjects demonstrated positive slopes, with two $R^2$ values of .43 and .26, and the remaining values less than .10. Again, these results suggest high levels of group and individual variability. The data were normally distributed, and the group’s mean slope was -.56, 95% CI =-17.9- 16.8, SD = 27.28.

In the final phase, the slopes representing absolute power of the low frequency component of heart rate variability are negative four times and positive eight times. All $R^2$ values were less than or equal to .14, except for two of the positive slopes which had $R^2$ values of .60 and .34, indicating good to fair model fits. The data were not normally distributed, and the group’s mean slope was -1.73, 95% CI = -19.00-15.52, SD = 27.15.

The high degree of variability may be seen in the individual graphs, figures 149-159 below.

FIGURES 149-159. AR Model Low Frequency – Individual Subject Graphs.
Figure 149. AR Model Low Frequency – Subject 101 Graph

Figure 150. AR Model Low Frequency – Subject 102 Graph

Figure 151. AR Model Low Frequency – Subject 103 Graph
Figure 152. AR Model Low Frequency – Subject 104 Graph

Figure 153. AR Model Low Frequency – Subject 105 Graph

Figure 154. AR Model Low Frequency – Subject 106 Graph
Figure 155. AR Model Low Frequency – Subject 107 Graph

Figure 156. AR Model Low Frequency – Subject 108 Graph

Figure 157. AR Model Low Frequency – Subject 109 Graph
Figure 158. AR Model Low Frequency – Subject 110 Graph

Figure 159. AR Model Low Frequency – Subject 111 Graph
TABLE 9. High Frequency Absolute Power

<table>
<thead>
<tr>
<th>ID</th>
<th>High Frequency Milliseconds Squared Resting Phase</th>
<th>High Frequency Milliseconds Squared First 5 Minutes</th>
<th>High Frequency Milliseconds Squared Final 5 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope R²</td>
<td>Slope R²</td>
<td>Slope R²</td>
</tr>
<tr>
<td>101</td>
<td>-15.87 .15</td>
<td>6.98 .19</td>
<td>1.16 .00</td>
</tr>
<tr>
<td>102</td>
<td>2.39 .01</td>
<td>10.59 .10</td>
<td>-8.23 .07</td>
</tr>
<tr>
<td>103</td>
<td>-.51 .00</td>
<td>-9.00 .30</td>
<td>-11.83 .06</td>
</tr>
<tr>
<td>104</td>
<td>1.99 .04</td>
<td>4.49 .15</td>
<td>-.96 .01</td>
</tr>
<tr>
<td>105</td>
<td>4.48 .19</td>
<td>2.44 .14</td>
<td>5.49 .21</td>
</tr>
<tr>
<td>106</td>
<td>.91 .05</td>
<td>1.68 .08</td>
<td>.61 .02</td>
</tr>
<tr>
<td>107</td>
<td>-.12 .00</td>
<td>-3.61 .12</td>
<td>4.72 .17</td>
</tr>
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<td>108</td>
<td>-10.54 .17</td>
<td>.16 .00</td>
<td>-2.08 .02</td>
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<tr>
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<td>-38.98 .18</td>
<td>-12.36 .06</td>
<td>5.89 .00</td>
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<td>-5.93 .10</td>
<td>-.12 .00</td>
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<tr>
<td>112</td>
<td>53.60 .30</td>
<td>22.75 .21</td>
<td>-7.23 .03</td>
</tr>
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</table>

The high frequency component of heart rate variability represents parasympathetic modulation of the heart, with more heartbeat-to-heartbeat variability. In most health conditions, increased variability or higher frequency suggests higher levels of cardiac health (Malik 1996).

In the resting phase, 50% of the sample experienced an increase in the high frequency, while 50% had a decrease. Only one of the six positive slope R² values suggests a fair model fit at .30. The remainder of the R² values were less than or equal to .19. As mentioned before, the data suggest a high rate of inter and intra-individual variability. The data were not normally distributed, and the group’s mean slope was 0.36, 95% CI = -13.17- 13.88, SD = 21.28.

In the second phase, after five minutes of acupuncture, seven subjects had positive slopes, but only one R² value approached a fair model fit at .21. Of the remaining five
subjects who experienced negative slopes, the $R^2$ values were less than or equal to .12, with one $R^2$ value of .30 suggesting a fair model fit. As stated previously, these results suggest that there is substantial model variability with few convincing fits. The data were normally distributed, the mean slope was 0.45, 95% CI = -6.46- 7.35, SD = 10.87.

The third phase, the last five minutes of acupuncture (results after a twenty-minute session) shows five subjects with positive slopes, with the highest $R^2$ value at .21. The remaining seven subjects had negative slopes with $R^2$ values less than or equal to .17. Again, this data reflects the high rate of variability in the data with no clear trends. The data were not normally distributed, and the group’s mean slope was -2.94, 95% CI = -8.23- 2.36, SD = 8.33.

The high degrees of variability may be seen in the individual graphs, figures 160-171, below.

Figure 160. AR Model High Frequency Absolute Power – Subject 101 Graph

Figure 161. AR Model High Frequency Absolute Power – Subject 102 Graph

Figure 162. AR Model High Frequency Absolute Power – Subject 103 Graph
Figure 163. AR Model High Frequency Absolute Power – Subject 104 Graph

Figure 164. AR Model High Frequency Absolute Power – Subject 105 Graph

Figure 165. AR Model High Frequency Absolute Power – Subject 106 Graph
Figure 166. AR Model High Frequency Absolute Power – Subject 107 Graph

Figure 167. AR Model High Frequency Absolute Power – Subject 108 Graph

Figure 168. AR Model High Frequency Absolute Power – Subject 109 Graph
Figure 169. AR Model High Frequency Absolute Power – Subject 110 Graph

Figure 170. AR Model High Frequency Absolute Power – Subject 111 Graph

Figure 171. AR Model High Frequency Absolute Power – Subject 112 Graph
TABLE 10. Low Frequency Normalized Units

<table>
<thead>
<tr>
<th>ID</th>
<th>Resting Phase</th>
<th>First 5 Minutes</th>
<th>Final 5 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>$R^2$</td>
<td>Slope</td>
</tr>
<tr>
<td>101</td>
<td>-2.65</td>
<td>.14</td>
<td>-.93</td>
</tr>
<tr>
<td>102</td>
<td>-.59</td>
<td>.01</td>
<td>1.47</td>
</tr>
<tr>
<td>103</td>
<td>2.29</td>
<td>.12</td>
<td>.42</td>
</tr>
<tr>
<td>104</td>
<td>3.45</td>
<td>.65</td>
<td>.82</td>
</tr>
<tr>
<td>105</td>
<td>-2.75</td>
<td>.01</td>
<td>-3.67</td>
</tr>
<tr>
<td>106</td>
<td>3.95</td>
<td>.28</td>
<td>3.86</td>
</tr>
<tr>
<td>107</td>
<td>-2.05</td>
<td>.04</td>
<td>-3.16</td>
</tr>
<tr>
<td>108</td>
<td>2.81</td>
<td>.06</td>
<td>-1.36</td>
</tr>
<tr>
<td>109</td>
<td>.29</td>
<td>.01</td>
<td>.02</td>
</tr>
<tr>
<td>110</td>
<td>1.42</td>
<td>.08</td>
<td>1.71</td>
</tr>
<tr>
<td>111</td>
<td>-.97</td>
<td>.03</td>
<td>-.50</td>
</tr>
<tr>
<td>112</td>
<td>-5.06</td>
<td>.27</td>
<td>.88</td>
</tr>
</tbody>
</table>

This next measure of heart rate variability, examines low frequency in normalized units, which differs from the absolute power low frequency by subtracting out the very low frequency component. To date, physiologists are still unsure what ultra low and very low frequencies of heart rate variability represent (Malik 1996; Julien 2006). In the resting phase, 50% of the sample experienced positive slopes and 50% had negative slopes. In two of the subjects with positive slopes, one $R^2$ suggests a good model fit at .65, the next closest $R^2$ is .28. Of the negative slopes, only one $R^2$ approaches a fair model fit at .27. The remainder of $R^2$ values are less than or equal to .14, suggesting high rates of variability with few trends. The data were normally distributed, and the group’s mean slope was 0.11, 95% CI = -1.79- 1.81, SD = 2.84.

In the second phase, after five minutes of acupuncture, seven subjects experienced positive slopes, while five subjects had negative slopes. The highest $R^2$ value was .29 in a
negative slope. The remainder of the $R^2$ values are less than or equal to .18. This further supports evidence of high levels of variation and lack of trends. The data were normally distributed, and the group’s mean slope was -0.037, 95% CI = -1.36- 1.29, SD = 2.09.

The final phase, the final five minutes of acupuncture, four subjects demonstrated negative slopes, while eight had positive slopes. Two $R^2$ values were suggestive of a good model fit occurred with positive slopes at .32 and .53. The remainder of the $R^2$ values were less than or equal to .17. These results continue to suggest high rates of individual and group variability. The data were normally distributed, and the group’s mean slope was 1.22, 95% CI = -.56- 2.99, SD = 2.8.

The high degree of variability may be seen in the individual graphs, figures 172-183 below.

FIGURES 172-183. Low Frequency Normalized Units – Individual Subject Graphs.
Figure 172. Low Frequency Normalized Units – Subject 101 Graph

Figure 173. Low Frequency Normalized Units – Subject 102 Graph

Figure 174. Low Frequency Normalized Units – Subject 103 Graph
Figure 175. Low Frequency Normalized Units – Subject 104 Graph

Figure 176. Low Frequency Normalized Units – Subject 105 Graph

Figure 177. Low Frequency Normalized Units – Subject 106 Graph
Figure 178. Low Frequency Normalized Units – Subject 107 Graph

Figure 179. Low Frequency Normalized Units – Subject 108 Graph

Figure 180. Low Frequency Normalized Units – Subject 109 Graph
Figure 181. Low Frequency Normalized Units – Subject 110 Graph

Figure 182. Low Frequency Normalized Units – Subject 111 Graph

Figure 183. Low Frequency Normalized Units – Subject 112 Graph
In the first phase of the final component to be analyzed, the high frequency component of heart rate variability measured in normalized units, 50% of the sample exhibited positive slopes, the hypothesized direction, while 50% of the sample had negative slopes. Among the positive slopes, two suggest good model fits with \( R^2 \) values of .32 and .46. Among the negative slopes, the highest \( R^2 \) values were .23, .24 and .25. The remainder of the \( R^2 \) values were less than or equal to .17. The data suggest high levels of inter and intra-individual variability. The data were normally distributed, and the group’s mean slope was 1.22, 95% CI = -.56- 2.99, SD = 2.80.

In the second phase, after five minute of acupuncture, eight subjects demonstrated positive slopes, and four had negative slopes. Among those with positive slopes, two had \( R^2 \) values suggesting fair model fits at .39 and .28. The remaining \( R^2 \) values are less than or equal to .18. Again, these data suggest high levels of variability. The data were not
normally distributed, and the group’s mean slope was 0.17, 95% CI = -.84- 1.18, SD = 1.59.

In the third phase, the last five minutes of acupuncture, four subjects demonstrated positive slopes, while eight subjects had negative slopes. One R² value was seen among subjects with positive slopes that approached a fair model fit at .28. Those with negative slopes had R² values at .26, .28 and .33, with the last suggesting a fair model fit. The remaining eight R² values were less than or equal to .15. As with the above data, these values suggest high levels of variability. The data were normally distributed, and the group’s mean slope was -.54, 95% CI = -1.76- .68, SD = 1.9.

The high degree of variability may be seen in the individual graphs, figures 184-195 below.

FIGURES 184-195. High Frequency Normalized Units – Individual Subject Graphs
Figure 184. AR Model High Frequency Normalized Units – Subject 101 Graph

Figure 185. AR Model High Frequency Normalized Units – Subject 102 Graph

Figure 186. AR Model High Frequency Normalized Units – Subject 103 Graph
Figure 187. AR Model High Frequency Normalized Units – Subject 104 Graph

Figure 188. AR Model High Frequency Normalized Units – Subject 105 Graph

Figure 189. AR Model High Frequency Normalized Units – Subject 106 Graph
Figure 190. AR Model High Frequency Normalized Units – Subject 107 Graph

Figure 191. AR Model High Frequency Normalized Units – Subject 108 Graph

Figure 192. AR Model High Frequency Normalized Units – Subject 109 Graph
Figure 193. AR Model High Frequency Normalized Units – Subject 110 Graph

Figure 194. AR Model High Frequency Normalized Units – Subject 111 Graph

Figure 195. AR Model High Frequency Normalized Units – Subject 112 Graph
TABLE 12. Heart Rate Variability Summary of the Mean Slopes

<table>
<thead>
<tr>
<th>Phase of Acupuncture and Heart Rate Variability Component</th>
<th>Mean Slope</th>
<th>95% Confidence Interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting Phase LF:HF Ratio</td>
<td>0.9</td>
<td>0.03-0.05</td>
</tr>
<tr>
<td>First 5 Minutes of Acupuncture LF:HF Ratio</td>
<td>0.13</td>
<td>0.30-0.05</td>
</tr>
<tr>
<td>Final 5 Minutes of acupuncture LF:HF Ratio</td>
<td>0.0024</td>
<td>-0.30-0.31</td>
</tr>
<tr>
<td>Resting Phase Low frequency ms²</td>
<td>9.45</td>
<td>-7.69-26.59</td>
</tr>
<tr>
<td>First 5 Minutes Acupuncture Low frequency ms²</td>
<td>-0.56</td>
<td>-17.9-16.8</td>
</tr>
<tr>
<td>Final 5 Minutes of acupuncture Low frequency ms²</td>
<td>-1.73</td>
<td>-19.0-15.52</td>
</tr>
<tr>
<td>Resting Phase High frequency ms²</td>
<td>0.36</td>
<td>-13.17-13.88</td>
</tr>
<tr>
<td>First 5 Minutes Acupuncture High frequency ms²</td>
<td>0.45</td>
<td>-6.46-7.35</td>
</tr>
<tr>
<td>Final 5 Minutes of acupuncture High frequency ms²</td>
<td>-2.94</td>
<td>-8.23-2.36</td>
</tr>
<tr>
<td>Resting phase Low frequency, normalized units</td>
<td>0.11</td>
<td>-1.79-1.81</td>
</tr>
<tr>
<td>First 5 minutes of acupuncture Low frequency, normalized units</td>
<td>-0.037</td>
<td>-1.36-1.29</td>
</tr>
<tr>
<td>Final 5 minutes of acupuncture Low frequency, normalized units</td>
<td>1.22</td>
<td>-0.56-2.99</td>
</tr>
<tr>
<td>Resting phase High frequency, normalized units</td>
<td>-0.29</td>
<td>-1.76-1.18</td>
</tr>
<tr>
<td>First 5 minutes of acupuncture High frequency, normalized units</td>
<td>0.17</td>
<td>-0.84-1.18</td>
</tr>
<tr>
<td>Final 5 minutes of acupuncture High frequency, normalized units</td>
<td>-0.54</td>
<td>-1.76-0.68</td>
</tr>
</tbody>
</table>

Heart Rate Variability Components Measured in Absolute Power Values

Absolute Power values in milliseconds squared or normalized units at treatments 1 and 10 are provided to facilitate examination of correlations between these absolute values and serum interleukin 6 values.
<table>
<thead>
<tr>
<th>HRV Component</th>
<th>Phase of treatment:</th>
<th>Mean Value</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF=Low Frequency</td>
<td>A=Resting</td>
<td>MS^2 or NU &amp; Temporal directionality</td>
<td></td>
</tr>
<tr>
<td>HF=High Frequency</td>
<td>B= 1st 5 Minutes</td>
<td>↑= increased</td>
<td></td>
</tr>
<tr>
<td>MS^2= Absolute Power</td>
<td>C= Final 5 Minutes</td>
<td>↓= decreased</td>
<td></td>
</tr>
<tr>
<td>NU=Normalized Units</td>
<td>1=First Treatment</td>
<td>10=Final Treatment</td>
<td></td>
</tr>
<tr>
<td>LF MS^2 1</td>
<td>A</td>
<td>356.2 baseline</td>
<td>121.9-590.5</td>
</tr>
<tr>
<td>LF MS^2 1</td>
<td>B</td>
<td>460.3 ↑</td>
<td>193.2-727.5</td>
</tr>
<tr>
<td>LF MS^2 1</td>
<td>C</td>
<td>514.8 ↑</td>
<td>213.6-816.1</td>
</tr>
<tr>
<td>HF MS^2 1</td>
<td>A</td>
<td>227.6 baseline</td>
<td>81.5-373.7</td>
</tr>
<tr>
<td>HF MS^2 1</td>
<td>B</td>
<td>203.7 ↓</td>
<td>36.0-371.4</td>
</tr>
<tr>
<td>HF MS^2 1</td>
<td>C</td>
<td>254.2 ↑</td>
<td>75.6-432.8</td>
</tr>
<tr>
<td>LF NU 1</td>
<td>A</td>
<td>47.5 baseline</td>
<td>25.4-69.6</td>
</tr>
<tr>
<td>LF NU 1</td>
<td>B</td>
<td>55.5 ↑</td>
<td>36.3-74.6</td>
</tr>
<tr>
<td>LF NU 1</td>
<td>C</td>
<td>54.5 ↓</td>
<td>36.1-72.9</td>
</tr>
<tr>
<td>HF NU 1</td>
<td>A</td>
<td>38.8 baseline</td>
<td>23.8-53.8</td>
</tr>
<tr>
<td>HF NU 1</td>
<td>B</td>
<td>28.9 ↓</td>
<td>9.9-47.8</td>
</tr>
<tr>
<td>HF NU 1</td>
<td>C</td>
<td>32.1 ↑</td>
<td>14.3-49.9</td>
</tr>
<tr>
<td>LF:HF 1</td>
<td>A</td>
<td>3.34 baseline</td>
<td>0.14-6.53</td>
</tr>
<tr>
<td>LF:HF 1</td>
<td>B</td>
<td>5.21 ↑</td>
<td>2.07-8.36</td>
</tr>
<tr>
<td>LF:HF 1</td>
<td>C</td>
<td>3.59 ↓</td>
<td>1.50-5.67</td>
</tr>
<tr>
<td>LF MS^2 10</td>
<td>A</td>
<td>286.9 baseline</td>
<td>134.1-439.7</td>
</tr>
<tr>
<td>LF MS^2 10</td>
<td>B</td>
<td>463.8 ↑</td>
<td>200.3-727.2</td>
</tr>
<tr>
<td>LF MS^2 10</td>
<td>C</td>
<td>389.8 ↓</td>
<td>217.8-561.9</td>
</tr>
<tr>
<td>HF MS^2 10</td>
<td>A</td>
<td>207.0 baseline</td>
<td>58.0-356.0</td>
</tr>
<tr>
<td>HF MS^2 10</td>
<td>B</td>
<td>209.5 ↑</td>
<td>93.8-325.2</td>
</tr>
<tr>
<td>HF MS^2 10</td>
<td>C</td>
<td>171.3 ↓</td>
<td>67.4-275.1</td>
</tr>
<tr>
<td>LF NU 10</td>
<td>A</td>
<td>48.5 baseline</td>
<td>32.8-64.2</td>
</tr>
<tr>
<td>LF NU 10</td>
<td>B</td>
<td>58.6 ↑</td>
<td>44.2-72.9</td>
</tr>
<tr>
<td>LF NU 10</td>
<td>C</td>
<td>58.6 same</td>
<td>47.0-70.1</td>
</tr>
<tr>
<td>HF NU 10</td>
<td>A</td>
<td>31.2 baseline</td>
<td>16.3-46.0</td>
</tr>
<tr>
<td>HF NU 10</td>
<td>B</td>
<td>27.9 ↓</td>
<td>15.1-40.7</td>
</tr>
<tr>
<td>HF NU 10</td>
<td>C</td>
<td>27.1 ↓</td>
<td>14.1-40.1</td>
</tr>
<tr>
<td>LF:HF 10</td>
<td>A</td>
<td>3.71 baseline</td>
<td>0.44-6.99</td>
</tr>
<tr>
<td>LF:HF 10</td>
<td>B</td>
<td>3.90 ↑</td>
<td>1.46-6.34</td>
</tr>
<tr>
<td>LF:HF 10</td>
<td>C</td>
<td>3.97 ↑</td>
<td>1.72-6.23</td>
</tr>
</tbody>
</table>
**Exploratory Aim 4, Relationships Between the Variables**

**Spearman’s Correlation Coefficients Between Absolute Values of Heart Rate Variability Components and Interleukin 6**

There were fourteen significant correlations between the values of interleukin 6 and the various phases and components of heart rate variability. Eleven of these correlations involved the interleukin 6 value calculated from the first pre-post difference. Table 14 presents the correlations.

<table>
<thead>
<tr>
<th>TABLE 14. Correlations Between Absolute Values of Heart Rate Variables &amp; IL6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HRV Segment and Component (Correlated variable)</strong></td>
</tr>
<tr>
<td>LF MS², last 5 minutes of acupuncture</td>
</tr>
<tr>
<td>HF MS², resting phase, before acupuncture</td>
</tr>
<tr>
<td>HF MS², last 5 minutes of acupuncture</td>
</tr>
<tr>
<td>LF NU first 5 minutes of acupuncture</td>
</tr>
<tr>
<td>LF NU final 5 minutes of acupuncture</td>
</tr>
<tr>
<td>LF NU first 5 minutes of acupuncture</td>
</tr>
<tr>
<td>HF NU first 5 minutes of acupuncture</td>
</tr>
</tbody>
</table>
**TABLE 14. Correlations Between Absolute Values of Heart Rate Variables & IL6 - Continued**

<table>
<thead>
<tr>
<th>HRV Segment and Component (Correlated variable)</th>
<th>Directional Change of HRV component</th>
<th>Measure of IL 6 (Correlated variable)</th>
<th>Treatment 1 or 10</th>
<th>Spearman’s coefficient</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF NU final 5 minutes of acupuncture</td>
<td>Increased</td>
<td>Pre – Post 1</td>
<td>1</td>
<td>-.872</td>
<td><em>p = .000</em></td>
</tr>
<tr>
<td>HF NU first 5 minutes of acupuncture</td>
<td>Decreased</td>
<td>Pre – Post 1</td>
<td>10</td>
<td>-.720</td>
<td><em>p = .008</em></td>
</tr>
<tr>
<td>HF NU final 5 minutes of acupuncture</td>
<td>Decreased</td>
<td>Pre – Post 1</td>
<td>10</td>
<td>-.594</td>
<td><em>p = .042</em></td>
</tr>
<tr>
<td>HF NU first 5 minutes of acupuncture</td>
<td>Decreased</td>
<td>Pre – Post 10</td>
<td>10</td>
<td>.706</td>
<td><em>p = .010</em></td>
</tr>
<tr>
<td>LF:HF Ratio first 5 minutes of acupuncture</td>
<td>Increased</td>
<td>Pre – Post 1</td>
<td>1</td>
<td>.844</td>
<td><em>p = .001</em></td>
</tr>
<tr>
<td>LF:HF Ratio final 5 minutes of acupuncture</td>
<td>Decreased</td>
<td>Pre – Post 1</td>
<td>1</td>
<td>.837</td>
<td><em>p = .001</em></td>
</tr>
<tr>
<td>LF:HF Ratio first 5 minutes of acupuncture</td>
<td>Increased</td>
<td>Pre – Post 1</td>
<td>10</td>
<td>.825</td>
<td><em>p = .001</em></td>
</tr>
<tr>
<td>LF:HF Ratio final 5 minutes of acupuncture</td>
<td>Increased</td>
<td>Pre – Post 1</td>
<td>10</td>
<td>.615</td>
<td><em>p = .033</em></td>
</tr>
</tbody>
</table>

*Exploratory Aim 4, Relationships Between the Temporal Slopes of Symptoms Interleukin 6 and Heart Rate Variability*

The final data analysis consisted of a Spearman’s correlation between the slopes (temporal change) for each outcome variable: between each phase of heart rate variability, symptom changes, and absolute values in terms of levels of and changes within serum interleukin 6. Table 15 presents a summary of the findings with a narrative afterwards.
TABLE 15. Summary of Correlations Between Temporal Change Measures

<table>
<thead>
<tr>
<th>Correlated Variable</th>
<th>Correlated Variable</th>
<th>Spearman’s coefficient</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot Flash Slope</td>
<td>Baseline IL 6</td>
<td>( r_s = .68 )</td>
<td>( p = .015 )</td>
</tr>
<tr>
<td>Hot Flash Slope</td>
<td>Post Treatment 1 IL 6</td>
<td>( r_s = .93 )</td>
<td>( p = .000 )</td>
</tr>
<tr>
<td>Depressed Mood Slope</td>
<td>Post Treatment 1 IL 6</td>
<td>( r_s = -.58 )</td>
<td>( p = .049 )</td>
</tr>
<tr>
<td>Depressed Mood Slope</td>
<td>Baseline minus Pre-10th treatment IL 6</td>
<td>( r_s = -.63 )</td>
<td>( p = .029 )</td>
</tr>
<tr>
<td>Resting HF MS(^2) Slope</td>
<td>Pre-tenth Treatment IL 6</td>
<td>( r_s = -.69 )</td>
<td>( p = .014 )</td>
</tr>
<tr>
<td>Resting HF MS(^2) Slope</td>
<td>Post 10(^{th}) Treatment IL 6</td>
<td>( r_s = -.66 )</td>
<td>( p = .020 )</td>
</tr>
<tr>
<td>First 5 Minutes LF:HF Ratio Slope</td>
<td>Pre minus Post treatment 1 IL 6</td>
<td>( r_s = -.73 )</td>
<td>( p = .008 )</td>
</tr>
<tr>
<td>Global Distress Slope</td>
<td>LF:HF Ratio Final 5 Minutes of Acupuncture</td>
<td>( r_s = -.64 )</td>
<td>( p = .024 )</td>
</tr>
<tr>
<td>Depressed Mood Slope</td>
<td>LF MS(^2) Slope, Final 5 Minutes of Acupuncture</td>
<td>( r_s = .79 )</td>
<td>( p = .002 )</td>
</tr>
<tr>
<td>Irritability Slope</td>
<td>LF MS(^2) Slope, Final 5 Minutes of Acupuncture</td>
<td>( r_s = .65 )</td>
<td>( p = .023 )</td>
</tr>
<tr>
<td>Depressed Mood Slope</td>
<td>HF MS(^2) Slope, First 5 Minutes of Acupuncture</td>
<td>( r_s = -.62 )</td>
<td>( p = .032 )</td>
</tr>
<tr>
<td>Irritability Slope</td>
<td>HF MS(^2) Slope, First 5 Minutes of Acupuncture</td>
<td>( r_s = -.59 )</td>
<td>( p = .042 )</td>
</tr>
<tr>
<td>Anxiety Slope</td>
<td>HF NU Slope, First 5 Minutes of Acupuncture</td>
<td>( r_s = -.63 )</td>
<td>( p = .028 )</td>
</tr>
<tr>
<td>Bladder Dysfunction Slope</td>
<td>HF MS(^2) Slope, First 5 Minutes of Acupuncture</td>
<td>( r_s = .59 )</td>
<td>( p = .045 )</td>
</tr>
<tr>
<td>Depressed Mood Slope</td>
<td>LF NU Slope, Final 5 Minutes of Acupuncture</td>
<td>( r_s = .71 )</td>
<td>( p = .009 )</td>
</tr>
<tr>
<td>Irritability Slope</td>
<td>LF NU, Final 5 Minutes of Acupuncture</td>
<td>( r_s = .64 )</td>
<td>( p = .025 )</td>
</tr>
<tr>
<td>Anxiety Slope</td>
<td>LF NU, Final 5 Minutes of Acupuncture</td>
<td>( r_s = .62 )</td>
<td>( p = .031 )</td>
</tr>
<tr>
<td>Depressed Mood Slope</td>
<td>HF NU, First 5 Minutes of Acupuncture</td>
<td>( r_s = -.86 )</td>
<td>( p = .000 )</td>
</tr>
<tr>
<td>Irritability Slope</td>
<td>HF NU, First 5 Minutes of Acupuncture</td>
<td>( r_s = -.83 )</td>
<td>( p = .001 )</td>
</tr>
<tr>
<td>Anxiety Slope</td>
<td>HF NU, Final 5 Minutes of Acupuncture</td>
<td>( r_s = -.64 )</td>
<td>( p = .024 )</td>
</tr>
</tbody>
</table>
Narrative Correlation Results

The first analysis looks at correlation between interleukin 6 and symptom changes.

There were four significant relationships between symptom change slopes and serum interleukin 6.

There was a positive relationship between hot flash change slopes and baseline serum levels of interleukin 6, \( r_s = .68, p = .015 \).

There was a positive relationship between hot flash change slopes and serum levels of interleukin 6 after the first acupuncture treatment, \( r_s = .93, p = .000 \).

There was a significant inverse relationship between mood change slopes and the change in the first post-treatment serum interleukin 6 levels, and pre-treatment tenth session, serum interleukin 6 levels, \( r_s = -.58, p = .049 \).

There was an inverse relationship between mood change slopes and the difference between the baseline serum levels of interleukin 6 and levels prior to the tenth acupuncture treatment, \( r_s = -.63, p = .029 \).

Correlations Between Interleukin 6 and Heart Rate Variability Slopes

There were five significant correlations between interleukin 6 and the various phases and frequencies of temporal change in heart rate variability.

There was a significant inverse relationship between interleukin 6 levels before the final treatment (pre-tenth) and the resting phase of high frequency slope, measured in absolute power, \( r_s = -.69, p = .014 \).
There was a significant inverse relationship between post-tenth treatment serum interleukin 6 levels and the resting phase of high frequency, measured in absolute power, $r_s = -.66, p = .020$.

There was a significant inverse relationship between the difference in baseline and post-treatment-one serum interleukin 6 levels and the low frequency to high frequency ratio slope in the first five minutes of acupuncture, $r_s = -.73, p = .008$.

**Correlations Between Symptom Changes and Heart Rate Variability**

There were fifteen significant relationships between symptom changes and the various frequencies and phases of heart rate variability.

There was a significant inverse relationship between global distress slope changes and the low to high frequency ratio slope in the final 5 minutes of acupuncture, $r_s = -.64, p = .024$.

There was a significant relationship between depressed mood slope changes and the low frequency slope, measured in absolute power, in the final five minutes of acupuncture, $r_s = .79, p = .002$.

There was a significant relationship between irritability slope changes and the low frequency slope, measured in absolute power, in the final five minutes of acupuncture, $r_s = .65, p = .023$.

There was a significant relationship between bladder dysfunction slope changes and the high frequency, measured in absolute power, in the first five minutes of acupuncture, $r_s = .59, p = .045$. 
There was a significant inverse relationship between depressed mood slope changes and the high frequency slope, measured in absolute power, in the first five minutes of acupuncture, $r_s = -.62, p = .032$.

There was a significant inverse relationship between irritability slope changes and the high frequency slope, measured in absolute power, in the first five minutes of acupuncture, $r_s = -.59, p = .042$.

There was a significant inverse relationship between anxiety slope changes and the high frequency slope, measured in normalized units, in the first five minutes of acupuncture, $r_s = -.63, p = .028$.

There was a significant relationship between bladder dysfunction slope changes and the high frequency slope, measured in absolute power, in the first five minutes of acupuncture, $r_s = .59, p = .045$.

There was a significant relationship between depressed mood slope changes and the low frequency slope, measured in normalized units in the last five minutes of acupuncture, $r_s = .71, p = .009$.

There was a significant relationship between irritability slope changes and the low frequency slope, measured in normalized units in the last five minutes of acupuncture, $r_s = .64, p = .025$.

There was a significant relationship between anxiety slope changes and the low frequency slope, measured in normalized units in the last five minutes of acupuncture, $r_s = .62, p = .031$. 
There was a significant inverse relationship between depressive mood disorder slope changes and the high frequency slope, measured in normalized units in the first five minutes of acupuncture, \( r_s = -0.86, p = 0.000 \).

There was a significant inverse relationship between irritability slope changes and the high frequency slope, measured in normalized units in the first five minutes of acupuncture, \( r_s = -0.83, p = 0.001 \).

There was a significant inverse relationship between anxiety slope changes and the high frequency slope, measured in normalized units in the first five minutes of acupuncture, \( r_s = -0.63, p = 0.028 \).

There was a significant inverse relationship between anxiety slope changes and the high frequency slope, measured in normalized units in the last five minutes of acupuncture, \( r_s = -0.64, p = 0.024 \).

Results of the Descriptive Qualitative Collection

From the transcripts and emic coding (Spradley 1979) two over-arching taxonomies were identified: Context, which refers to the life experiences that each subject brought into the study, and Outcomes, which were the perceived results of the acupuncture intervention. Within the Context taxonomy, stress was frequently mentioned. Within the Outcomes taxonomy, two domains were identified, Process Outcomes and Treatment Outcomes. Process Outcomes were further parsed out into Research Process Outcomes and Treatment Process Outcomes. The only clearly stated Research Outcome was one subject’s statement, “I did not like the phlebotomy process.” Treatment Outcome processes were further broken down into positive experiences and negative experiences.
with the acupuncture process itself. Treatment Outcomes were also divided into positive and negative treatment outcomes.

Summary

The specific aim one, feasibility, was established with all a priori criteria met. Menopausal symptoms improved with statistical significance.

Interleukin 6 declined in seven subjects between the first acupuncture treatment and the tenth, specifically between the post-first treatment levels and the pre-tenth levels, but did not reach statistical significance.

The heart rate variability showed no trends and demonstrated high levels of variability. Although the sample size was small, mean slope changes in the various phases of acupuncture treatment and the various components of heart rate variability, were calculated and found to be strikingly close to flat lines with only one exception, the resting phase of low frequency measured in milliseconds squared. The hypothesized direction of this slope was negative, while the actual direction was positive.

Spearman’s correlation revealed significant correlations between hot flash and depressed mood symptom slopes and levels of interleukin 6. In some correlations, contrary to prior reports, symptoms such as depressed mood and anxiety improved, yet interleukin 6 levels remained elevated or increased at the tenth treatment, or final acupuncture treatment. The phases and measures of heart rate variability also correlated with changes in interleukin 6, with precedent literature to support the findings (Carney 2007). Moreover, the symptoms that were seen to correlate most frequently with phases and measures of heart rate variability included anxiety (4 correlations), depressed mood
(6 correlations) and irritability (4 correlations). The most frequently correlated heart rate variability measure were the combined measures of high frequency, with eleven correlations.

Finally, responses to the final qualitative question that explored other aspects of women’s experience with acupuncture could be categorized into two major taxonomies, outcomes and contextual experiences of the subjects’ lives during the study. Two taxonomies were identified, Context and Outcomes. Under the taxonomy of outcomes, two major domains were identified, Process and Treatment Outcomes, among which perceptions of positive and negative outcomes were identified.
CHAPTER FIVE: DISCUSSION

Specific Aim 1, Feasibility

The primary aim of this project was to evaluate overall feasibility of capturing each variable or outcome of interest. The project was deemed feasible with a few modifications. During recruitment, in order to minimize a therapeutic expectation response, a form of placebo response, the ideal subject was to have been acupuncture naïve, and someone who may not have originally considered acupuncture for menopausal symptom management. Thus, initial recruitment materials were vague and did not mention that acupuncture was to be the intervention. This strategy was a dismal failure, as was using physician offices as sites for recruitment, for neither sparked interest nor subjects. Once the recruitment strategy was amended to allow women who had prior experience of acupuncture, but not within the past six months for any reason or for menopausal symptoms in the past twelve months, and recruitment advertisements were modified to specifically mention an acupuncture intervention via radio and newspaper advertisements, recruitment flourished. This suggests that women experiencing menopausal symptoms are interested in acupuncture as an intervention. Finally, during screening, additional exclusion criteria items were identified and should be added, no cigarette smoking or alcohol during the study, a general exclusion of significant mental illness or participation in concurrent studies, no caffeine ingestion 24 hours prior to ECG collection.

In general, the study went better than expected. As mentioned in the results section, recruitment was 100% in four months, there was 100% retention, and all data
were collected with no missing data points. Collected data points included 348 symptom scores, 150 heart rate variability scores and 8 interleukin 6 values (4 absolute values and 4 calculated values) for 506 unique data points per subject, 6072 for the study.

Specific Aim 1a, Menopausal Symptom Experience

This small study was not intended to demonstrate improvement in menopausal hot flashes or vasomotor symptoms; rather we sought to explore whether or not acupuncture could improve a wider array menopausal symptoms. The majority of prior acupuncture-for-menopausal-symptom studies focused solely on vasomotor symptoms (Wyon 1995; Porzio 2002; Cohen, Rousseau et al. 2003; Wyon, Wijma et al. 2004; Filshie, Bolton et al. 2005; Borud 2007; Vincent, Barton et al. 2007; Avis 2008). This project was unique in that we examined a more inclusive symptom constellation using the Menopausal Rating Scale, which looked at eleven symptoms, and we added a measure of global distress to ensure that we would understand to what degree these symptoms were troubling to the women experiencing them. Despite the small sample size, the results did reach statistical significance. Unfortunately, the lack of a control group prevents any valid comparison. Thus, these results should be cautiously interpreted as menopausal symptoms, especially hot flashes, seem susceptible to improvement in both acupuncture as well as guided relaxation groups (Nedstrand, Wijma et al. 2004; Zaborowska, Brynhildsen et al. 2007), and susceptibility to placebo effects have been described (Avis, et al.2008). The important question in this type of research is to uncover specific versus non-specific effects regarding symptom improvement between symptomatic subjects relaxing on a massage table, or meditating, versus experiencing acupuncture. Accordingly, the focus of
this project was to begin to identify physiologic changes that might shed light on the mechanism of acupuncture.

**Exploratory Aim 2, Effects of Acupuncture on Interleukin 6**

Due to the small sample size, statistical significance in serum changes was not expected. In this sample, a mild, subclinical elevation of serum interleukin 6 was expected, due to age and menopausal status (Papanicolaou, Wilder et al. 1998; Rachon, Mysliwska et al. 2002) (Yasui, et al. 2007) and, among the twelve subjects, five had a baseline greater than or equal to 6.3 pg/ml. This seems to support the theory that menopausal women who were experiencing significant symptoms would tend to have an elevated serum interleukin 6. In every subject, however, serum interleukin 6 did change after the acupuncture, however, not in a consistent direction, in some cases it was increased and others it decreased. There were no significant correlations between ages, body mass index, weight and serum levels of interleukin 6. One significant outlier must be mentioned, however. Subject 112, 48 years old, had a baseline level of 18.29, which increased after acupuncture to 21.51. Exclusion criteria were in place to screen out subjects with inflammatory conditions, so after these results were obtained, further questioning revealed that for two days prior to beginning the study (Christmas Eve and Christmas day) she had 4 or more alcoholic drinks on each day. Although, alcohol had not been reported to influence interleukin 6 (Ho, Xue et al. 2004), the consulting immunologist considered this outlier was due to excessive alcohol intake before the sample was obtained (personal communication, H. Zwickey, March 5, 2009). Subsequent
interleukin 6 levels were more in the expected range (2.21 and 3.75 pcg/ml) for age, and there had been no further alcohol intake.

There were four significant correlations between symptom slopes and levels of serum interleukin 6. Significant correlations included a positive relationship between hot flashes and both the baseline interleukin 6 \( r_s = .68, \ p = .015 \) and first post-acupuncture levels of interleukin 6, \( r_s = .93, \ p = .000 \). Additionally, there was an inverse relationship between depressed mood and the difference between the first post treatment level and the level before the tenth treatment level of interleukin 6 \( r_s = -.58, \ p = .05 \), and again, between depressed mood and the difference between the baseline interleukin 6 and prior to the last treatment level of interleukin 6, \( r_s = -.63, \ p = .029 \). The interesting relationship is between the depressed mood slopes, which all declined during the intervention. This finding suggests that as mood improved, interleukin 6 remained the same or increased. The literature suggests that elevated levels of interleukin 6 are correlated with depressed mood (Motivala, Sarfatti et al. 2005; Wright, Strike et al. 2005; Andrei, Fraguas et al. 2007; Friedman, Hayney et al. 2007). Thus, the proposed physiologic model that suggests a relationship between elevation of interleukin 6 and depressed mood is supported. The paradoxical finding of improved mood and elevated interleukin 6 suggests that acupuncture may not affect symptoms by down-regulating interleukin 6, but perhaps, may influence interleukin 6 receptors, through down-regulation, or perhaps through the endogenous opioid system, completely over-riding effects of the circulating interleukin 6.

In light of this interesting finding, further research is warranted.
The sample size was not intended to verify the physiologic models, and this is confirmed with these results. There is no evidence of a strong effect of acupuncture on serum levels of interleukin 6, however, this assertion cannot be made with confidence in such a small sample size, and without a control group. Other studies have demonstrated a response by interleukin 6 to acupuncture in stronger immunologic models, such as allergy and asthma (Joos, Schott et al. 2000; Jeong, Kim et al. 2002). Menopausal women, however, may not be an ideal model to assess how effectively acupuncture modulates the immune-inflammatory response, as menopause does not usually produce a strong inflammatory response, but rather a minimal response (Yasui, T., Maegawa, M., Tomita, J., Miyatani, Y., Yamada, M., Uemura, H., Matsuzaki, T., Kuwahara, A., Kamada, M., Tsuchiya, N., Yuzurihara, M., Takeda, S., and Irahara, M. 2007).

Exploratory Aim 3, Effects of Acupuncture on Heart Rate Variability

This study presents new, previously unpublished findings in the area of the heart rate variability response to acupuncture in a symptomatic population. The unique aspect was a longitudinal, repeated measures design, which measured each symptomatic subject as they experienced a series of acupuncture treatments, and simultaneously reporting symptom response. Additionally, instead of using only one or two acupuncture points, a therapeutic acupuncture protocol was used. These factors coupled with the capture of five spectral components of heart rate variability at each intervention in three phases of treatment, resting- before acupuncture, after five minutes of acupuncture and the final five minutes of acupuncture, allowed the researcher to see the heart rate variability
response as the subject experienced an entire treatment. The results of this project describe a complex biopsychosocial response to acupuncture.

Prior studies that have examined the response of heart rate variability to acupuncture have been limited in that they have only examined the effects of one or two acupuncture needles inserted into major acupuncture points (a non-therapeutic intervention) while measuring immediate effects on heart rate variability in one or two sessions. Four studies used healthy volunteers with a control group that used some form of placebo acupuncture device, most commonly superficial needle insertion, off identified acupuncture points (Carpenter 2006; Dhond, R. P., Yeh, C., Park, K., Kettner, N., and Napadow, V. 2008; Haker, E., Egervist, H. and Bjerring, P. 2000; Streitberger, K., Steppan, J., Maier, C., Hill, H., Backs, J. and Plaschke, K. 2008). Li (2005) looked at healthy, but fatigued truck drivers and also used a placebo control group that involved superficial needle insertion, off-point (Li, Z., Wang, C. Mak, A.F.T. and Chow, D.H.K. 2005), and two studies looked at symptomatic subjects, migraine headaches (Backer, M, Grossman, P., Schneider, J., Michalsen, A., Knoblauch, N., Tan, L., Niggemeyer, C., Linde, K., Melchart, D. and Dobos, G.J. 2008) and anxiety disorder (Agelink, M. W., Sanner, D., Eich, H., Pach, J., Bertling, R., Lemmer, W., Klieser, E. and Lehmann, E. 2003 [abstract only]). Both studies that used a symptomatic sample also used off-point, superficial needle insertion as a placebo control group. In the studies that examined healthy volunteers, either a single point in time measure of heart rate variability was performed, or only one or two needles were used, or both of these situations described the research effort. Backer, et al. (2008) and Agelink, et al. (2003), however, not only used a
treatment protocol with therapeutic intent, but they also looked at the effects of acupuncture on heart rate variability at baseline and final treatment. Unfortunately, no interim measures were performed.

The prior studies reported mixed results. In the verum acupuncture group, Agelink, et al. (2003) found trends towards an increased high frequency, with significant reduction of the low to high frequency ratio, with no significant results reported for the placebo group. Backer, et al. (2008) reported a decrease in low frequency in both verum and placebo groups, but only in those with at least 50% migraine reduction (responders). Those subjects who did not report headache improvement showed no changes in HRV. Backer, et al (2008) also reported a significant decrease in the high frequency component, but noted that neither verum nor placebo acupuncture demonstrated changes in the low frequency component of HRV, and group assignment did not predict migraine headache improvement. Hacker, et al. (2000), reported an increase in high frequency with verum auricular acupuncture, and an increase in both high and low frequencies after verum acupuncture in the thenar muscle of the hand, in addition to an increase in the low to high frequency ratio sixty minutes after placebo acupuncture. Li, et al. (2005) reported a decrease in low frequency, absolute power, an increase in the high frequency, measured in normalized units, and a decrease in the low to high frequency ratio in the fatigued group. However, in those drivers not fatigued, after verum acupuncture, Li and colleagues reported an increase in both low frequency and high frequency measured in absolute power. There were no significant HRV changes in those fatigued drivers who received placebo (superficial needle insertion, off-point). Li, et al. (2005) concluded that
the subject’s functional state had an impact on how verum acupuncture affected heart rate variability. A subject’s functional state may prove to be an important concept to understand in acupuncture research. An emerging body of literature suggests that acupuncture may be an adaptogenic or modulating intervention in which the physiology is not consistently affected one way or another, but rather, depends on the state of the physiology at the time of the intervention (Arranz, L. Guayerbas, N., Siboni, L. and De la Fuente, M.2007; Kung, Y.Y., Chen, F.P, and Hwang, S.J 2006; Yamaguchi, N., Takahashi, T. Sakuma, M., Sugita, T., Uchikawa, K., Sakaihara, S., Kanda, T., Arai, M., and Kawakita, K. 2007).

Streitberger, et al (2008), examined twenty healthy subjects and used one acupuncture point on two separate occasions, had findings most consistent with findings of this project. Streitberger and colleagues reported a large amount of variability in all parameters of heart rate variability. The only significant finding was a short-term increase in the low to high frequency ratio, in the verum acupuncture, but not the placebo group, and a tendency towards a transiently increased low frequency in the verum acupuncture group. These results may be interpreted as a normal physiologic response to an unpleasant sensation, and a sympathetic nervous system response to the painful stimuli of the acupuncture needles, however, this seems unlikely given that there are usually very few complaints of pain reported during acupuncture procedures.

Ultimately, all prior studies have only captured brief snapshots of heart rate variability response to acupuncture, which lacks a more complete picture of what heart
rate variability does through time and treatment exposure. This may have led to incorrect
perceptions of the effects of acupuncture on heart rate variability.

In this project, the high degree of variability seen in both intra and inter-
individual data, from treatment to treatment, in all measures, mirrors all findings reported
to date. Additionally, this large amount of variability seen both intra and inter-subject,
lends support to a theory of acupuncture stimulating physiologic modulating influences
rather than a consistent physiologic impact e.g. consistently increasing overall beat-to-
beat variability. One example of this was subject 110, who had a dramatically high level
of variability at baseline. The acupuncture converted her to a state with less variability,
quite the opposite of what was hypothesized or intended, yet she also reported significant
symptom improvement. Examination of individual slopes for each phase and component
of HRV suggests particular trends may be occurring among subpopulations in this
symptomatic sample. HRV slope means, however, do not support the notion of
significant trends. However, in this small sample size, it is worth noting that some
subjects did demonstrate definite trends, thus, further research is warranted.

A larger sample with a control group may provide the amplification and
comparison needed to clarify observations in this feasibility study. In all but one set of
mean slopes, changes were very close to zero, which translates into no real increase or
derecrease. For example, the mean low to high frequency ratio slopes increased in all three
phases of acupuncture, albeit minimally, in most instances. The mean slope for low
frequency, measured both in absolute power and normalized units, increased during the
baseline/resting phase, but decreased in both the first five minutes and the final twenty
minutes of acupuncture. The mean slope for high frequency, measured both in absolute power and normalized units, went up at the baseline/resting phase, as well as during the first five minutes, but then decreased during the last five minutes of acupuncture. The shift in both low and high frequencies seen in the final phase of acupuncture may be reflecting physiologic shifts or modulation occurring as the nervous system accommodates to the sensory stimuli of the acupuncture needles or to the neurohormonal response of a profound state of relaxation.

Examination of temporal individual, intra-treatment change also shows dramatic and unpredictable variability. In some instances, there are dramatic changes in a particular measure of HRV from baseline to completion of acupuncture, while on other days, in the same subject, there are virtually no changes from baseline in any given measure. Again, this may be reflective of the functional state of the subject at that particular time. It bears re-stating that HRV measures do exhibit circadian features (Guo and Stein 2002); however, this was controlled for as all subjects were treated at the same time of day, plus or minus 2 hours. One possible physiologic interpretation for this high level of intra-individual variability could be that the acupuncture needles initially increase the sympathetic nervous system stimuli (seen as increased low frequency), followed by a parasympathetic response as the subject relaxes with the needles in place (seen as increased high frequency). That this is not reflected in the LF:HF ratio, which remains elevated through out all three phases, is most likely due to the decreased LF (negative slopes) in the final two phases, and increased HF during the baseline and first five minutes of acupuncture (positive slopes). The final decrease seen in the high
frequency during the last five minutes of acupuncture is most likely the reason for the static elevation of the LF:HF ratio. The decreased high frequency may be a reaction to the mid-treatment needle stimulation, which triggers a slight discomfort. Reports of the sensation of ‘de qi,’ include heavy cramping sensations, sensations of warmth, an electrical sensation or a sharp pain. However, the mean slopes suggest that there were no consistent increases or decreases in the LF: HF ratio, or in either measure of LF or HF across the time phases. Physiologically, it is difficult to explain this paradoxical response, because once a relaxation response was in place, it would be expected to continue. However, it is important to understand that the low frequency component of HRV reflects both sympathetic and parasympathetic cardiac modulation, while high frequency only reflects parasympathetic modulation. Thus, an increase in low frequency most certainly represents an increase of both sympathetic as well as parasympathetic activity. Additionally, a host of other complex interactions of neural, hormonal, mechanical and thermoregulatory effects modulates the cardiac autonomic nervous system (Lombardi, Malliani et al. 1996). It is tempting to oversimplify complex biological systems in an attempt to describe them, however, a simplistic, mechanistic model or explanation may be insufficient to explain the effects of acupuncture seen in individuals, on the various frequencies seen in spectral analysis of heart rate variability. What was seen in this project, in each measure of heart rate variability, and in various phases of acupuncture, was a delineated dynamical change model as the temporal physiology responded to acupuncture. This evidence strongly suggests acupuncture may exert a modulating effect, shifting the physiology depending on the current functional state. Thus, sometimes the
high frequency may be increased by acupuncture, while at other times the high frequency may be decreased or unaffected by acupuncture. A larger study with a control group may be able to clarify these ideas.

The initial physiologic model of acupuncture has neither been confirmed nor disproved by this small study. An acupuncture model that emphasizes neurologic physiology seems to be the most accurate explanation of acupuncture outcomes. In this project, the ear needles were placed in the fossa area of the pinna, which is innervated by the third branch (mandibular) of the trigeminal nerve, as well as second cervical spinal nerves. These nerves have a junction with the vagus nerve via the superior ganglion of the vagus nerve (a.k.a. jugular ganglion), thus, the parasympathetic nervous system would be stimulated and activated by direct needle stimulation. The other acupuncture points used in this protocol stimulated the median and ulnar nerves, bilaterally, which directly affect cervical spinal nerves 5-8 and the first thoracic spinal nerves. Because nerve impulses travel to the central nervous system (CNS) as well as target viscera via the peripheral nervous system, it is reasonable that stimulation from an acupuncture needle would affect not only the CNS, but those organs also innervated by the spinal nerves, such as in this case the heart. Acupuncture points in the lower extremities are positioned on the fourth and fifth lumbar dermatome, and thus may have exerted influence on the genitourinary system via the lumbar spinal nerves.
Exploratory Aim 4, Relationships Between Symptoms, Interleukin 6 and Heart Rate Variability

The Spearman correlations between symptoms and interleukin 6 are described above. There were also interesting and significant correlations between the various components and phases of heart rate variability and symptoms, as well as heart rate variability presented as slopes, and absolute values and pre-post acupuncture changes in serum levels of interleukin 6. These findings will be discussed at the end of this section.

The most frequent significant correlations occurred between anxiety, depression and irritability slopes and heart rate variability slopes. For this project, there was not an hypothesized direction proposed for low or high frequency and mood, though there have been correlations between elevated low frequency components in patients with post traumatic stress disorder (Cohen, H., Kotler, M., Matar, M.A., Kaplan, Z., Miodownik, H. and Cassuto, Y. 1997) and women who suffer social isolation, depression and difficulties managing anger (Horsten, M., Ericson, M., Perski, A., Wamala, S.P., Schenck-Gustafsson, K. and Orth-Gomer, K. 1999). In this project there was a significant relationship ($r_s=.79, p=.002$) between the depressed mood slope and the low frequency slope, absolute power, in the final 5 minutes of acupuncture. This suggests that as that mood improved, marked by a negative slope, that the low frequency (LF) slopes decreased or in physiologic terms, heart rate variability increased. As mentioned earlier, an increased LF is implicated in both cardiac parasympathetic and sympathetic modulation, but in relationship to high frequency, which predominantly reflects parasympathetic modulation, LF represents a relative sympathetic or an excitatory, flight
or flight mode. Thus, higher LF may translate as increased levels of emotional distress. The irritability slope follows suit, as the slope decreases and symptoms improve, specifically, the LF slope decreases in the final 5 minutes of acupuncture ($r_s = .65, p = .023$). This makes physiologic sense that as mood improves, the physiology becomes more relaxed. A nice correspondence was demonstrated for both of these symptoms when the high frequency slope increased in the first 5 minutes of acupuncture (a marker for increased parasympathetic cardiac modulation) as the symptom slopes decreased, $r_s = - .62, p = .032$ for depressed mood, and $r_s = - .59, p = .042$ for irritability. These correlations were also seen in the low and high frequency slopes, measured in normalized units (NU), further supporting the relationship, as the depressed mood slope decreased, the LF NU slope in the last 5 minutes decreased ($r_s = .71, p = .009$), and the HF NU slope in the first 5 minutes increased ($r_s = -.86, p = .00$). While the irritability slope decreased, the LF NU slope in the last 5 minutes decreased ($r_s = .64, p = .025$), and the HF NU slope in the first 5 minutes increased ($r_s = -.83, p = .00$). There were also correlations seen in the anxiety slopes, which as the slope decreased, again, indicating symptom improvement, LF NU slope in the last 5 minutes decreased ($r_s = .62, p = .031$), and the HF NU in the first 5 minutes increased ($r_s = -.63, p = .028$), as well as in the last 5 minutes ($r_s = -.64, p = .024$). These results suggest that modulation of the cardiac sympathetic and parasympathetic influences are occurring, but whether this is due to a direct effect on the nervous system by the acupuncture needles, or the relaxed state induced by the needles, e.g. an opioid response, remains to be determined.
One unexpected correlation was seen between the bladder dysfunction slopes and the high frequency slope in absolute power measured in the first 5 minutes of acupuncture. As bladder symptoms improved, the high frequency slope increased \((r_s = .59, p = .045)\). Whether this reflects a dynamic in the nervous system or is a reflection of the mood is difficult to tease out. If a woman experienced improved bladder control, her anxiety and irritability may be improved. However, Emmons, et al (2005) demonstrated that acupuncture improved bladder symptoms such as urgency, frequency and incontinence, but the relationship between the parasympathetic modulation associated with the high frequency slope component of heart rate variability and bladder function remains to be determined.

Acupuncture may have directly affected all of these symptoms via the aforementioned neurophysiologic model. While there were no clear or consistent trends in changes in the various phase of heart rate variability or interleukin 6 levels, the heart rate variability did change at almost every treatment. Thus, it does seem highly suggestive that acupuncture affected the physiology in this project resulting in improved symptoms.

**Correlations Between Interleukin 6 and Heart Rate Variability Slopes**

There was a significant inverse relationship observed between both the baseline and pre-tenth levels of interleukin 6 values and the resting phase of the high frequency slope, measured in absolute power, \(r_s = -.69, p = .014\), suggesting that as the high frequency slope increased (representing increased parasympathetic cardiac modulation) interleukin 6 levels declined or vice versa. Additionally, there was a significant inverse relationship
between the post-tenth treatment interleukin 6 level and the resting phase high frequency slope measured in absolute power, $r_s = -0.66$, $p = 0.020$.

Finally, there was a significant inverse relationship between the difference or temporal change between the baseline and first post-acupuncture interleukin 6 level and the LF:HF ratio in the first 5 minutes of acupuncture, $r_s = -0.73$, $p = 0.008$, which suggests that as the ratio increased interleukin 6 decreased. When the LF:HF ratio increases, it can do so if both the LF or the HF increases, or if only the LF increases. The ratio is considered the major marker for the overall balance of cardiac sympathetic-parasympathetic modulation, thus an increased ratio suggests more sympathetic modulation, which counters the argument for increased well-being or predominantly parasympathetic modulation corresponding with lower levels of inflammatory markers.

There is reason to be cautious in the interpretation of these correlations because comparing temporal changes between two variables is considered by some to be a weak analysis. The only statement that can be made with confidence is that there may be a relationship. As mentioned earlier, a larger sample size and control group should provide the amplification needed for clarification of these relationships.

**Correlations Between Interleukin 6 and Absolute Values of HRV Components**

The final set of correlations was between interleukin 6 and the absolute values for the components of the heart rate variability on the days that the interleukin 6 sample was obtained (refer to table 14). Initially, the idea that components of heart rate variability would have any relationship to interleukin 6 seemed to be a completely spurious correlation. However, a closer look at the literature found that these correlations have
been identified and examined in several samples, both healthy and those with identified pathology. Carney, et al. (2007) looked at a sample of patients with coronary heart disease and depression, and found that levels of fibrinogen and interleukin 6 did correlate with HRV. Specifically, Carney, et al. (2007) writes that there were significant correlations between elevated serum interleukin 6 and decreased heart rate variability (primarily increased low frequency). Carney, et al. also mentions, that as a group, this particular sample had higher than average serum interleukin 6 and lower heart rate variability (expected due to co-morbidities of clinical depression and significant heart disease), thus these results may not generalize to a healthy population. Researchers have attempted to correlate interleukin 6 with heart rate variability in an attempt to risk stratify and predict outcomes for those with coronary heart disease. Aronson et al. (2001), however, did not find any correlations between interleukin 6 and spectral analysis of heart rate variability in sixty-four patients with decompensated heart failure. In women with a history of coronary heart disease and history of myocardial infarction (Aronson, D., Mittleman, M. A. and Burger, A. J. 2001), Janszkey and colleagues, however, did find significant inverse correlations between interleukin 6 and both low and high frequency components (Janszky, I., Ericson, M., Lekander, M., Blom, M., Buhlin, K., Georgiades, A. and Ahnve, S. 2004). In a review of thirteen studies that examined heart rate variability and inflammatory markers, Haensel et al. (2008) concluded that evidence suggests that a decrease in heart rate variability is associated with ongoing subclinical inflammation (Haensel, A., Mills, P. J., Nelesen, R. A., Ziegler, M. G. and Dimsdale, J. E. 2008). At the other end of the spectrum, in a group of healthy adults who participate in
the ongoing Coronary Artery Risk Development in Young Adults (CARDIA) study, which is a prospective, bi-ethnic, multicenter epidemiological study begun in 1985-6 to understand cardiovascular risk development in young adults, Sloan, et al. (2007) found a weak, but significant inverse correlation between interleukin 6 and the low frequency component of HRV (Sloan, R.P., McCreath, H., Tracey, K.J., Sidney, S., Liu, K. and Seeman, T. 2007).

Current understanding of the relationship between the physiology of the autonomic nervous system (ANS) and the immune system is that the ANS is activated by cytokines, yet the ANS also controls the release of cytokines (Andersson 2005; Czura and Tracey 2005; Libert 2003; Tracey 2007). Concurrently, the low frequency component of heart rate variability is modulated by both the parasympathetic as well as the sympathetic nervous system, while the high frequency is predominately a result of parasympathetic modulation. Thus, when the low frequency component changes, it is not possible to clearly understand which branch of the ANS is dominant. For these reasons, some researchers concentrate on the low to high frequency ratio to establish dominance of ANS cardiac modulation, but the complete picture only comes into focus by examining each component. At this point, the literature is sparse regarding causality between cytokine level changes and changes in heart rate variability.

In this project, when the absolute values of the components of heart rate variability were correlated with interleukin 6 on the day of interleukin 6 sampling, the results were interesting. These significant correlation results were maintained even when dropping the outlier subject 112.
Discussion of Results from the Descriptive Qualitative Data

From the transcripts and emic coding (Spradley 1979) two over-arching taxonomies were identified: Context, which refers to the life experiences that each subject brought into the study, and Outcomes, which were the perceived results of the acupuncture intervention. Within the Context taxonomy, stress was frequently mentioned. Stressors in the subjects’ lives were captured in the transcript as well as during informal conversations with the subjects during the study.

Most notable were three women who experienced unusually stressful events during their time in the study. In the second week of the study, one subject’s daughter attempted suicide and was hospitalized in the intensive care unit. During the same week, this subject had learned that the man she had known as her father was not her biological father, and that her mother had been raped by an acquaintance, who was this subject’s father. Concurrently, during this period, the same subject’s parents were involved in a bitter domestic violence situation and the mother was living with her daughter, the subject. This subject reported that she felt that the acupuncture had not only helped her with the menopause symptoms, but had also helped her deal with these unusual life stressors.

In the third week of her four-week course of treatment, a second subject witnessed the unexpected death of a very close friend. Additionally, she had recently lost most of her business and income due to the recent economic downturn. She, too, reported that the acupuncture seemed to help her with these unusually stressful events. Finally, one subject reported such significant depression, that before enrollment she was referred to her
primary care provider for depression and thyroid screening. Only after she was medically cleared was she allowed to participate in the study. Her mood, sleep and energy improved dramatically, which she attributed solely to the acupuncture intervention. As a traditional Chinese medicine provider, this researcher had witnessed dramatic improvements such as these in private practice. However, I was surprised to see such dramatic results in a research setting where the patient-practitioner relationship was much more succinct and clinical.

Some propose that the effectiveness of Complementary and Alternative Medicine (CAM) therapies depend heavily on the patient-practitioner relationship (Ernst, 2004), and from this relationship, healing is thought to occur as the result of a non-specific or ‘placebo’ response. It should be noted however, that the patient-practitioner response has been reported to have significant beneficial clinical effects in conventional biomedical therapies, as well (Maddigan, S. L., Majumdar, S. R. and Johnson, J. A. 2005; Radosevich, D. M., McGrail, M.P., Jr, Lohman, W. H., Gorman, R., Parker, D. and Calasanz, M. 2001).

In this small study, which did not control for a placebo effect, it was interesting to find one subject (107) who did not respond to the acupuncture intervention. She reported that the treatments were pleasant and relaxing, but her symptoms did not improve. When asked by this researcher if she wanted to continue, she reported that she did not want to stop the study because she felt that her experience was valuable to try to answer the question about the effectiveness of acupuncture for these particular symptoms. It was interesting to note that her symptom slopes were not always flat (see figures 7, 24, 36, 66,
77, 87, 95, 117, 106), but vaginal dryness, sexual dysfunction, pain, hot flashes and global distress did not show the improvements that most of the other women experienced. Interestingly, her interleukin 6 decreased between the first and tenth treatment (figure 129), and her heart rate variability showed an increase in the high frequency and decreased low frequency (figures 141, 153, 165, 177 and 189) illustrating that although these parameters moved in the hypothesized directions, her symptoms did not improve. This evidence argues for another mechanism by which acupuncture may exert an effect for menopausal symptom improvement, perhaps the endogenous opioid theory put forth by Pomeranz and colleagues (Pomeranz, as cited in Stux and Hammerschlag 2001). One mechanism by which some people may not respond to acupuncture analgesia may be related to one or several genetic single nucleotide polymorphisms (SNP) in the central nervous system neurotransmitter system or opioid receptors, such as, but not limited to the serotonin transporter gene or the mu opioid receptor gene.

On the other extreme from subject 107’s minimal-response, was subject 112 who seemed to be a ‘super-responder’ in that she experienced dramatic and significant improvement in her symptoms, and did not request a referral for more acupuncture because she had experienced such improvement. In her response to the final question asking for her experiences of acupuncture she reported sensations of the needles ‘humming and singing to each other,’ indicating that she may have had a more heightened experience to the sensory stimulation induced by the acupuncture needles. Examination of her symptom slopes shows definite improvements (figures 12, 29, 41, 52, 71, 82, 99, 122). Her interleukin 6 also showed dramatic improvements (figure 134),
however, in the days before her sample was taken, she had indulged in holiday alcohol consumption, more than her normal amounts, thus, this particular measure remains suspect for a true interleukin 6 baseline. Her baseline or resting heart rate variability did move in the hypothesized direction with the ratio decreasing (figure 146), the low frequency in both measures decreasing (figures 158, 182) and her high frequency increasing (figures 170, 194). In light of these measures also improving in the non-responder, this researcher believes that as subjects felt better, the heart rate variability improved as a response to less anxiety and discomfort from symptom distress. At this stage, these musings are merely conjecture; more research is needed for confirmation.

Implications for Traditional Chinese Medicine

As a provider of traditional Chinese medicine (TCM), one of the more interesting findings revolved around the TCM pattern diagnosis. Menopause is a biomedical definition, made retrospectively, that describes a woman who has not had a menstrual period for twelve months. The symptoms that are associated with menopause are believed to be related to the relative estrogen deficiency. Many TCM schools in the west stress the notion that menopause and the accompanying symptoms are most commonly related to the TCM pattern diagnosis Kidney Yin deficiency (Maciocia 1994). TCM pattern diagnoses rely on a presentation that includes the patient’s subjective reports of heat, insomnia, restlessness, thirst, sweat patterns, energy levels, and other subjective symptoms. In addition, the quality and strength of the radial pulse on both the left and right wrist are palpated, and the body of the tongue is observed for color, in addition to the color and quantity of the tongue coat, and quality and color of the sublingual veins
All of this information is considered, and the TCM clinician forms a clinical impression and defines the TCM pattern.

In this project, each subject was evaluated and assigned a TCM pattern diagnosis. Contrary to what many TCM students are taught, the majority of subjects presented with excess as opposed to deficient patterns. Eleven of the twelve women presented with the commonly seen excess pattern Liver Qi Stagnation. The only subject that presented with a true Kidney Yin deficiency was 107, the non-responder. In TCM, acupuncture works very well to relieve excess conditions, but for deficient patterns herbal formulas are more effective (Bensky and Barolet 1990). This supports Scheid’s (2006) argument that contemporary TCM textbooks may have oversimplified ‘traditional’ Chinese medicine. Moreover, the idea that TCM as conceptualized in the west, a 3000-year-old system of medicine that has remained intact through the ages is far from how this system came into being. More accurately, TCM is a political construct that came about in the early 1950s as a result of Chairman Mao’s decision merge the ‘best of western medicine’ with the ‘best of Chinese medicine’ (Taylor, K. 2004). This view of TCM has denied the rich diversity and plurality that ethnic Chinese medicine represents in its cultural context. Thus, the most significant implications of this small study for TCM practitioners is to treat the presenting pattern, not the biomedical diagnosis, as well as to remember how culture shapes medical beliefs and traditions.
Summary

Strengths of the Study

The project met all criteria for feasibility and the data suggested avenues for future research. The physiologic models neither were supported nor refuted, specifically, the model of how fluctuating estrogen levels may influence interleukin 6 and the sympathetic nervous system. Serum interleukin 6 was not consistently elevated, and there were no clear trends that women entered the study with significantly disordered cardiac sympathetic modulation, nor that acupuncture changed women from predominantly sympathetic to predominantly parasympathetic cardiac modulation. One item that was consistent was the modifiers and moderators of how women experienced menopausal symptoms. While not directly measured directly, anecdotally, the women who participated in this study reported very high stress levels, which, by self-report, exacerbated their symptoms. The model of how acupuncture may work was neither supported nor refuted. There was no evidence that acupuncture increased parasympathetic cardiac modulation or directly lowered interleukin 6 levels; however, the sample size was small.

Unique findings included examples of the variability in heart rate variability in response to a ten-treatment acupuncture intervention. This was the first study to replicate a traditional Chinese medicine acupuncture protocol, and look at the heart rate variability at each treatment. However promising some of the correlations looked, though, small samples are especially prone to random data with subsequent spurious correlations and over-optimistic interpretation. Complementary and alternative medicine research may be
especially vulnerable given the lack of large funding opportunities, which may generate many small pilot or feasibility studies that often produce conflicting information.

Though not an aim for this project, findings did confirm those of prior studies, that acupuncture seems to improve menopausal symptoms, the unique aspect of this study was that acupuncture improved symptoms other than vasomotor symptoms. In light of this, larger, multi-center studies to examine acupuncture for menopausal symptoms would be warranted; however, pursuing the effects of acupuncture on interleukin 6 in a menopausal population is probably not the best use of resources.

No clear trends were seen in the heart rate variability alone, but significant correlations with improvements in anxiety, irritability and depression also warrant further study, not only in those with menopausal symptoms, but other syndromes in which these distressing symptoms manifest.

Limitations of the Study

There were many limitations in this small feasibility study. Primarily, this project was not designed to gather statistically significant data; rather, it was to better understand how a study such as this could be conducted in the sense of appropriate biomarkers, timing and number of sample collections and willingness of subjects to participate. The data generated from a very small sample size, such as this was, tend to be very unreliable due to the high likelihood of random data showing artificially inflated effects (Field 2005). Additionally, without a control group, there was nothing with which to compare the active intervention group. Moreover, there were trends and correlations, suggesting that maybe in a larger sample with more disordered physiology, such as coronary heart
disease or an autoimmune disorder, findings may be more impressive. The subjects in this project were very healthy, and menopause may not be the ideal population in which to study a response to either the immune-inflammatory system, or disordered heart rate variability. Finally, the researcher performed all interventions and analyses, which can lead to a high risk of conscious or unconscious bias.

**Implications for Future Research**

This study has launched a research career that will look at acupuncture as an intervention for symptom management, in addition to measuring physiologic changes that may be attributed to acupuncture. Future directions of study include larger sample sizes with control groups, and looking at symptom management in populations with more disordered physiology in order to see a more robust response in pro-inflammatory cytokines. While menopausal women, as a model for inflammation and disordered HRV were not robust, the next population will be fibromyalgia where cytokines and HRV will be examined. Additionally, this study generated more interest in how scientists model and evaluate complex biological systems such as the dynamic changes that were seen as the heart rate variability responded to acupuncture in this project. A more complete understanding of chaos, complexity and systems theory will be undertaken.

Finally, there was a steep learning curve for the heart rate variability aspect of this study. Learning how to present and manage the data is an important factor in heart rate variability research. Most of the literature cited performed natural logarithmic data transformation. This sample was so small, that when the data was transformed did not
change results, thus raw data was presented. Future research will incorporate natural logarithmic transforms.
APPENDIX A:

IRB HUMAN SUBJECT APPROVALS, ADDENDUMS AND CONSENT FORMS
Subject’s Consent Form

Project Title: Acupuncture for menopausal symptom management: An exploration of mechanism and experience.

You are being asked to read the following material to ensure that you are informed of the nature of this research study and of how you will participate in it, if you consent to do so. Signing this form will indicate that you have been so informed and that you give your consent. Federal regulations require written informed consent prior to participation in this research study so that you can know the nature and risks of your participation and can decide to participate or not participate in a free and informed manner. If you choose not to participate, your refusal will involve no penalty or loss of benefits which you would normally experience.

PURPOSE
You are being asked to participate voluntarily in the above-identified research project. The purpose of this research is to evaluate acupuncture for the treatment of menopausal symptoms which include hot flashes, sweats, difficulty sleeping, depressed mood, irritability, anxiety, skin rash, fatigue, mood swings, changes in body fluids, and discomfort with sexual intercourse. Some of these symptoms may be due to how the menopause change of life affects both the nervous system as well as the immune system. In addition to how effectively acupuncture works for these symptoms, the researcher will also be measuring your blood and heart rate for any changes that could be caused by acupuncture. Acupuncture is thought to act on chemicals in the brain that carry messages between the nerves, which may cause changes in the nervous system as well as the immune system. By counting and measuring your heartbeats and measuring the cytokine in your blood, we hope to see if there are changes that might be caused by acupuncture. We are also interested to see if the acupuncture has an effect on your menopausal symptoms. Past studies indicate that acupuncture may be very effective for hot flashes and sweats, but we are interested in some of the other symptoms that women experience. Acupuncture has not been formally approved for this condition, however, acupuncture is a safe procedure and will be performed by a licensed acupuncturist with 5 years of experience. The needles used are sterile, single-use only and disposable.

SELECTION CRITERIA
The next investigator will discuss the requirements for participation in this study with you. To participate you must meet certain criteria:

1. Have gone through or completed a natural menopause, which means at least 12 months...
APPENDIX B:

IRB APPROVED RECRUITMENT AND ADVERTISEMENT MATERIALS
“Are you a healthy 48-65 years old woman experiencing significant menopausal symptoms such as fatigue, low energy, depressed mood, hot flashes and/or sweats?

The University of Arizona, College of Nursing is recruiting healthy menopausal women for a research study to evaluate a non-hormonal, non-drug approach to control menopausal symptoms.

If you are interested, please call Cheryl Wright, RN, MSN at 520 312 7171”

Approved Copy For Radio and Newspaper:

“The University of Arizona College of Nursing is currently recruiting healthy women to participate in a study: Acupuncture for menopausal symptom management: An exploration of mechanism and experience. If you are 48-65 years old and experiencing significantly bothersome menopausal symptoms such as fatigue, low energy, depressed mood, hot flashes and/or sweats, please call Cheryl Wright at 312 7171 for more information.”
APPENDIX C:

MENOPAUSAL RATING SCALE
Menopause Rating Scale (MRS)

Which of the following symptoms apply to you at this time? Please, mark the appropriate box for each symptom. For symptoms that do not apply, please mark 'none'.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>none</th>
<th>mild</th>
<th>moderate</th>
<th>severe</th>
<th>very severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

1. Hot flushes, sweating (episodes of sweating) ........................................... □ □ □ □ □
2. Heart discomfort (unusual awareness of heart beat, heart skipping, heart racing, tightness) .................. □ □ □ □ □
3. Sleep problems (difficulty in falling asleep, difficulty in sleeping through, waking up early) ........... □ □ □ □ □
4. Depressive mood (feeling down, sad, on the verge of tears, lack of drive, mood swings) ................... □ □ □ □ □
5. Irritability (feeling nervous, inner tension, feeling aggressive) ............... □ □ □ □ □
6. Anxiety (inner restlessness, feeling panicky) ............................................ □ □ □ □ □
7. Physical and mental exhaustion (general decrease in performance, impaired memory, decrease in concentration, forgetfulness) ........................................... □ □ □ □ □
8. Sexual problems (change in sexual desire, in sexual activity and satisfaction) ............................. □ □ □ □ □
9. Bladder problems (difficulty in urinating, increased need to urinate, bladder incontinence) ............. □ □ □ □ □
10. Dryness of vagina (sensation of dryness or burning in the vagina, difficulty with sexual intercourse) .......... □ □ □ □ □
11. Joint and muscular discomfort (pain in the joints, rheumatoid complaints) ................................... □ □ □ □ □
Global Distress question added at the end of the MRS:

“On a scale of 1-10, with 10 being the worst imaginable, how distressing were your symptoms today?

1 2 3 4 5 6 7 8 9 10”
University of Arizona College of Nursing

Acupuncture for Symptom Management in a Menopausal Population: An Exploration of Mechanism & Experience

Baseline MEASURES
Cheryl Wright, MSN, MTOM, Principle Investigator
3rd Year Doctoral Student
Dissertation Chair: Judith Berg, PhD, RNC, WHNP
Phone: (520) 312 7171

WASHINGTON WOMEN'S HEALTH QUESTIONNAIRE
The following questions ask you to tell us about your symptoms which relate to midlife. Please answer all questions that apply by circling the correct answer or filling in the appropriate blank. All information will be held in complete confidence by the staff of this research project.

ID# _________________________
BIRTHDATE _________________________
LAST MENSTRUAL PERIOD _________________________
HEIGHT IN INCHES______________________
WEIGHT IN POUNDS__________________
CALCULATED BMI__________________
TODAY'S DATE ___________________________

BACKGROUND INFORMATION
1. What was the highest grade or year of school you completed?
   1 Less than 7th grade
   2 Junior high school
   3 Partial high school (10th or 11th grade)
   4 High school graduate
   5 Partial college or specialized training
   6 College graduate
   7 Graduate degree
   8 Unsure or decline to state
APPENDIX D:

TRANSCRIPT FROM THE AUDIOTAPED RECORDED FINAL QUESTION
Transcription (or verbal report from those who declined to be taped) for study re: “Is there anything you would like to share about your experience during your acupuncture treatment?”

101: “Well, I think that some days I feel a lot better, uh, I, actually am going through other stress, uh, so it’s kind of, maybe, hard to tell, uh, the benefits, of. I did feel relaxed when I was getting the acupuncture, for the most part. Um, I didn’t like the drawing blood or the tape or the needle, that was the worst part of it. I, that’s all, that’s about all I can really think of to say.” Note: During this 4-week study period, a very close friend became suddenly ill and died precipitously. In addition, due to the severe economic downturn, she lost significant means of income and was feeling very anxious about her financial situation.

102 ‘Well, after, I don’t know how many treatments, three or four maybe, um, I started experiencing, uh, I guess I would call it a clarity of thought I haven’t had for a while. Um, a feeling of, um, hope came back, and um, my energy got better. I had more energy to do things, and, um, and them, um, and, the week that followed that, um, I started having dreams which I don’t remember, um, having for a long time, for probably, maybe a couple times a year I’ll remember a dream and, um, this happened twice in one week, so… And, then the other experience has been, um, being able to wake up at a, at a, um, normal time instead of sleeping in a long time and then not feeling rested. So, um, [laughter], I guess that’s, um, about it, just an overall feeling of more energy, and um, [laughter] thank you.”

103: “This is Nancy, and I just finished my last treatment, and um in the beginning I felt better after the acupuncture, but I didn’t see, um, anything right away, as far as, uh, different on my, uh, symptoms. Um but gradually I noticed that the hot flashes were diminishing in intensity and frequency, and um I’m still having a little trouble sleeping, but I think that that has also um diminished. I’m able to go to sleep quicker after I wake up in the middle of the night than I was before. And, um, definitely less anxiety and irritability. um I’ve I have had a lot of stress uh different things happening during the study, but I think I’ve been able to handle it um better than I normally would have. Um, nothing, uh, profound, um, has happened with me as far as, um, any major changes, um, just an overall
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